Chapter

Gastrointestinal Delivery of APIs from Chitosan Nanoparticles

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Abstract

Successful clinical treatment outcomes rely on achieving optimal systemic delivery of therapeutics. The oral route of administering Active Pharmaceutical Ingredients (API) remains formidable because of ease to the patient and convenience. Yet, the gastrointestinal tract (GIT) poses several barriers that need to be surmounted prior to systemic availability, especially for Class IV type drugs. Drug delivery systems in the form of nanoparticles (NP), can be appropriately formulated to alter the physicochemical properties of APIs, thereby addressing constraints related to absorption from the GIT. Polymers offer amenability in the fabrication of NP due to their diversity. Chitosan has emerged as a strong contender in orally deliverable NP because it is biocompatible, biodegradable and muco-adhesive. Due to the positively charged amine moieties within chitosan (NH_3^+), interactions with the negatively charged sialic acid of mucin within the mucosa is possible, which favors delayed GI transit and epithelial uptake. This ultimately results in improved systemic bioavailability. Thus, we expect research in the use of chitosan in oral NP delivery to intensify as we transcend the frontier toward clinical testing of viable formulations.

Keywords: chitosan, gastrointestinal, cellular uptake, nanoparticles, drug delivery, formulation

1. Introduction

According to the US National Institute of Health, drug delivery is a process that permit the influx of therapeutic substances in to the body. Drug delivery systems are designed to enhance the efficiency and safety of therapeutics by regulating the rate, time and place of release in the body [1, 2]. Drug delivery technology has emerged as an essential tool for the improvement of drug bioavailability, reduction in the side effects of medication, all of which generate remarkable clinical outcomes [3]. Drugs may be administered to the body via local application, enterally or parenterally. The parenteral route typically relates to administration that excludes absorption from the gastrointestinal tract (GIT). It consists of administration by injection, inhalation and via transdermal routes. The enteral route is associated with the absorption of the drug via the GIT, this includes oral, sublingual, and rectal administration. Aptly, the mode of drug administration depends on the disease, the desired therapeutic effect and the nature of the product available [4]. Moreover, each delivery route has inherent benefits and constraints. Nevertheless, the majority of manufactured medicines in the pharmaceutical industry are delivered orally, owing to the distinctive advantages offered by this route, including versatility in

accommodating various types of drugs, simplicity of administration and accessibility, patient compliance and safety profiles [5–7]. Additionally, the intestinal epithelium is an ideal platform for drug absorption due to the viscous mucosal layer lined with abundant enterocytes, goblet cells, and Peyer's patches that trap drug molecules within the mucus as they transit the GIT. [8, 9].

In comparison to other routes of administration, the oral route is exceptionally complex in expression of anatomical features physiology throughout the GIT [10]. Furthermore, these expressions vary along the GIT in both intensity structure. For example, the mucus layer varies in composition and physical properties along the GIT and the pH varies significantly in the main sections of the GIT. The gastrointestinal motility also varies in intensity and form along the GIT and also depends on food status [11]. Even though, these features can impede drug delivery across the GIT, through careful interplay between formulation science and GIT physiology, scientists have been able to exploit this variance for improved drug delivery. In this regard, nanoparticle formulations have immerged as strong contenders able to surmount some of the constraints associated with GIT absorption. Nanoparticles have gained great interest by researchers in recent years as they can be used to improve drug solubility and bioavailability in the harsh GIT environment due to increased surface area to volume ratio, thus provide a rapid onset of therapeutic action [12]. They can also be used to targeting specific sites within the GIT and hence reduce the effects of enzymatic degradation, all of which can improve the safety and effectiveness of drugs [12, 13].

Nanoparticle formulations may be presented in various forms however, polymeric nanoparticles present the versatility of polymers and can be tailored to achieve superior drug stability, enhanced drug payload capacity, longer circulation times and controlled drug release capabilities, when compared with other their colloidal counterparts [14, 15]. In this regard, chitosan-based nanoparticle formulation have been shown to present several of the desirable attributes listed above in addition to being biodegradable, having low toxicity, amenable to tuneable physical properties and bio-adhesive properties [16, 17].

In this chapter we will be discussing the interplay between the GIT physiology/anatomy and drug physicochemical/biopharmaceutical factors in the absorption process that influence oral therapeutics. We, will also review the physicochemical properties of chitosan relevant for effective GIT delivery, including methods of formulation. The most utilised nanoparticle formulation methods used for chitosan-based nanoparticles are also examined. Finally, we will highlight the recent developments on chitosan-based nanoparticles used in the oral delivery of different drugs.

2. The GIT

The GIT, also known as the digestive tract or alimentary canal, is approximately 9 meters long and can be functionally divided into two parts, the upper and the lower GIT (**Figure 1**). The upper GIT; consisting of mouth, pharynx, oesophagus, stomach and small intestine, play a major role in the transport of the swallowed food bolus, enzymatic digestion and absorption of nutrients [18]. The lower GIT is usually referred to the large intestine and is responsible for the adsorption of water, fermentation of undigested sugars and the storage and evacuation of stool [19]. Following oral dosing, the drug traverses several semipermeable cell membranes through its trajectory to absorption and eventually enters the general circulatory system. Drugs cross cell membranes, which comprise of bimolecular lipid matrix, either by passive diffusion or active transport.

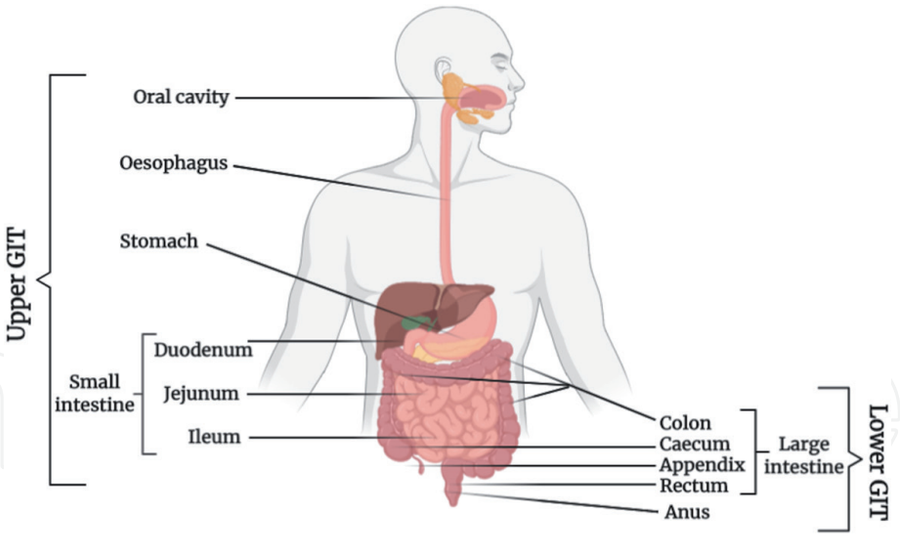

Figure 1.
The anatomy of the human gastrointestinal tract.

2.1 Passive diffusion

The most prevalent form of absorption of the majority of orally administered drugs is by passive diffusion across cell membranes. This process comprises of a three-step process, whereby the permeant first transverses into the membrane, disperses across it and then is released into the cytosol [20]. Typically, drug molecules move down a concentration gradient, from a region of high concentration (e.g., GI fluids) to one of low concentration (e.g., blood), without the expenditure of energy [21]. Usually, a concentration gradient is manifested as a disparity in concentration of a substance within an area and is linearly related to the diffusion rate. The latter is also governed by the lipid solubility, size and polarity of the drug species.

Most drugs are either weak acids or bases and occur either in the unionized or ionized form as a function of pH [22]. For lipophilic drugs, the unionized form of drug, may penetrate cell membranes easily as the membrane is lipoidal. On the other hand, hydrophilic drugs, present an ionized form of the drug, which has high electrical resistance and thus cannot traverse the cell membranes easily but may diffuse through the para-cellular spaces. However, it is worth noting that the para-cellular junctions contribute to less than 0.01% of the entire GIT surface area and furthermore, the permeability of these junctures diminishes down the GIT [23]. Additionally, the capability of drugs to traverse a membrane also relies on the acid–base dissociation constant (pKa) of the drug in question. The pKa is the pH at which concentrations of ionized and unionized forms are equivalent [24]. So, if the pH is less than the pKa, the unionized form of a weak acid prevails, and *vice versa* for weak bases. Henceforth, when a weak acid is administered orally, nearly all the drug in the stomach remains unionized, preferring diffusion via the gastric mucosa. On the other hand, for a weak bases with a pKa = 4.4, majority of the drug in the stomach will be ionized [21].

Overall, the process by which molecules traverse cell membranes is by passive diffusion, down the concentration gradient. However large hydrophilic ionic molecules and charged molecules cannot freely traverse the phospholipid bilayer cell membrane passively. Their transport may be confined to protein channels and distinct transport mechanisms present within the membrane [25]. Such drugs gain access through the membrane by facilitated diffusion whereby molecules integrate with embedded protein carriers to shuttle them across the membrane. This process does not expend energy and is also down the concentration gradient though quicker than

would be anticipated by diffusion alone [26]. A frequent case of facilitated diffusion is the migration of glucose into cells during the production of adenosine triphosphate (ATP). Glucose is both large and polar thereby unable to pass the lipid bilayer via simple diffusion. Hence, glucose molecules are delivered into the cell via a unique carrier protein (glucose transporter) to promote its internalisation in cells [27].

2.2 Active Transport

Active transport is an energy-dependent process that translocates drug molecules against their concentration gradient by a molecular pump [20]. Carrier-mediated active transport demand energy via ATP hydrolysis or by accompanying the co-transport of counter ions down its electrochemical gradient (e.g., Na^+, H^+, Cl^-) [28]. The most common active transport system is the sodium-potassium pump and receptor-mediated endocytosis. Energy can either be directly provided to the ion pump or indirectly by connecting a pump-action to an activated ionic gradient. It is often encountered in the gut mucosa, the liver, renal tubules and the blood–brain barrier [22]. Active transport is typically restricted to drugs that structurally resemble endogenous substances; e.g., vitamins and amino acids, and that are absorbed via specific sites in the small intestine. Targeting drugs to these transporters can enhance their bioavailability and distribution [21].

The sodium-potassium pump system (Na^+/K^+ ATPase), utilises ATP to move Na^+ and K^+ in and out of the cell. It is a vital ion pump located in the membranes of various cell types, such as the Na+/amino acid symport in the mucosal cells of the small bowel [22, 29].

Cells control the endocytosis of certain substances via receptor-mediated endocytosis. The use of this form of endocytosis in the GIT is crucial for oral delivery of drugs because it delays the transit of drugs in GIT. Receptor-mediator endocytosis involves the internalisation of macromolecules by binding the latter to receptors considered as membrane-associated protein [30]. There are more than 20 different receptors involved in the internalisation of macromolecules [31]. Following binding to the receptor on the cell surface, the cell will endocytose the portion of the cell membrane enclosing the receptor-ligand complex via a clathrin-dependent endocytic process [28]. Clathrin plays a significant role in the formation of clathrin-coated pits; invaginated regions of the plasma membrane, and pinch off to form clathrin-coated vesicles that transport molecules within cells [31].

In summary, drug adsorption may occur passively or via active transport. In either case, absorption occurs predominantly in the small intestine due to its more permeable membrane and larger surface area provided by the microvilli. Even though, the stomach has a relatively broad epithelial surface, yet the dense mucus layer and transient transit times expended by dosage forms contribute to an impeded absorption. Moreover, the colon with an absorptive surface area of about $5m^2$ has negligible contribution to drug absorption in GIT, due to slow caecal arrival times of dosage forms, the presence of numerous gut bacteria and solid stool that impede lateral diffusion. All in all, absorption of oral drugs is interlinked and controlled by various intrinsic factors; like drug solubility, dissolution and permeability across the mucosal barriers, and physiological factors; such as gastrointestinal transit time, pH and gut microbiome [13, 32].

2.3 Drug dissolution, solubility and permeability

Drug dissolution, solubility and permeability are the three fundamental parameters used in the Biopharmaceutics Classification System (BCS) to predict the factors limiting drug absorption from GIT [33]. The BCS is recognised as a useful

tool for designing drug delivery systems and is adopted by the US Food and Drug Administration (FDA), the European Medicines Agency (EMA) and the World Health Organization (WHO) [34]. According to the BCS, all drug substances are classified into four categories: class I—high soluble and high permeable, class II—low soluble and high permeable, class III—low soluble and high permeable and class IV—low soluble and low permeable [35].

Drug solubility is crucial outcome in pharmaceutical dosage form. In the BCS system, a drug is deemed highly soluble when the maximal dose strength is soluble in 250 mL of aqueous media across the pH range of 1 to 7.5 [35]. However, more than 40% of the established new chemical entities in the pharmaceutical sector are considered insoluble in water, causing inadequate bioavailability [36]. This makes solubility amongst the most important rate limiting parameters in GIT absorption.

Drug dissolution reflects a dynamic consequence to drug absorption [33], whereby drug is released, dissolved and made accessible for absorption. With the exception of enteric formulations and drugs with low acid solubility, the dissolution process for majority of drugs starts in the stomach where the volume of gastric fluid is sufficient to attain effective drug dissolution [37]. Thus, the gastric fluid containing the disintegrated immediate-release dosage forms brings the solubilized drug into contact with the absorptive surface of the small intestine as absorption in the stomach is generally minimal.

Drug permeability represents the final frontier in the sequence of rate-liming steps to systemic drug availability. It is a measure of the ease of permeation of the drug across the intestinal wall. There is a positive association between the intestinal permeability and drug solubility GI milieu, which in turn depends on the physicochemical characteristics of the drug [38], including the pKa, particle size, lipophilicity, as discussed in the sections below. The ultimate amount of drug absorbed from the GIT also bears dependence on its transit time in the GIT [39].

2.4 Gastrointestinal pH

The GI pH influences the extent of ionization of drug molecules and thereby impacts on its absorption across the epithelium. Variations in pH across the GIT can be exploited for delayed drug release in desired section of the GIT in order to achieve efficient absorption. The fasted stomach is acidic, with pH range of 1–3, which increases upon food or liquid intake. Food is known to buffer the acidic content of the stomach. A rise in pH resumes in response to the continual gastric secretion and then finally, the pH reverts to the original levels due to gastric emptying of content; [40]. The gastric emptying rate significantly affects the rate of drug absorption because it regulates arrival in the duodenum, where the epithelial surface is suited for absorption [41]. Moreover, the disparity in gastric pH conditions affects the drug delivery behaviour of modified release dosage forms such as enteric coated products, where the onset of release along with the overall release kinetics may be changed [42].

The arrival of orally administered dosage forms into the small intestine is met by a pH of about 6 in the duodenum through to pH 7.4 at the terminal ileum [43]. This high pH variability is due to duodenal secretion of alkaline bicarbonate. During postprandial state, the initial intestinal pH drops due to the influx of acidic chyme, which is buffered by bicarbonate secretion as it travels distally [13]. Besides, the mean pH in proximal small intestine during the first hour of transit is usually 6.6, which is further decreased to 5–6 in the distal duodenum [44].

Typically, the pH in the caecum drops to just below pH 6 owing to the fermentation processes of the colonic microbiota and then rises to pH 7 at the rectum [42]. The drop in the amount of short chain fatty acids at the distal colon causes the

secretion of colonic mucosal bicarbonate that leads to a neutral pH. Short chain fatty acids are the end products of fermentation of dietary fibres by the anaerobic intestinal microbiota [45]. As a consequence of the neutral pH of the colonic luminal fluid, the solubilisation of drug is the rate-limiting factor in colonic drug absorption [46]. The unspecific interactions of drugs with colonic content (e.g. dietary residues, intestinal secretions or faecal matter) all adds to the odds of effective adsorption across the colon [47].

2.5 GIT transit time

Generally, the GIT transit time of most orally administered doses through buccal cavity and oesophagus is transient. The stomach is naturally the first segment of the GIT, wherein disintegration and dissolution of solids such as drugs and formulations occur [42]. The period required for a dosage form to exit the stomach is inconstant and relies on several physiological factors, such as age, body posture, gender and food intake [48]. Gastric transit can span from 0 to 2 h in the fasted state and can be extended up to 6 h after food intake [47]. The small intestine is the region of choice for drug absorption with a transit time ranging from 2 to 6 h in healthy individuals. The dissolution of poorly soluble, weakly acidic compounds and lipophilic compounds is greatly enhanced in this region [13]. In colon-specific drug delivery, the drug has to cross the whole GIT prior to arrival at the colon. Thus, the transit time across the colon can be highly variable, and ranges from 20 to 56 h in healthy humans, although higher variations are also reported in literature amounting up to 72 h [42, 49, 50]. Variations in colonic transit time are affected by dosing time, bowel movements as well as gender, whereby females generally have longer colonic transit times than males [51, 52].

2.6 Gut microbiome

Enzymatic and microbial degradation of GIT content affects the amount ultimately made available for absorption. The active sites for most endogenous enzymes are the stomach and small intestine. Even though these enzymes may affect the stability of orally administered drugs, it is possible to exploit this property for regional drug delivery of formulations in the GIT [47]. On the other hand, the intestinal microbiome which includes 500–1000 bacterial species is also important for the digestion of food and the metabolism of drugs [53]. Gastrointestinal microbiome is found in both upper and lower GIT, whereby, a lower bacterial number (10^{13}–10^{14} bacteria mL^{-1} of intestinal content) is in the upper GIT because of the fast luminal flow, intestinal fluid volume, and the secretion of bactericidal compounds in this part of the GIT, and highest bacterial community (10^{10}–10^{11} bacteria mL^{-1} of intestinal content) is in the colon, in which the redox potential is low and the transit time is long [54, 55]. Therefore, greater number of the intestinal microbiome exists in the anaerobic colon, in which the fermentation of carbohydrates contributes to their nourishment. Usually, orally administered drugs are transformed to bioactive, bio-inactive, or toxic metabolites by the gut microbial population, all of which can impede the bioavailability of drug. However, gut microflora can improve drug bioavailability by eliminating polar moiety from derived conjugates and thereby promoting biliary recycling of compounds [13].

Thus, formulation scientist must be cognizant of the interplay between drug and physiological and anatomical manifestations within the GIT when designing orally administered dosage forms. For example, enteric coating can be applied to dosage forms to delay the release of the API in the acidic gastric fluid until pH above 5.0 [56]. Enteric coating may also be used to shield acid-labile drugs from gastric

distress, and upon arrival to the alkaline pH milieu, the enteric polymer coating disintegrates within the intestinal fluid, releasing the drug [57]. Despite employing such coatings and other conventional interventions, numerous pharmaceuticals still display insufficient bioavailability through the oral route of administration. This necessitates the use of alternate strategies. One area of research that is gaining traction more recently is the employment of nanoparticles.

3. Nanoparticle technology

Nanoparticle technology is a multidisciplinary field that utilizes principles from chemistry, biology, physics and engineering to design and fabricate submicronic (< 1 μm) colloidal systems [58]. Nanotechnology has several pharmaceutical and medical applications wherein nanoparticles (NPs), with sizes comparable to large biological molecules such as enzymes can be employed in the delivery of therapeutic agents [59]. The effectiveness of the nanoscale drug delivery vehicles lies on their ability to attain the following key attributes [60]:

- The NP must be able to bind or contain the appropriate drug.
- The nanocarrier must stay stable in the serum to allow systemic delivery of the therapeutics and only release the drug once at the required site.
- The NP-drug complex has to reach the required site either via receptor-mediated interactions or by the enhanced permeability and retention (EPR) effect.
- The residual NP carrier should ideally be made of a biological or biologically inert material with a limited lifespan to allow safe degradation.

There are several types of NP drug delivery systems, which may be broadly divided as organic and inorganic NPs [61]. Their particle size, surface charge (ζ potential), hydrophilicity/hydrophobicity, composition, etc. can be tailored for a diverse applications [62]. The primary consideration when designing orally administered NP drug delivery system is to maximise drug concentration in the GI therapeutic window.

3.1 Oral organic NP

Organic NP (**Figure 2**) are solid particles comprised of organic compounds (usually lipidic or polymeric) ranging from 10 nm to 1 μm [63]. They can be formulated by simple techniques to encapsulate therapeutic agents. Preferably, compounds used in formulation of organic NPs should be biodegradable and biocompatible [61]. Manifestations of organic NP include liposomal, polymeric and solid lipid NP, each system possessing requisite features that addresses physiological and anatomic constraints addressed in sections above. In addition, others systems such as micelles, dendrimers etc. have been also explored as effective nanocarriers for effective deployment of APIs in the GIT [14, 64].

3.2 Oral inorganic NP

Inorganic NP represent a wide spectrum of systems synthesized from metals, metal oxides, and metal sulphides [65]. Gold, silica and superparamagnetic oxide NP are among the long list of inorganic NP (**Figure 3**). They have been studied for

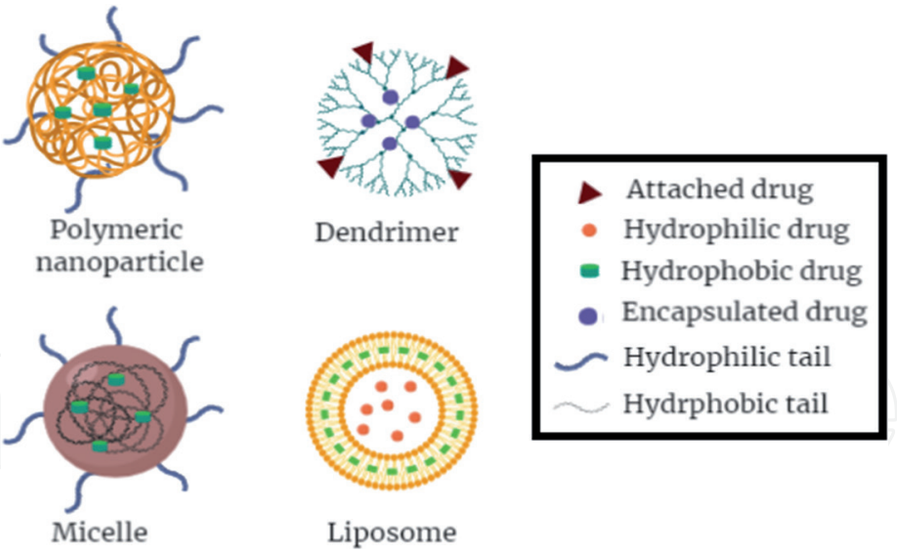

Figure 2.
Examples of organic nanoparticle platforms for drug delivery.

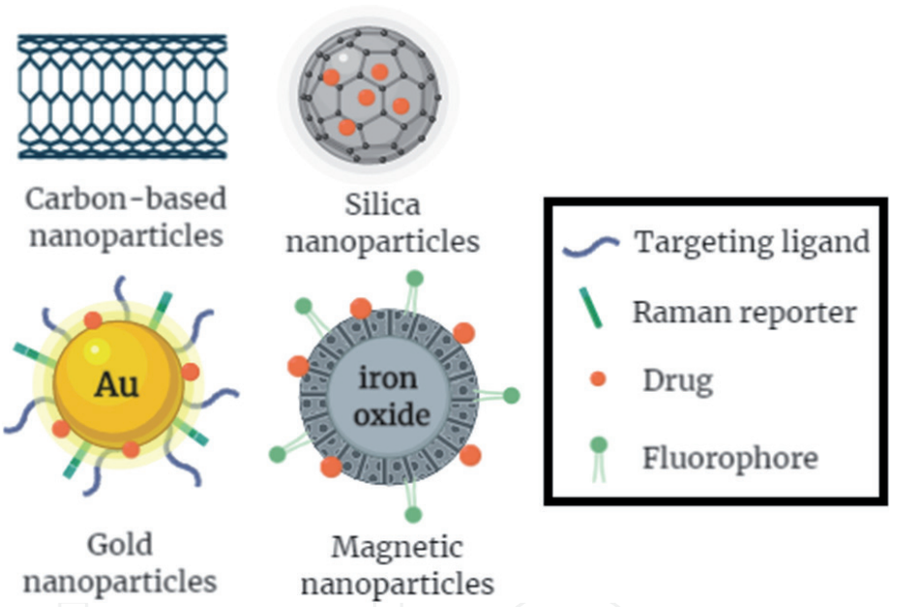

Figure 3.
Examples of inorganic NP platforms for drug delivery.

use in imaging on nuclear magnetic resonance and high-resolution superconducting quantum interference devices, and their intrinsic properties have been utilised for therapy [66]. Inorganic NP can easily be conjugated to ligands for tumour targeting and/or with chemotherapeutics for tumour therapy. Additionally, their surface composition can be feasibly manipulated to create NP that can escape the reticulo-endothelial system [67]. Even though inorganic NP present good stability characteristics, they have not been the focus of attention in oral NP research, possibly due to concerns on the degradation and elimination end products, which can be potentially toxic [68].

Generally, inorganic NPs differ conceptually from organic NPs in terms of fabrication principles. Inorganic NPs can be formed by the precipitation of inorganic salts, which are linked within a matrix, whilst, most organic NPs are formed by several organic molecules through self-organization or chemical binding [61]. Notwithstanding, both types of NP are very promising in the formulation of oral

delivery system and forms part of the evolutional success in several clinical applications. Polymeric NP arguably presents more desirable attributes as orally delivered NP because of their higher stability, enhanced drug payload and controlled drug release capabilities compared with their colloidal counterparts [14, 69].

3.3 Polymeric NP

According to Alexis F. et al., polymeric NP represent the most effective nanocarrier system for prolonged drug delivery [70]. 'Polymeric NPs' include any type of polymer formed as NP. Nanospheres are solid spherical NP with molecules attached or adsorbed to their surface, whilst nanocapsules are vesicular systems with substances confined within a cavity consisting of a liquid core (either water or oil) surrounded by a solid shell [71]. Characteristic properties of polymers such as molecular weight, hydrophobicity and crystallinity can be explored to manifest controlled drug release kinetics and entrapment of therapeutic agents [72]. Polymers also provide significant flexibility in the design of oral NP and many exhibit biodegradability [73]. In this regard, synthetic and natural variants have been studied. For example poly-lactic-co-glycolic-acid (PLGA) and poly-lactic-acid (PLA) are synthetic whilst natural polymers include gelatine, dextran, and chitosan [74]. The use of natural polymers is preferred over the synthetic ones as the former usually exhibit less toxicity, widely available and have lower production costs [75]. Chitosan is arguable one of the most studied polymer in NP formulation in view of its distinctive properties. In orally administered NP, chitosan offers added desirability including muco-adhesiveness, augmenting the dissolution rate of poorly water-soluble drugs; useful in drug targeting in the GIT [76].

4. Chitosan polymer

Chitosan is a hydrophilic, cationic polysaccharide soluble in dilute acids such as acetic acid and formic acid, due to protonated amine groups ($NH3^+$) [75]. It is an N-acetylated derivative of chitin, a natural polysaccharide found in the shells of marine crustaceans. Chitin is chemically inert and thus has fewer applications that chitosan [77]. The acetamido group of chitin, (C_2H_4NO) can be turned into amino group to yield chitosan by the alkaline deacetylation of chitin. Chitosan is approved as safe by the United States Food and Drug Administration (US-FDA) for dietary use and wound dressing applications, but its toxicity increases with electrical charge and degree of deacetylation [17]. Chemically, it comprises of β- [1–4] -linked D-glucosamine and N-acetylated units (**Figure 4**).

The amine group has pKa of 6.2–6.5 [78]. At slightly acidic pH values, the amine groups ($NH3^+$) become protonated, hence possessing the ability to effectively form electrostatic interactions with negatively charged species within mucin in the GIT [75]. Positively charged moieties of chitosan also interact with the tight junctions of the intestinal epithelial cells and thus modulate drug permeation and absorption through the interstitial space between epithelial cells [79]. Moreover, the existence of both hydroxyl and amino groups offers various possibilities for chemical modification. Chemical modifications give rise to different functional derivatives of chitosan like carboxylation, thiolation, alkylation, acylation etc. that further imparts desirable physiochemical and biopharmaceutical properties, such as solubility, adsorption and pH sensitivity in oral drug delivery [80]. For example, N-trimethyl chitosan chloride is developed to amplify the intestinal solubility of chitosan; thiolated chitosan is produced to augment the mucoadhesiveness of chitosan;

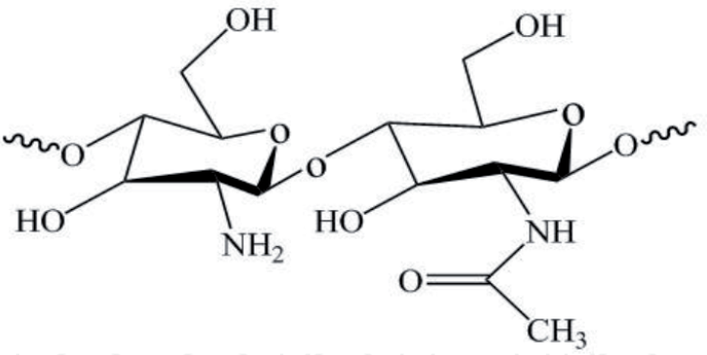

Figure 4.
Chemical structure of chitosan, comprising N-acetyl-D-glucosamine (right) and D-glucosamine (left) units.

quaternization of chitosan reinforces its impact on the tight junctions of the GIT epithelium whilst grafting carboxylated chitosan with poly(methyl methacrylate) imparts increased pH sensitivity [81]. Physical modification through blending with other polymers may be used to enhance desirable physical properties. For example, blending of chitosan with polyethylene glycol (PEG) and polyvinyl alcohol (PVA) ameliorate the hydrophilic property of chitosan, while blending of chitosan with cellulose improves its antibacterial properties [82].

4.1 Mucoadhesion from chitosan

Some of the key desirable features in orally administered dosage forms is delayed GI transit in the duodenum and ability to traverse the epithelium effectively. In this regard, chitosan-based NP have been shown to possess these attributes. Mucoadhesion refers to the adhesion between two materials, one of which is mucosal [83]. It can be utilised to prolong the GI transit of dosage forms in the duodenum, thereby improving bioavailability. Delayed transit results from interactions of positively charged moieties in chitosan with negatively charged moieties in sialic acid within mucin [81]. Chitosan is also capable of physically penetrating the mucous network. Prolonged GI residence results in higher net drug flux across the GIT membrane. Drug flux is a combination of passive diffusion and uptake of whole NP by Peyer's patches [84]. Moreover, chitosan offers controlled drug release capabilities via diffusion from the matrix. Yin et al. prepared thiolated trimethyl chitosan NP for the oral delivery of insulin, where increase in the mucoadhesion resulted in increased insulin transport through rat intestine and uptake by Peyer's patches compared to controls. They attributed these results to the disulfide bond formation between the NP and mucin [85]. Overall, to achieve the desired properties of interest such as particle size, particle size distribution and area of application, the mode of preparation of chitosan NP plays an essential role.

4.2 Fabrication methods for chitosan NP

The preparation of chitosan NP is principally divided into two approaches. The first approach is based on a two-step procedure, where an emulsification system is carried out to generate nanodroplets in which organic compounds (polymer, monomer, and lipid) are solubilized, followed by precipitation or polymerisation into NP [61]. The second approach involves a one-step procedure where the NP are directly generated via different mechanisms such as nanoprecipitation or ionic gelation [86]. An example of each of the two general approaches is summarized in the following.

4.2.1 Chitosan NP by ionic gelation

Ionic gelation, also known as ionotropic gelation or polyelectrolyte complexation involves the gradual addition of a cross-linking agent (tripolyphosphate, glutardehyde etc.) into an aqueous solution of chitosan under continuous stirring to form hydrogels [87]. The polyanions from the cross-linker forms a meshwork of structures by interacting with the polyvalent cations within chitosan, leading to gelation [88]. APIs can be loaded into these hydrogels during the production where it becomes encapsulated or added to the formed NP, where it can be adsorbed into the matrix. The choice of the cross-linker should be matched to the desired physical characteristics of the NP, such as mechanical strength, as well as safety profiles. For example, glutardehyde reported to be toxic when used in high concentrations and results in NP with low mechanical strength. This has been attributed to its double bond (–C=N–) association with the amine group in chitosan [89]. Genipin is a natural cross-linker obtained from iridoid glucoside (geniposide) and present in gardenia fruits that can be cross-linked with chitosan. It displays slower degradation rate than glutaraldehyde and possess higher biocompatibility. Sodium tripolyphosphate (STPP) displays better crosslinker characteristics than each of the above because of its inorganic nature and consequently, results in production of chitosan NP with better mechanical stability. The size dimension derived from STPP gelled chitosan NP is of lower order as well. Another attractive feature of STPP is that it is nontoxic, relatively inexpensive, multivalent, has quick gelling property and thus, widely utilised as a crosslinker in chitosan-based NP [90–92].

4.2.2 Emulsion evaporation

Polymeric nano-emulsions are formulated whereby organic solvent is added to a solution of chitosan with surfactant and mixed via sonication [93]. Basically, the emulsion droplets are converted into NP suspension as the organic solvent evaporates by continuous magnetic stirring at room temperature. The NP suspensions are then centrifuged, washed with distilled water to remove additives such as surfactants and finally lyophilized [94]. Poovi et al. encapsulated the poorly water-soluble drug, repaglinide, into chitosan NP using the emulsion evaporation for sustained release. They proved that the NP exhibited a controlled release of repaglinide and obtained a high drug loading (11.22% w/w) and encapsulation efficiency (97.0%) [95]. In another study, Lee et al. employed solvent evaporation method to formulate polymeric NP from chitosan derivatives fluorescein isothiocyanate (FITC) - conjugated glycol CSs (FGCs) using diluted chloroform as the solvent. Size range of 150–500 nm were obtained and the NP remained stable in phosphate buffered saline for 20 days at 37°C [96].

4.3 In vitro drug release from chitosan NP

In vitro drug release studies give us insights on the response of formulated delivery systems to challenges in *in vivo*. The rate and extent of *in vitro* drug release from chitosan-based NP is influenced by a host of factors, notably, shape and size of the of the delivery system, physicochemical properties of the drug and external media [97]. Three primary mechanisms govern the drug release from chitosan NP, which includes desorption (release of drug from surface), diffusion, and erosion/degradation of the particle matrix [98]. Erosion or degradation of polymers lead to successive physical depletion of the polymer as chains and bonds break [99]. Drug release from the chitosan NP matrix is often pH dependent because of the solubility of chitosan in acidic media [100]. In acidic media, the matrix swells or disentangles

and may act as an effective barrier to drug diffusion. The extent of drug diffusion through this gelled matrix depends on the diffusivity of the drug [99]. In alkaline media, the polymer matrix does not swell and drug release is controlled mainly by passive diffusion into the medium and the polymer plays an insignificant role in the drug release profile. If the drug is weakly bound to the surface of the NP, an initial burst release occurs [97]. In vitro drug release from chitosan NP usually show a two-step pattern with an initial rapid release followed by sustained release [101]. Patel et al. observed that rifampicin- chitosan NP presents a burst effect in the early stages followed by slow sustained drug release in which 90% of rifampicin was released in the range of 28–34 h. A good correlation fit was obtained between the cumulative drug released and square root of time, signifying that the drug release from the NP is diffusion-controlled as described by the Higuchi model. They concluded that rifampicin release from chitosan NP is pH dependent, i.e., faster at a lower pH than around neutral pH [102]. Similarly, Avadi et al. observed that insulin-loaded gum arabic/chitosan NP present a burst effect release in acidic medium, relating it to high solubility of both chitosan and insulin. No burst release was observed at higher pH values of 6.5 and 7.2. They concluded that the release followed a non-Fickian transport, governed by diffusion and/or swelling of the chitosan chains [103]. The performance of chitosan NP in the GIT depends on its response to the external milieu as discussed above. Equally important is how the GIT responds to the presence of NP. The following section describes the consequence of NP deployment in the GIT in the management of selected diseases and expected responses.

4.4 Chitosan as an oral delivery vehicle for therapeutics

As mentioned in sections 4.1 and 4.2, extensive research presented the potential of chitosan as an oral absorption enhancer owing to its mucoadhesive properties and ability to loosen tight junctions within the GI epithelia, hence permitting the passage of macromolecular therapeutics across a "well-organised" epithelia [100]. Moreover, due to various characteristics; i.e. non-toxic, biodegradable, biocompatible, antimicrobial property etc. [104], chitosan NP hold promise as a suitable oral delivery vehicle for a wide spectrum of therapeutics including, anti-cancer drugs, antibacterial agents, polyphenolic compounds and protein drugs.

4.4.1 Anti-cancer drug delivery

Chemotherapeutic APIs usually exhibit low bioavailability following oral administration. Several studies have investigated chitosan-based NP as a possible delivery system to address this issue. For example, doxorubicin (Dox), broadly employed to treat breast, bladder and other cancers, is typically delivered intravenously. The oral bioavailability of Dox is low due to efflux transporter P-glycoprotein, which identifies Dox as a substrate, restraining its cellular uptake [105]. In 2013, Feng et al. developed chitosan/o-carboxymethyl chitosan (CS/CMCS) NP as a pH responsive carrier for the oral delivery of Dox. They investigated the bioavailability of orally administered Dox-CS/CMCS NP and free Dox drug on Sprague–Dawley rats. Negligible Dox was detected in plasma after the oral dosage of free Dox, representing its poor absorption. On the other hand, 2.3-folds increase in plasma concentration of Dox was registered after an oral dose of Dox-CS/CMCS NP. Moreover, accumulation of Dox in the liver, spleen and lungs were demonstrated in rats treated with oral Dox- CS/CMCS NP, as opposed to DoX solution which was more concentrated in the kidneys. They concluded that the NP matrix improved the intestinal absorption of Dox and thus improved oral bioavailability [106].

Gemcitabine (Gem) is a widely prescribed anticancer agent used in pancreatic, lung and advanced colon cancer. Oral administration of Gem results in low oral bioavailability, high first-pass clearance gastrointestinal toxicity, such as nausea, vomiting and diarrhoea [107]. Hosseinzadeh et al. synthesised and characterised chitosan/Pluronic® F-127 (Gem-Chi/PF) NP in oral delivery of Gem for the treatment of colon cancer. *In vitro* studies showed that the NP presented enhanced cytotoxicity effects against HT-29 cell line and concluded that Gem-Chi/PF NP is a potential candidate for colon cancer treatment [108].

4.4.2 Anti-bacterial agent delivery

Chitosan impedes the growth of bacteria, fungi, and yeast [109]. It exhibits potential antimicrobial properties at pH below 6.0 because of the positively charged – NH_3^+ at the C-2 position within the glucosamine. Low molecular weight chitosan derived NP integrate with bacterial DNA, impeding mRNA synthesis. Conversely, the NH_3^+ in high molecular chitosan derived NP interact with the negatively-charged cell wall in microorganisms and subsequently amend cell permeability [110]. Alqahtani et al. formulated chitosan NP from high and low molecular weight variants to encapsulate the non-antibiotic diclofenac sodium (DIC). The antibacterial properties of NP from low and high molecular weight of chitosan on *Staphylococcus aureus* and *Bacillus subtilis* was significantly higher than from DIC alone. The antibacterial activity of chitosan was higher from the high molecular weight chitosan at pH = 5.5 [111]. In another *in vitro* study, Qi et al., investigated the antibacterial activity of chitosan NP and copper-loaded chitosan NP against various microorganisms (*E. coli, S. choleraesuis, S. typhimurium and S. aureus*). The antibacterial activity of chitosan NP and copper-loaded chitosan NP were significantly higher than from chitosan and doxycycline alone. Furthermore, copper-loaded NP indicated higher antibacterial activity against microorganism compared to chitosan NP void of copper. They concluded that this is due to the higher surface charge density of copper-loaded NP that improves the affinity of the cargo with the negatively charged bacteria membrane. Clearly, the antimicrobial property of chitosan is demonstrable and may augment the antibacterial effects of therapeutic antimicrobial when administered orally.

4.4.3 Polyphenolic compounds delivery

Secondary plant metabolites in the form of polyphenolic compounds have gained wide attention by scientists due to their wide spectrum of pharmacological activities, including antioxidant, antimicrobial and anticancer properties. Most however suffer from poor systemic bioavailability following oral administration due to low solubility and susceptibility to GI degradation. To overcome this constraint, chitosan-based NP have been proposed as a possible delivery intervention, which not only protect these APIs from GI degradation but also improves bioavailability [112]. Curcumin (CUR) is a polyphenol that has been studied extensively. It is derived from the rhizomes of *Curcuma longa* and active against a range of cancers in *in vitro* setups [113, 114]. However, preclinical and clinical data indicate that oral administration of CUR results in poor systemic bioavailability and high susceptibility to metabolic degradation [115]. In a study by AlKhader et al., the pharmacokinetic and anti-colon cancer properties of curcumin-containing chitosan-pectinate NP (CUR-CS-PEC-NPs) were evaluated. The cellular uptake and subsequent anti-proliferative effects of the CUR-CS-PEC-NPs were boosted at low CUR concentration after 48 and 72 hours of treatment compared to free CUR at equivalent dose. Besides, the carrier provided protection to CUR from acidic degradation. After oral administration of CUR-CS-PEC-NPs and free CUR at

10 mg/ml in rats a 4-fold increase in CUR concentration was detected compared to that of free CUR. Their findings indicated a null release of CUR in the upper GIT and a successful delivery of CUR to the colon with increased bioavailability of delivered CUR with time from CUR-CS-PEC-NPs for 24 h. Hence, rapid degradation metabolism of free CUR was noticed at the same duration. They concluded that this formulation may serve as a suitable delivery system for CUR to the colon in which CUR will be available on site for its chemotherapeutic activity against tumour [116].

4.4.4 Protein drug delivery

Proteins are the building blocks of life and required in replicating organisms. Their high molecular weight, chemical and enzymatic susceptibility in the GIT, low diffusion rate through the mucosa barrier and fast systemic clearance, limit their delivery via oral route. As a result, most proteins are administered parenterally. Fortunately, chitosan-based NP are emerging as promising means for the delivery of protein drugs by the oral route through a combination of shielding GI pH, enzymatic degradation and facilitation of epithelial uptake [117]. In a study by He et al., chitosan-STPP insulin NP (CS/STPP/insulin) were orally administered to Type I diabetic rat models in comparison to free insulin solution. Free insulin solution failed to elicit any difference in the blood glucose level, whilst CS/STPP/insulin NP distinctly reduced the blood glucose levels by up to 59% within 8 hours. Crucially, CS/TPP/insulin NP allowed for a fast recovery of blood sugar level when fasting was halted. Moreover, the CS/TPP/insulin NP exhibited negligible toxicity to liver enzymes, confirming the safety profile of the orally delivered CS/TPP/insulin NP. They concluded that CS/TPP NP are an effective oral delivery vehicle for insulin [118]. In another study, Tan et al. demonstrated better *in vitro* uptake and safety profile from amphotericin B-containing chitosan coated nanostructured lipid carrier (ChiAmpB NLC) than from uncoated NLC [119]. The same authors later demonstrated better *in vivo* uptake from ChiAmpB NLC in rats than from uncoated NCL [120]. They attributed the observed increase in systemic bioavailability to increased mean absorption and mean residence times (MAT and MRT) from ChiAmpB NLC than from naked NLC. This was prompted by the mucoadhesive effect imposed by chitosan.

5. Conclusion

The oral route of administration remains formidable in the systemic delivery of therapeutics. It affords patient compliance, ease of administration and flexibility and remains the favourite choice for administration by patients. However, orally administered therapeutics may undergo premature release in the upper GIT which may render them to enzymatic or pH degradation. Therapeutics that are delivered to the absorptive window are susceptible to efflux pump and metabolic enzymes (e.g., cytochrome P450 enzymes) within the GIT epithelia, which is itself a structural barrier. Scientist involved in the design of therapeutics intended for GI delivery must be cognizant of the above constraints and balance these with the physicochemical properties of the therapeutic. Recent evidence attest to the fact that appropriately formulated NP may be fit for this pursuit. In this regard, chitosan NP is the subject of intense interest because it is readily available, biocompatible, biodegradable, mucoadhesive and influences traversing of therapeutics across the GI epithelia. We expect to see more evidence on the application of chitosan in the oral delivery of therapeutics, especially in the form of NP. Further studies on toxicity related issues *in vivo* will assist in discerning any unanticipated effects in humans. These will pave the way for running clinical trials in humans in near future.

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References

[1] M. Rashid, Q. Zaid Ahmad, and Tajuddin, "Trends in Nanotechnology for Practical Applications," in *Applications of Targeted Nano Drugs and Delivery Systems*, Elsevier, 2019, pp. 297-325.

[2] C. Witharana and J. Wanigasekara, "Drug Delivery Systems: A New Frontier in Nano-technology," *Int. J. Med. Res. Heal. Sci.*, vol. 6, no. 9, pp. 11-14, 2017, [Online]. Available: http://www.ijmrhs.com/medical-research/drug-delivery-systems-a-new-frontier-in-nanotechnology.pdf.

[3] L. Kulinsky and M. J. Madou, *BioMEMs for drug delivery applications*, vol. 2015. Woodhead Publishing Limited, 2012.

[4] K. K. Jain, "Drug Delivery Systems - An Overview," in *Methods in Molecular Biology*™, vol. 437, Humana Press, 2008, pp. 1-50.

[5] P. Shrivastava *et al.*, "Nanotechnology for oral drug delivery and targeting," in *Nanoengineered Biomaterials for Advanced Drug Delivery*, Elsevier, 2020, pp. 473-498.

[6] A. El-Kattan and M. Varm, "Oral Absorption, Intestinal Metabolism and Human Oral Bioavailability," in *Topics on Drug Metabolism*, InTech, 2012, pp. 1-37.

[7] J. Zhang, Z. Xie, N. Zhang, and J. Zhong, "Nanosuspension drug delivery system: preparation, characterization, postproduction processing, dosage form, and application," in *Nanostructures for Drug Delivery*, Elsevier, 2017, pp. 413-443.

[8] G. Kaur, M. Arora, and M. N. V. R. Kumar, "Oral Drug Delivery Technologies-A Decade of Developments," J. Pharmacol. Exp. Ther., vol. 370, pp. 529-543, 2019, doi: 10.1124/jpet.118.255828.

[9] B. Homayun, X. Lin, and H.-J. Choi, "Challenges and Recent Progress in Oral Drug Delivery Systems for Biopharmaceuticals," Pharmaceutics, vol. 11, no. 3, pp. 1-29, 2019, doi: 10.3390/pharmaceutics11030129.

[10] L. S. Schanker, "Absorption of Drugs from the Gastrointestinal Tract," in *Concepts in Biochemical Pharmacology*, Berlin, Heidelberg: Springer Berlin Heidelberg, 1971, pp. 9-24.

[11] A. A. Date, J. Hanes, and L. M. Ensign, "Nanoparticles for oral delivery: Design, evaluation and state-of-the-art," J. Control. Release, vol. 240, pp. 504-526, 2016, doi: 10.1016/j.jconrel.2016.06.016.

[12] W. Cui *et al.*, "Application of the Nano-Drug Delivery System in Treatment of Cardiovascular Diseases," *Front. Bioeng. Biotechnol.*, vol. 7, no. 489, pp. 1-18, 2020, doi: 10.3389/fbioe.2019.00489.

[13] P. Viswanathan, Y. Muralidaran, and G. Ragavan, "Challenges in oral drug delivery: A nano-based strategy to overcome," in *Nanostructures for Oral Medicine*, Andronescu Ecaterina and G. A. Miha, Eds. Elsevier Inc., 2017, pp. 173-201.

[14] F. Din *et al.*, "Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors," Int. J. Nanomedicine, vol. 12, pp. 7291-7309, 2017, doi: 10.2147/IJN.S146315.

[15] J. K. Patra *et al.*, "Nano based drug delivery systems: recent developments and future prospects," J Nanobiotechnol, vol. 16, no. 71, pp. 1-33, 2018, doi: 10.1186/s12951-018-0392-8.

[16] A. Rampino, M. Borgogna, P. Blasi, B. Bellich, and A. Cesàro, "Pharmaceutical nanotechnology

Chitosan nanoparticles: Preparation, size evolution and stability," *Int. J. Pharm.*, vol. 455, pp. 219-228, 2013, doi: 10.1016/j.ijpharm.2013.07.034.

[17] M. A. Mohammed, J. T. M. Syeda, K. M. Wasan, and E. K. Wasan, "An overview of chitosan nanoparticles and its application in non-parenteral drug delivery," Pharmaceutics, vol. 9, no. 4, pp. 1-26, Dec. 2017, doi: 10.3390/pharmaceutics9040053.

[18] P. M. Treuting, M. J. Arends, and S. M. Dintzis, "Upper Gastrointestinal Tract," in *Comparative Anatomy and Histology*, Elsevier, 2018, pp. 191-211.

[19] P. M. Treuting, M. J. Arends, and S. M. Dintzis, "Lower Gastrointestinal Tract," in *Comparative Anatomy and Histology*, Elsevier, 2018, pp. 213-228.

[20] N. J. Yang and M. J. Hinner, "Getting across the cell membrane: an overview for small molecules, peptides, and proteins," *Methods Mol. Biol.*, vol. 1266, pp. 29-53, 2015, doi: 10.1007/978-1-4939-2272-7_3.

[21] Le Jennifer, "Drug Absorption ," *MSD Manual Professional Edition*, May 2019. https://www.msdmanuals.com/professional/clinical-pharmacology/pharmacokinetics/drug-absorption# (accessed Sep. 26, 2020).

[22] T. E. Peck, S. A. Hill, T. E. Peck, and S. A. Hill, "Drug passage across the cell membrane," in *Pharmacology for Anaesthesia and Intensive Care*, Cambridge University Press, 2014, pp. 1-8.

[23] H. F. Helander and L. Fändriks, "Surface area of the digestive tract-revisited," Scand. J. Gastroenterol., vol. 49, no. 6, pp. 681-689, 2014, doi: 10.3109/00365521.2014.898326.

[24] S. Kumar, "Local anesthetics," in *Anesthesia Secrets*, Elsevier Inc., 2011, pp. 105-111.

[25] OpenStax, "The Cellular Level of Organization," in *Anatomy and Physiology*, OpenStax, 2013, pp. 1-18.

[26] K. S. Ramos, "Introduction to Molecular Toxicology," in *Comprehensive Toxicology: Second Edition*, Second., vol. 2, Elsevier Inc., 2010, pp. 1-8.

[27] A. M. Navale and A. N. Paranjape, "Glucose transporters: physiological and pathological roles," Biophys. Rev., vol. 8, no. 1, pp. 5-9, Mar. 2016, doi: 10.1007/s12551-015-0186-2.

[28] J. H. Hamman, P. H. Demana, and E. I. Olivier, "Targeting receptors, transporters and site of absorption to improve oral drug delivery.," *Drug Target Insights*, vol. 2, pp. 71-81, 2007, Accessed: Oct. 07, 2020. [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/21901064.

[29] J. F. Collawn, "Unlocking the mysteries of Na+-K+-ATPase endocytosis: Phosphorylation is the key," Am. J. Respir. Cell Mol. Biol., vol. 35, no. 1, pp. 1-2, Jul. 2006, doi: 10.1165/rcmb.f317.

[30] W. Stillwell, "Moving Components Through the Cell," in *An Introduction to Biological Membranes*, Elsevier, 2016, pp. 369-379.

[31] R. J. Kaufman and L. Popolo, "Protein Synthesis, Processing, and Trafficking," in *Hematology: Basic Principles and Practice*, Elsevier Inc., 2018, pp. 45-58.

[32] P. B. Shekhawat and V. B. Pokharkar, "Understanding peroral absorption: regulatory aspects and contemporary approaches to tackling solubility and permeability hurdles," Acta Pharm. Sin. B, vol. 7, no. 3, pp. 260-280, May 2017, doi: 10.1016/j.apsb.2016.09.005.

[33] J. Alsenz, "The Impact of Solubility and Dissolution Assessment on Formulation Strategy and Implications

for Oral Drug Disposition," in *Encyclopedia of Drug Metabolism and Interactions*, Hoboken, NJ, USA: John Wiley & Sons, Inc., 2012, pp. 1-70.

[34] A. Beig, M. Markovic, and A. Dahan, "Solubility, Permeability, and Their Interplay," in *Methods and Principles in Medicinal Chemistry*, John Wiley & Sons, Ltd, 2018, pp. 171-202.

[35] K. T. Savjani, A. K. Gajjar, and J. K. Savjani, "Drug Solubility: Importance and Enhancement Techniques," ISRN Pharm., vol. 2012, pp. 1-10, 2012, doi: 10.5402/2012/195727.

[36] S. Sareen, L. Joseph, and G. Mathew, "Improvement in solubility of poor water-soluble drugs by solid dispersion," *Int. J. Pharm. Investig.*, vol. 2, no. 1, p. 12, 2012, doi: 10.4103/2230-973x.96921.

[37] A. L. Golub, R. W. Frost, C. I. Betlach, and M. A. Gonzalez, "Physiologic considerations in drug absorption from the gastrointestinal tract," J Allergy Clin Immunol., vol. 78, pp. 689-94, 1986.

[38] A. Dahan and J. M. Miller, "The solubility-permeability interplay and its implications in formulation design and development for poorly soluble drugs," AAPS J., vol. 14, no. 2, pp. 244-251, Jun. 2012, doi: 10.1208/s12248-012-9337-6.

[39] T. Kimura and K. Higaki, "Gastrointestinal Transit and Drug Absorption," Biol. Pharm. Bull., vol. 25, no. 2, pp. 149-164, Feb. 2002, doi: 10.1248/bpb.25.149.

[40] D. M. Mudie, G. L. Amidon, and G. E. Amidon, "Physiological Parameters for Oral Delivery and In vitro Testing," Mol. Pharm., vol. 7, no. 5, pp. 1388-1405, 2010, doi: 10.1021/mp100149j.

[41] S. Riley, F. Sutcliffe, M. Kim, M. Kapas, M. Rowland, and L. Turnberg, "The influence of gastrointestinal transit on drug absorption in healthy volunteers.," Br. J. Clin. Pharmacol., vol. 34, no. 1, pp. 32-39, 1992, doi: 10.1111/j.1365-2125.1992.tb04104.x.

[42] M. Koziolek *et al.*, "Investigation of pH and Temperature Profiles in the GI Tract of Fasted Human Subjects Using the Intellicap® System," J. Pharm. Sci., vol. 104, no. 9, pp. 2855-2863, Sep. 2015, doi: 10.1002/jps.24274.

[43] J. Bratten and M. P. Jones, "New Directions in the Assessment of Gastric Function: Clinical Applications of Physiologic Measurements," Dig. Dis., vol. 24, no. 3-4, pp. 252-259, Jul. 2006, doi: 10.1159/000092878.

[44] D. F. Evans, G. Pye, R. Bramley, A. G. Clark, J. Dyson, and J. D. Hardcastle, "Measurement of gastrointestinal pH profiles in normal ambulant human subjects," Gut, vol. 29, pp. 1035-1041, 1988, doi: 10.1136/gut.29.8.1035.

[45] G. Den Besten, K. Van Eunen, A. K. Groen, K. Venema, D. J. Reijngoud, and B. M. Bakker, "The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism," J. Lipid Res., vol. 54, no. 9, pp. 2325-2340, Sep. 2013, doi: 10.1194/jlr.R036012.

[46] S. Amidon, J. E. Brown, and V. S. Dave, "Colon-Targeted Oral Drug Delivery Systems: Design Trends and Approaches," AAPS PharmSciTech, vol. 16, no. 4, pp. 731-741, Aug. 2015, doi: 10.1208/s12249-015-0350-9.

[47] S. Hua, "Advances in Oral Drug Delivery for Regional Targeting in the Gastrointestinal Tract - Influence of Physiological, Pathophysiological and Pharmaceutical Factors," *Frontiers in Pharmacology*, vol. 11. Frontiers Media S.A., p. 524, Apr. 28, 2020, doi: 10.3389/fphar.2020.00524.

[48] L. Kagan and A. Hoffman, "Systems for region selective drug delivery in the gastrointestinal tract:

Biopharmaceutical considerations," Expert Opin. Drug Deliv., vol. 5, no. 6, pp. 681-692, Jun. 2008, doi: 10.1517/17425247.5.6.681.

[49] B. R. Southwell, M. C. C. Clarke, J. Sutcliffe, and J. M. Hutson, "Colonic transit studies: Normal values for adults and children with comparison of radiological and scintigraphic methods," Pediatr. Surg. Int., vol. 25, no. 7, pp. 559-572, Jul. 2009, doi: 10.1007/s00383-009-2387-x.

[50] A. H. Maurer *et al.*, "The SNMMI and EANM Practice Guideline for Small-Bowel and Colon Transit 1.0*," *J. Nucl. Med.*, vol. 54, no. 11, pp. 2004-2013, Nov. 2013, doi: 10.2967/jnumed.113.129973.

[51] G. Sathyan, S. Hwang, and S. K. Gupta, "Effect of dosing time on the total intestinal transit time of non-disintegrating systems," Int. J. Pharm., vol. 204, no. 1-2, pp. 47-51, Jun. 2000, doi: 10.1016/S0378-5173(00)00472-5.

[52] S. Buhmann, C. Kirchhoff, R. Ladurner, T. Mussack, M. F. Reiser, and A. Lienemann, "Assessment of colonic transit time using MRI: A feasibility study," Eur. Radiol., vol. 17, no. 3, pp. 669-674, Mar. 2007, doi: 10.1007/s00330-006-0414-z.

[53] S. Tuddenham and C. L. Sears, "The intestinal microbiome and health," Curr. Opin. Infect. Dis., vol. 28, no. 5, pp. 464-470, Jan. 2015, doi: 10.1097/QCO.0000000000000196.

[54] E. T. Hillman, H. Lu, T. Yao, and C. H. Nakatsu, "Minireview Microbial Ecology along the Gastrointestinal Tract," Microbes Env., vol. 32, no. 4, pp. 300-313, 2017, doi: 10.1264/jsme2.ME17017.

[55] S. E. Aidy, B. van den Bogert, and M. Kleerebezem, "The small intestine microbiota, nutritional modulation and relevance for health," Curr. Opin. Biotechnol., vol. 32, pp. 14-20, Apr. 2015, doi: 10.1016/j.copbio.2014.09.005.

[56] D. Kapoor, R. Maheshwari, K. Verma, S. Sharma, P. Ghode, and R. K. Tekade, "Coating technologies in pharmaceutical product development," in *Drug Delivery Systems*, Elsevier, 2019, pp. 665-719.

[57] M. Long and Y. Chen, "Dissolution Testing of Solid Products," in *Developing Solid Oral Dosage Forms*, Elsevier Inc., 2009, pp. 319-340.

[58] I. Brigger, C. Dubernet, P. Couvreur, and C. Couvreur, "N anoparticles in cancer therapy and diagnosis," *Adv. Drug Deliv. Rev.*, vol. 54, pp. 631-651, 2002, [Online]. Available: www.elsevier.com/locate/drugdeliv.

[59] Y. Xing, J. Zhao, P. S. Conti, and K. Chen, "Radiolabeled Nanoparticles for Multimodality Tumor Imaging," Theranostics, vol. 4, no. 3, pp. 290-306, 2014, doi: 10.7150/thno.7341.

[60] A. S. Thakor and S. S. Gambhir, "Nanooncology: The future of cancer diagnosis and therapy," Cancer J. Clin., vol. 63, no. 6, pp. 395-418, Nov. 2013, doi: 10.3322/caac.21199.

[61] G. Romero and S. E. Moya, "Synthesis of organic nanoparticles," in *Frontiers of Nanoscience*, vol. 4, no. 1, Elsevier Ltd, 2012, pp. 115-141.

[62] N. Ma *et al.*, "Influence of nanoparticle shape, size, and surface functionalization on cellular uptake," J. Nanosci. Nanotechnol., vol. 13, no. 10, pp. 6485-6498, Oct. 2013, doi: 10.1166/jnn.2013.7525.

[63] J. Allouche, "Synthesis of organic and bioorganic nanoparticles: An overview of the preparation methods," in *Nanomaterials: A Danger or a Promise? A Chemical and Biological Perspective*, Brayner R., Fiévet F., and Coradin T., Eds. Springer, London, 2013, pp. 27-74.

[64] S. Gurunathan, M.-H. Kang, M. Qasim, and J.-H. Kim, "Nanoparticle-Mediated Combination Therapy: Two-in-One Approach for Cancer," Int. J. Mol. Sci., vol. 19, pp. 1-37, 2018, doi: 10.3390/ijms19103264.

[65] S. Bhattacharyya, R. A. Kudgus, R. Bhattacharya, and P. Mukherjee, "Inorganic Nanoparticles in Cancer Therapy," Pharm. Res., vol. 28, no. 2, pp. 237-259, 2011, doi: 10.1007/s11095-010-0318-0.

[66] H. C. Huang, S. Barua, G. Sharma, S. K. Dey, and K. Rege, "Inorganic nanoparticles for cancer imaging and therapy," J. Control. Release, vol. 155, no. 3, pp. 344-357, Nov. 2011, doi: 10.1016/j.jconrel.2011.06.004.

[67] Z. P. Xu, Q. H. Zeng, G. Q. Lu, and A. B. Yu, "Inorganic nanoparticles as carriers for efficient cellular delivery," Chem. Eng. Sci., vol. 61, no. 3, pp. 1027-1040, Feb. 2006, doi: 10.1016/j.ces.2005.06.019.

[68] S. J. Soenen, P. Rivera-Gil, J. M. Montenegro, W. J. Parak, S. C. De Smedt, and K. Braeckmans, "Cellular toxicity of inorganic nanoparticles: Common aspects and guidelines for improved nanotoxicity evaluation," Nano Today, vol. 6, no. 5, pp. 446-465, Oct. 2011, doi: 10.1016/j.nantod.2011.08.001.

[69] W. Rao *et al.*, "Thermally responsive nanoparticle-encapsulated curcumin and its combination with mild hyperthermia for enhanced cancer cell destruction," Acta Biomater., vol. 10, no. 2, pp. 831-842, Feb. 2014, doi: 10.1016/j.actbio.2013.10.020.

[70] F. Alexis, E. M. Pridgen, R. Langer, and O. C. Farokhzad, "Nanoparticle technologies for cancer therapy," in *Handbook of Experimental Pharmacology*, vol. 197, Springer Berlin Heidelberg, 2010, pp. 55-86.

[71] S. S. Guterres, M. P. Alves, and A. R. Pohlmann, "Polymeric Nanoparticles, Nanospheres and Nanocapsules, for Cutaneous Applications," *Drug Target Insights*, vol. 2, pp. 147-157, Jul. 2007, doi: 10.1177/117739280700200002.

[72] J. M. Chan, P. M. Valencia, L. Zhang, R. Langer, and O. C. Farokhzad, "Polymeric nanoparticles for drug delivery.," in *Cancer Nanotechnology. Methods in molecular biology (Methods and Protocols)*, vol. 624, Grobmyer S. and Moudgil B., Eds. Clifton, N.J.: Humana Press, 2010, pp. 163-175.

[73] J. P. Rao and K. E. Geckeler, "Polymer nanoparticles: Preparation techniques and size-control parameters," Prog. Polym. Sci., vol. 36, no. 7, pp. 887-913, Jul. 2011, doi: 10.1016/j.progpolymsci.2011.01.001.

[74] A. Sionkowska, "Current research on the blends of natural and synthetic polymers as new biomaterials: Review," Prog. Polym. Sci., vol. 36, no. 9, pp. 1254-1276, Sep. 2011, doi: 10.1016/j.progpolymsci.2011.05.003.

[75] K. Kavitha, M. Rupesh Kumar, and S. Jagadeesh Singh, "Novel Mucoadhesive Polymers-A Review," J. Appl. Pharm. Sci., vol. 01, no. 08, pp. 37-42, 2011.

[76] T. Ahmed and B. Aljaeid, "Preparation, characterization, and potential application of chitosan, chitosan derivatives, and chitosan metal nanoparticles in pharmaceutical drug delivery," Drug Des. Devel. Ther., vol. 10, pp. 483-507, Jan. 2016, doi: 10.2147/DDDT.S99651.

[77] M. W. Anthonsen, K. M. Vårum, and O. Smidsrød, "Solution properties of chitosans: conformation and chain stiffness of chitosans with different degrees of N-acetylation," Carbohydr. Polym., vol. 22, no.

3, pp. 193-201, Jan. 1993, doi: 10.1016/0144-8617(93)90140-Y.

[78] Q. Z. Wang *et al.*, "Protonation constants of chitosan with different molecular weight and degree of deacetylation," Carbohydr. Polym., vol. 65, no. 2, pp. 194-201, Jul. 2006, doi: 10.1016/j.carbpol.2006.01.001.

[79] A. Muheem *et al.*, "A review on the strategies for oral delivery of proteins and peptides and their clinical perspectives," *Saudi Pharmaceutical Journal*, vol. 24, no. 4. Elsevier B.V., pp. 413-428, Jul. 01, 2016, doi: 10.1016/j.jsps.2014.06.004.

[80] N. Islam, I. Dmour, and M. O. Taha, "Degradability of chitosan micro/nanoparticles for pulmonary drug delivery," *Heliyon*, vol. e01684, pp. 1-9, May 2019, doi: 10.1016/j.heliyon.2019.e01684.

[81] U. I. Garg, S. I. Chauhan, U. I. Nagaich, and N. Jain, "Current Advances in Chitosan Nanoparticles Based Drug Delivery and Targeting," Tabriz Univ. Med. Sci., vol. 7, no. 2, pp. 113-117, 2019, doi: 10.15171/apb.2019.023.

[82] E. A. Elhefian, M. Nasef, and A. H. Yahaya, "Chitosan-Based Polymer Blends: Current Status and Applications," J. Chem. Soc. Pakistan, vol. 36, no. 1, pp. 11-27, 2014.

[83] R. Shaikh, T. Raj Singh, M. Garland, A. Woolfson, and R. Donnelly, "Mucoadhesive drug delivery systems," J. Pharm. Bioallied Sci., vol. 3, no. 1, pp. 89-100, Jan. 2011, doi: 10.4103/0975-7406.76478.

[84] B. M. Boddupalli, Z. N. K. Mohammed, R. Nath A., and D. Banji, "Mucoadhesive drug delivery system: An overview," *J. Adv. Pharm. Technol. Res.*, vol. 1, no. 4, pp. 381-387, Oct. 2010, doi: 10.4103/0110-5558.76436.

[85] L. Yin, J. Ding, C. He, L. Cui, C. Tang, and C. Yin, "Drug permeability and mucoadhesion properties of thiolated trimethyl chitosan nanoparticles in oral insulin delivery," Biomaterials, vol. 30, no. 29, pp. 5691-5700, Oct. 2009, doi: 10.1016/j.biomaterials.2009.06.055.

[86] R. K. Shatrohan Lal, "Synthesis of Organic Nanoparticles and their Applications in Drug Delivery and Food Nanotechnology: A Review," *J. Nanomater. Mol. Nanotechnol.*, vol. 03, no. 04, Aug. 2014, doi: 10.4172/2324-8777.1000150.

[87] R. R, M. I. Y, A. U, V. V, R. V, and K. moorthy S.B, "CHITOSAN NANOPARTICLES - AN EMERGING TREND IN NANOTECHNOLOGY," *Int. J. Drug Deliv.*, vol. 6, no. 3, pp. 204-229, Sep. 2014, Accessed: Oct. 13, 2020. [Online]. Available: http://www.arjournals.org/index.php/ijdd/article/view/1452.

[88] B. Sarmento, D. Ferreira, F. Veiga, and A. Ribeiro, "Characterization of insulin-loaded alginate nanoparticles produced by ionotropic pre-gelation through DSC and FTIR studies," Carbohydr. Polym., vol. 66, no. 1, pp. 1-7, Oct. 2006, doi: 10.1016/j.carbpol.2006.02.008.

[89] T. Mitra, G. Sailakshmi, and A. Gnanamani, "Could glutaric acid (GA) replace glutaraldehyde in the preparation of biocompatible biopolymers with high mechanical and thermal properties?," *J. Chem. Sci*, vol. 126, no. 1, pp. 127-140, 2014, doi: https://doi.org/10.1007/s12039-013-0543-2.

[90] R. Sabra, N. Billa, and C. J. Roberts, "An augmented delivery of the anticancer agent, curcumin, to the colon," React. Funct. Polym., vol. 123, pp. 54-60, 2018, doi: 10.1016/j.reactfunctpolym.2017.12.012.

[91] A. Lange de Pinho Neves, C. Cardoso Milioli, L. Müller, H. Gracher Riella, N. Cabral Kuhnen, and H. Karine Stulzer, "Factorial design as tool in chitosan nanoparticles development by ionic gelation technique," *Physicochem. Eng. Asp.*, vol. 445, pp. 34-39, 2014, doi: 10.1016/j.colsurfa.2013.12.058.

[92] L. M. Zhao *et al.*, "Preparation and application of chitosan nanoparticles and nanofibers," *Brazilian* J. Chem. Eng., vol. 28, no. 3, pp. 353-362, 2011, doi: 10.1590/S0104-66322011000300001.

[93] N. B. V N and H. Ksyadav, "DIFFERENT TECHNIQUES FOR PREPARATION OF POLYMERIC NANOPARTICLES-A REVIEW," *Asian* J. Pharm. Clin. Res., vol. 5, no. 3, pp. 16-23, 2012.

[94] D. Bennet and S. Kim, "Polymer Nanoparticles for Smart Drug Delivery," in *Application of Nanotechnology in Drug Delivery*, InTech, 2014, pp. 257-310.

[95] G. Poovi, U. M. Dhana leks, N. Narayanan, and P. Neelakanta, "Preparation and Characterization of Repaglinide Loaded Chitosan Polymeric Nanoparticles," *Res.* J. Nanosci. Nanotechnol., vol. 1, no. 1, pp. 12-24, Jan. 2011, doi: 10.3923/rjnn.2011.12.24.

[96] M. Lee *et al.*, "Size control of self-assembled nanoparticles by an emulsion/solvent evaporation method," Colloid Polym. Sci., vol. 284, no. 5, pp. 506-512, Feb. 2006, doi: 10.1007/s00396-005-1413-3.

[97] A. Mitra and B. Dey, "Chitosan microspheres in novel drug delivery systems," *Indian* J. Pharm. Sci., vol. 73, no. 4, pp. 355-366, Jul. 2011, doi: 10.4103/0250-474X.95607.

[98] S. Naskar, K. Koutsu, and S. Sharma, "Chitosan-based nanoparticles as drug delivery systems: a review on two decades of research," J. Drug Target., vol. 27, no. 4, pp. 379-393, Apr. 2018, doi: 10.1080/1061186X.2018.1512112.

[99] M. A. Mohammed, J. T. M. Syeda, K. M. Wasan, and E. K. Wasan, "An Overview of Chitosan Nanoparticles and Its Application in Non-Parenteral Drug Delivery," Pharmaceutics, vol. 9, no. 4, pp. 1-26, 2017, doi: 10.3390/pharmaceutics9040053.

[100] V. T. Chivere, P. P. D. Kondiah, Y. E. Choonara, and V. Pillay, "Nanotechnology-Based Biopolymeric Oral Delivery Platforms for Advanced Cancer Treatment," Cancers (Basel)., vol. 12, no. 522, pp. 1-22, 2020, doi: 10.3390/cancers12020522.

[101] A. R. Dudhani and S. L. Kosaraju, "Bioadhesive chitosan nanoparticles: Preparation and characterization," Carbohydr. Polym., vol. 81, no. 2, pp. 243-251, Jun. 2010, doi: 10.1016/j.carbpol.2010.02.026.

[102] B. K. Patel, R. H. Parikh, and P. S. Aboti, "Development of Oral Sustained Release Rifampicin Loaded Chitosan Nanoparticles by Design of Experiment," J. Drug Deliv., vol. 2013, pp. 1-10, 2013, doi: 10.1155/2013/370938.

[103] M. R. Avadi *et al.*, "Ex Vivo Evaluation of Insulin Nanoparticles Using Chitosan and Arabic Gum," *Int. Sch. Res. Netw. Pharm.*, vol. 2011, pp. 1-6, 2011, doi: 10.5402/2011/860109.

[104] H. M. Ibrahim and E. M. R. El-Zairy, "Chitosan as a Biomaterial — Structure, Properties, and Electrospun Nanofibers," in *Concepts, Compounds and the Alternatives of Antibacterials*, InTech, 2015, pp. 81-101.

[105] A. E. Caprifico, E. Polycarpou, P. J. S. Foot, and G. Calabrese, "Fluorescein Isothiocyanate Chitosan Nanoparticles in Oral Drug Delivery Studies," Trends Pharmacol. Sci., vol. 41, no. 10, pp.

686-689, Oct. 2020, doi: 10.1016/j.tips.2020.07.005.

[106] C. Feng *et al.*, "Chitosan/o-carboxymethyl chitosan nanoparticles for efficient and safe oral anticancer drug delivery: In vitro and in vivo evaluation," Int. J. Pharm., vol. 457, no. 1, pp. 158-167, Nov. 2013, doi: 10.1016/j.ijpharm.2013.07.079.

[107] J. Ciccolini, C. Serdjebi, · Godefridus, J. Peters, and E. Giovannetti, "Pharmacokinetics and pharmacogenetics of Gemcitabine as a mainstay in adult and pediatric oncology: an EORTC-PAMM perspective," Cancer Chemother. Pharmacol., vol. 78, pp. 1-12, 2016, doi: 10.1007/s00280-016-3003-0.

[108] H. Hosseinzadeh, F. Atyabi, R. Dinarvand, and S. N. Ostad, "Chitosan–Pluronic nanoparticles as oral delivery of anticancer gemcitabine: preparation and in vitro study," Int. J. Nanomedicine, vol. 7, pp. 1851-1863, Apr. 2012, doi: 10.2147/IJN.S26365.

[109] G.-J. TSAI and W.-H. S. H.-C. C. C.-L. PAN, "Antimicrobial activity of shrimp chitin and chitosan from different treatments and applications of fish preservation," *Fish. Sci.*, vol. 68, pp. 170-177, 2002, doi: https://doi.org/10.1046/j.1444-2906.2002.00404.x.

[110] A. I. Barbosa, A. J. Coutinho, S. A. C. Lima, and S. Reis, "Marine Polysaccharides in Pharmaceutical Applications: Fucoidan and Chitosan as Key Players in the Drug Delivery Match Field," Mar. Drugs, vol. 17, no. 654, pp. 1-21, 2019, doi: 10.3390/md17120654.

[111] F. Y. Alqahtani *et al.*, "Preparation, characterization, and antibacterial activity of diclofenac-loaded chitosan nanoparticles," *Saudi* Pharm. J., vol. 27, no. 1, pp. 82-87, Jan. 2019, doi: 10.1016/j.jsps.2018.08.001.

[112] D. D. Milincic *et al.*, "Application of Polyphenol-Loaded Nanoparticles in Food Industry," Nanomaterials, vol. 9, no. 1629, pp. 1-21, 2019, doi: 10.3390/nano9111629.

[113] R. Sabra, C. J. Roberts, and N. Billa, "Courier properties of modified citrus pectinate-chitosan nanoparticles in colon delivery of curcumin," Colloid Interface Sci. Commun., vol. 32, no. 100192, pp. 1-9, 2019, doi: 10.1016/j.colcom.2019.100192.

[114] R. Sabra, N. Billa, and C. J. Roberts, "Cetuximab-conjugated chitosan-pectinate (modified) composite nanoparticles for targeting colon cancer," Int. J. Pharm., vol. 572, no. 118775, pp. 1-11, 2019, doi: 10.1016/j.ijpharm.2019.118775.

[115] P. Anand, A. B. Kunnumakkara, R. A. Newman, and B. B. Aggarwal, "Bioavailability of curcumin: Problems and promises," Mol. Pharm., vol. 4, no. 6, pp. 807-818, Nov. 2007, doi: 10.1021/mp700113r.

[116] E. Alkhader *et al.*, "Pharmacokinetic and anti-colon cancer properties of curcumin-containing chitosan-pectinate composite nanoparticles," J. Biomater. Sci. Polym. Ed., vol. 29, no. 18, pp. 2281-2298, Dec. 2018, doi: 10.1080/09205063.2018.1541500.

[117] C. Y. Wong, H. Al-Salami, and C. R. Dass, "The role of chitosan on oral delivery of peptide-loaded nanoparticle formulation," J. Drug Target., vol. 26, no. 7, pp. 551-562, Aug. 2017, doi: 10.1080/1061186X.2017.1400552.

[118] Z. He *et al.*, "Scalable fabrication of size-controlled chitosan nanoparticles for oral delivery of insulin," Biomaterials, vol. 130, pp. 28-41, Jun. 2017, doi: 10.1016/j.biomaterials.2017.03.028.

[119] J. S. Ling Tan, C. J. Roberts, and N. Billa, "Mucoadhesive

chitosan-coated nanostructured lipid carriers for oral delivery of amphotericin B," Pharm. Dev. Technol., vol. 24, no. 4, pp. 504-512, 2019, doi: 10.1080/10837450.2018.1515225.

[120] J. S. L. Tan, C. Roberts, and N. Billa, "Pharmacokinetics and tissue distribution of an orally administered mucoadhesive chitosan-coated amphotericin B-Loaded nanostructured lipid carrier (NLC) in rats," J. Biomater. Sci. Polym. Ed., vol. 31, no. 2, pp. 141-154, 2020, doi: 10.1080/09205063.2019.1680926.