

# Intestinal Absorption Enhancement *Via* the Paracellular Route by Fatty Acids, Chitosans and Others: A Target for Drug Delivery

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**Abstract:** Peroral delivery of hydrophilic drugs is one of the greatest challenges in biopharmaceutical research. Hydrophilic drugs usually present low bioavailability after oral administration. One of the causes of this low bioavailability is their poor intestinal permeation through the paracellular pathway. This pathway is actually restricted by the presence of tight junctions at the apical side of the enterocytes. In the last few years, great interest has been focused on the structure and cellular regulation of tight junctions, materializing in more in-depth knowledge of this intestinal barrier. Simultaneously, and on the basis of this understanding, continuous efforts are being made to develop agents that can modulate tight junctions and magnify the paracellular permeability of hydrophilic compounds without causing significant intestinal damage. This review focuses on strategies to improve the paracellular permeation of poorly absorbed drugs as a way to enhance their bioavailability after oral administration. Most of the research on this subject has been carried out using *in vitro* models (mainly Caco-2 cell monolayers), which yield useful information on the potential effects and mechanisms of action of absorption-enhancing compounds. However, *in vivo* studies, which are much more scarce, are needed to confirm the effects of potential enhancers and to evaluate the suitability of including these compounds as excipients in drug formulation. We review the *in vitro* and *in situ* studies involving the most promising paracellular permeation enhancers (e.g., medium chain fatty acids and chitosan and its derivatives), analyzing the degree of drug absorption enhancement achieved, as well as the potential associated toxicity. The few studies performed *in vivo* are also presented. In addition, the findings of recent absorption enhancers, such as zonula occludens toxin or thiolated polymers, are reviewed.

**Keywords:** Paracellular permeation enhancement, tight junctions, sodium caprate, chitosan, oral bioavailability, drug delivery.

## INTRODUCTION

The pharmaceutical industry invests great sums of money in the development of drugs that can be effective in application to pathologies that affect major population groups or in treating new diseases. In this challenging task, and apart from the pharmacological effects which represent the first development step, it is of great relevance to consider the biopharmaceutical and pharmacokinetics properties of the drug - particularly those concerning intestinal absorption and bioavailability (BA) after oral administration. The oral route is the preferred administration route, and it must be taken into account that some of the newer promising drugs cannot be developed as oral products because of their null or scarce bioavailability. In general, these are hydrophilic compounds, of medium to high-molecular weight, and sometimes containing strongly charged functional groups - implying that transport across the intestinal barrier occurs essentially via the paracellular pathway [1]. The contribution of the latter to intestinal absorption is considered to be small, since this pathway occupies less than 0.1% of the total surface area of the intestinal epithelium [2], and the presence of tight junctions (TJ) between the epithelial cells limits drug absorption. Therefore, for the above mentioned drugs, the

main cause of low bioavailability is their poor intestinal permeability.

In the last decade many efforts have been made to increase the paracellular transport of less absorbable drugs - the use of enhancers being the most promising and studied strategy [3,4]. Our understanding of tight junction physiology and regulation has increased in recent years [5], thus facilitating the search for compounds capable of enhancing absorption via the paracellular pathway. To obtain maximum benefit from such enhancing compounds it is necessary to confirm that reduced membrane permeability is the cause (or at least the main cause) of poor drug bioavailability. In this case, an increase in intestinal absorption would enhance drug bioavailability, which in turn would allow oral administration of the drug and also a reduction in inter- and intrasubject variability in plasma concentrations and therefore in therapeutics effects.

In the literature [3,4] an enormous variety of compounds have been tested as potential paracellular enhancers (Ca<sup>++</sup> chelators, medium-chain fatty acids, medium-chain glycerides, steroidal detergents, acylcarnitines, chitosans and other mucoadhesive polymers). According to Aungst *et al.* [3] there are several critical issues to consider in the selection of a compound as potential absorption enhancer for use in drug delivery: the degree of bioavailability enhancement achieved, the influence of formulation and physiological variables, the possible toxicity originated by

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the enhancing action and the mechanism of permeation enhancement.

This review is not intended to gain insight to either the physiology and regulation of tight junctions or the mechanism of action of paracellular permeation enhancers. The objective is rather to review the efficacy and toxicity of several promising enhancers, analyzing the different methodologies used and the different results obtained. Interest has focused on medium chain fatty acids and chitosan and its derivatives, since these are the most studied and effective enhancers. The medium chain fatty acid sodium caprate is being used as an absorption enhancer in the clinical setting in Japan, Denmark and Sweden. There have been no reports of serious side effects [6-8]. Additionally, the results obtained with the more recent paracellular enhancers will be presented. Previous reviews are recommended to address the different aspects not considered here [3-5].

### THE PARACELLULAR ROUTE

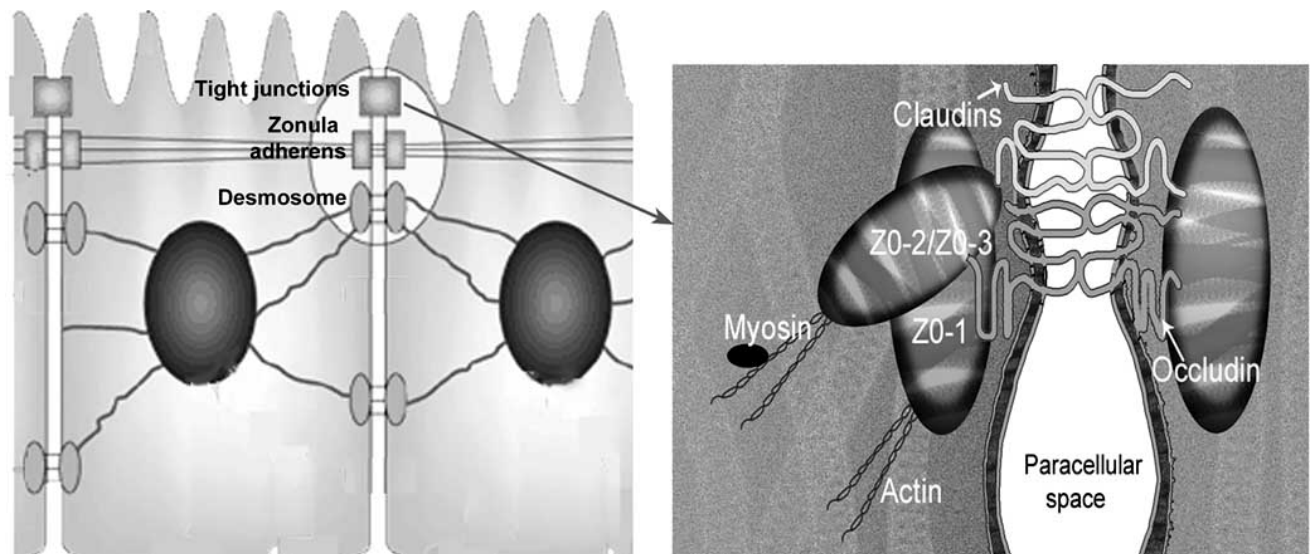
In general, solute absorption in the intestinal epithelium can occur via both transcellular and paracellular pathways. Whereas transcellular absorption has long been known and studied, paracellular transport was belittled for much of the last century, and was not considered as an effective pathway until the sixties, when it was discovered that molecules of different sizes can cross the epithelium via the paracellular route [9].

The paracellular route can be defined as the aqueous pathway along the intercellular space between adjacent cells, which is restricted by tight junctions at the most apical part of the cells (Fig. 1). The aqueous nature of this route makes it favorable for the absorption (through passive diffusion) of

small hydrophilic solutes (nutrients, ions, etc.) [10]. It is now known that the flux across the TJ can be considerable, and that the permeability and selectivity of the junctions can be regulated [11]. Tight junction permeability differs according to the type of permeant involved, its size and charge. Although the junctional complex found in the apical portion of adjacent cells is composed of three distinct regions (tight junction or zonula occludens, zonula adherens and macula adherens or desmosome) (Fig. 1), paracellular permeability is primarily regulated by the TJ, because this is the rate-limiting barrier of the transport pathway. TJ have been described as gates (selectively allowing the passage of small hydrophilic compounds) and as fences (forming an intermembrane diffusion barrier maintaining enterocyte polarity and excluding potentially toxic molecules). These two functions are not separate unrelated phenomena [5]. It is becoming increasingly clear that TJ in themselves constitute the product of a global polarizing process; their role therefore does not seem to correspond to that of a simple fence [12].

Manipulating paracellular permeability has been proposed and used as a way to enhance drug absorption [3,4]. To achieve this effect in an effective and safe manner, an understanding of the architecture and regulation of TJ is essential. In the last decades some light has been shed on this topic. Because a complete review of TJ structure, function and molecular regulation is beyond the scope of this article, and has recently been made [12-15], only a brief summary will be provided here of the more relevant aspects of the subject and its repercussions upon paracellular permeability.

Under the transmission electron microscope, tight junctions appear as a series of focal contacts between the plasma membrane of two adjacent cells. In early freeze-fracture electron microscopy sections, the tight junctional



**Fig. (1).** Left: The functional complex located in the apical part of the adjacent enterocytes is formed by tight junctions, zonula adherens and macula adherens or desmosomes. The tight junctions area is expanded at right. Right: schematic representation of the protein interactions at tight junctions. The strands at the paracellular space are formed by two tetra-spanning transmembrane proteins of the occludin and claudins family. The COOH terminals of both occludin and claudins bind to a family of highly related cytoplasmic proteins called ZOs (ZO-1, ZO-2, ZO-3). This interaction is only represented in one part of the illustration. Other proteins have been localized to the cytoplasmic surface of tight junction, though they are not represented.

complex appeared as a dense network of interdigitating strands or fibrils in the plane of the plasma membrane [16]. At some points the strands showed discontinuities which might correspond to “pores” [17], and which would imply channel-like permeability (i.e., fluctuating aqueous pores embedded in the fibrils would account for diffusion through TJ). The composition of the strands was still unknown. This early concept has progressed little since 1978, and it is presently also postulated that fibrils on one cell interact with fibrils on an adjacent cell to seal the paracellular space and define the permeability characteristics [13]. To date the commonly held view is that increases in paracellular permeability under perturbed or pathological conditions result from the dilatation of existing tight junction pores [18]. In contrast, the composition of the fibrils is now well known. Fibrils are formed by at least two types of tetra-spanning transmembrane proteins: occludin and claudin (Fig. 1). Occludin, a 65-kDa phosphoprotein, was first identified in different species in the mid-nineties [19-21]. The findings reported to date are consistent with a functional role for occludin in defining the barrier [22,23]. Claudins are a family of proteins, named from the Latin *claudere*, “to close”. Currently, 20 claudins, present in different tissues, can be identified in the GenBank database. They appear to represent the major structural components of tight junction strands, since they show an intrinsic ability to polymerize into linear fibrils [24], whereas occludin forms only short strand fragments. A role for claudins in barrier formation is thus evidenced. Recent studies have proposed that claudins are the pore-forming structures in TJ [25,26] – thus strongly supporting the idea that claudins confer specific selectivity to paracellular transport.

Furthermore, a dense cytoplasmic network of proteins has been described at the TJ. These are referred to as tight junction associated proteins (TJAPs), and are designated ZO-1, ZO-2 and ZO-3. As can be seen in (Fig. 1), these proteins interact among each other and also serve as a link between occludin and the actin filaments of the cytoskeleton [4]. The association of tight junctions with the apical perijunctional actomyosin ring seems to regulate global TJ permeability [15]. It is also known that the barrier assembly and permeability characteristics of TJ are influenced by many cellular signaling mechanisms - though these remain largely undefined [27]. In conclusion, despite rapid progress in the knowledge of TJ structure and molecular physiology, their function in the context of paracellular permeability is still far from fully clear.

Watson *et al.* [18] recently attempted to address this issue. These authors employed an approach that measures the paracellular permeability of several polyethylene glycol (PEG) oligomers, allowing detailed functional profiling and mathematical modeling of the paracellular route. Moreover, this methodology has been used by the authors to study possible mechanistic differences in the action of enhancers. They characterized two distinct permeation components for PEG oligomers: a restrictive component (pore radius < 4 Å) and a second component (pore radius > 4 Å) that is not restrictive and is responsible for the permeability of large compounds. From these results it is clear that important questions arise regarding the transport routes of small and large molecules across the intestinal barrier, and whether

such transport can be explained by simple dilatation of a single population of homogenous pores. According to the authors, additional studies are needed to answer these questions. Although this research has been done in colonic cell lines and using specific oligomers, the authors claim that this technique combined with molecular and morphological approaches should provide valuable insight to the nature of the paracellular pathway.

In summary, evidence now exists to suggest that the regulation of paracellular permeability by the TJ is a complex process, illustrated by the diversity in functional reactions of this structure. At present, apart from conventional permeability studies, several probes have become available for detecting changes in activation of intracellular proteins, such as the protein kinase C isotypes. Moreover, *in situ* imaging techniques are being used [14]. Studies in the field of diseases originated by paracellular permeability alterations (food allergies, malabsorption syndromes and inflammatory bowel diseases such as Crohn’s disease and ulcerative colitis) [28,29] also contribute to our understanding of paracellular transport. In future, all such intense research can contribute to secure in-depth knowledge of the control mechanisms implicated in paracellular transport and its modulation, which may have great repercussions for drug delivery.

## PERMEATION ENHANCERS

The present review focuses on medium chain fatty acids (MCFAs) and chitosan and its derivatives with the presentation of *in vitro-in situ* versus *in vivo* studies. Other recently proposed paracellular absorption enhancers have also been included.

### MEDIUM CHAIN FATTY ACIDS (MCFAs)

Based on the research conducted in the last decade it has become clear that several sodium salts of medium chain fatty acids [caprylate C8 (CH<sub>3</sub>-(CH<sub>2</sub>)<sub>6</sub>-COOH), caprate C10 (CH<sub>3</sub>-(CH<sub>2</sub>)<sub>8</sub>-COOH) and laurate C12 (CH<sub>3</sub>-(CH<sub>2</sub>)<sub>10</sub>-COOH)] are able to enhance the paracellular permeability of hydrophilic compounds. Lindmark *et al.* [30] carried out a comparative study with these three fatty acids and sodium caproate (C6). They showed that C8, C10 and C12 (but not C6) exhibit dose-dependent enhancing effects on mannitol transport across cell monolayers - C12 being the most effective enhancer. Interestingly, the lowest concentrations to enhance transport of the marker molecule were in the vicinity of their critical micellar concentration (CMC), which in turn differs considerably for each.

Among these MCFAs, sodium caprate is the most extensively studied and the only absorption enhancing agent included in a marketed drug product. It is added in a suppository formulation intended for human use in Sweden and Japan [8]. Since this fatty acid has a low molecular weight, it could be absorbed from the intestine even more quickly than the drug itself [3]. The numerous studies conducted with C10 have addressed different aspects such as its enhancing effect on the permeability of compounds with different molecular weights (MW), its concentration and time-dependent effects, its toxicity and mechanism of action. These points are analyzed below.

### ***In Vitro and In Situ Studies with Sodium Caprate***

Most published data on sodium caprate as an absorption enhancer have been obtained using *in vitro* and *in situ* techniques. The different experimental designs and settings involved, as well as the different marker molecules and drugs used, make it difficult to draw firm conclusions regarding the aforementioned aspects.

#### ***Effects on the Permeability of Marker Molecules***

In relation to the effect of C10 on the permeability of different compounds, it is convenient to differentiate between marker molecules and drugs. In the case of marker molecules, studies performed in Caco-cells with low MW marker compounds such as mannitol (MW 182), phenol red (MW 354.4) or sodium fluorescein (MW 376), have shown C10 to significantly enhance the intestinal permeability of these substances via the paracellular route [6,30-32]. Moreover, Sakai *et al.* [6], in a confocal laser scanning study, demonstrated that sodium caprate is also able to enhance the permeability of hydrophobic molecules (rhodamine 123 hydrate) via both the paracellular and transcellular route. Regarding the effect of C10 upon the permeability of high MW marker molecules, there appears to be wide agreement on the promoting effect of this fatty acid on the transport of compounds such as FITC-dextran (average MW 4000) [6,33-35], polysucrose (MW 15000) [36] and insulin [37]. Lindmark *et al.* [38], using marker molecules with a broad molecular weight range (up to 19,600), recorded increased permeability of the cells to all tested compounds in the presence of C10. However, the authors point out that the effect would be significant only for substances of MW 1200 g/mol, i.e., in the case of larger molecules, permeability enhancement would not result in a significant increase in the dose fraction absorbed [39].

#### ***Effects on Drug Permeability***

To date, most reported data on the influence of C10 upon drug intestinal permeability have been based on *in vitro* or *in situ* models. Tomita *et al.* [40], using an *in situ* loop technique, showed the jejunal absorption of cefmetazole, a poorly absorbed antibiotic, to be significantly enhanced by sodium caprate at a concentration of 0.25% (13 mM) - though to a lesser extent than colonic absorption. A similar regional difference has also been evidenced in the transport of ebitaride (a pentapeptide ACTH analog) and insulin. In these latter cases, by means of a modified Ussing chamber method, the authors found drug permeability to be improved at the colonic but not at the jejunal membrane [37,41]. Clearly, these regional differences cannot be detected when Caco-2 cell monolayers are used as experimental model, though other aspects - such as the possibility that C10 may act as a multidrug resistance (MDR) modulator - can easily be tested. For instance, according to the results recently obtained in Caco-2 cell monolayers by Lo *et al.* [42], C10 not only enhances the transcellular and paracellular absorption of epirubicin, an anticancer drug, but is also able to reduce basolateral-to-apical secretion of this compound. Thus, these authors suggested that C10 may exert MDR reversing effects which could have significant repercussions for cancer chemotherapy. Additionally, others authors [31,34] have studied the effect of sodium caprate on the

permeability of peptide drugs in Caco-2 cells. In both studies an improvement in drug permeability has been reported. Taken as a whole, it can be concluded that sodium caprate *in vitro* or *in situ* exerts a clear enhancing effect on the permeability of both paracellular marker compounds (even in the case of high MW compounds) and drugs (antibiotics and peptide drugs). It is worth pointing out that Raiman *et al.* [43] have recently found that C10 (10 mM) does not affect the permeability of the bisphosphonate drug clodronate across Caco-2 cell monolayers.

#### ***Concentration and Time-Dependent Effects***

In all of the studies mentioned above, the sodium caprate concentration used was in the vicinity of its CMC (i.e., 13 mM). Several research groups, using Caco-2 cells, have shown the C10 enhancement effect on membrane permeability to be concentration-dependent [6,30-32]. We have also recently observed this concentration-dependent effect *in vitro* (Caco-2 cell) using acamprostate, an alcoholic antirelapse drug (unpublished data). However, when we performed the experiments *in situ* [44] and *in vivo* with rats, this effect disappeared. Our results agree with those obtained *in vivo* by Raof *et al.* [45] in pigs. According to their data, the enhancing capacity of C10 is dose-independent.

Regarding the time-dependent effect of C10 on intestinal permeability, the studies carried out by Anderberg *et al.* [32], using Caco-2 cells, showed significant time-dependent effects at 13 mM and higher concentrations (16 mM). These experiments were performed after long incubations (approximately 1 h) with C10. Since the immediate effect of the enhancer is presumably more relevant than the long-term effect *in vivo*, Lindmark *et al.* [7], in Caco-2 cells monolayers, determined both the long- and short-term effects. According to their results, the time-dependent effect of sodium caprate 13 mM upon permeability can be separated into two phases: an initial phase (10-20 min.) in which a rapid increase in permeability was observed, and a later phase characterized by a slow but more prolonged enhancement in permeability. Kamm *et al.* [34] have also shown C10 absorption enhancement to be markedly dependent on incubation time in Caco-2 cells. Our experiments performed *in vitro* also confirm this exposure time-dependent effect, though *in situ* experiments failed to show such an effect (unpublished results).

#### ***Local Toxicity***

The local toxicity of sodium caprate in the small intestine is one of the main concerns in relation to the use of this fatty acid in pharmaceutical products. As mentioned before, sodium caprate is currently used as an absorption enhancer in ampicillin suppositories in some countries. This suppository formulation caused nonspecific damage to the rectal mucosa, according to the results obtained in a study conducted in humans [46]. However, the damage was reversible and was attributed not only to C10 but also to the triglyceride suppository base.

The toxicity of C10 has been extensively studied *in vitro*. Considering that cytotoxicity depends on the concentration and duration of exposure, comparisons among results obtained in experiments using different protocols may not always be valid. Cell damage can be assessed by several

methods (morphological observations, the release of biological markers, and the recovery of transepithelial electrical resistance (TEER)) - a fact that further complicates the drawing of firm conclusions. A closer examination of the reported data suggests that C10 at effective concentrations (around 13 mM) does not affect epithelial viability [36] and does not cause serious cytotoxicity - its effects moreover being reversible [31,33,37,41]. In this sense, Sakai *et al.* [47], in a systematic study of the toxicity of several enhancers, have reported that only high concentrations of this fatty acid (50 mM) are significantly cytotoxic for the plasma membrane. In contrast, the results obtained by Kamm *et al.* [34], involving C10 at a concentration of 10 mM, reflected serious and irreversible Caco-2 monolayer damage.

It should be mentioned that in general, Caco-2 monolayers are often seen to be more sensitive to the cytotoxic effects of permeation enhancers than whole intestinal tissue [3,31]. This is clearly the case with sodium caprate. According to the results reported by Chao *et al.* [31], C10 at a concentration of 50 mM is able to disrupt and detach the cell monolayer, whereas no perceptible evidence of mucosal irritation or damage was obtained after delivering a liquid formulation containing sodium caprate 100 mM to the rat ileum (*in vivo* experiment). The authors argue that this discrepancy could be partly explained by assuming that the enhancer is diluted *in vivo* to concentrations tolerable for the intestinal mucosa. In addition, the intact tissue produces a protective mucous layer not found in Caco-2 cell monolayers. Finally, the authors indicate that the *in vivo* intestinal mucosa possesses mechanisms allowing recovery over time which are not found in cell culture.

### Mechanism of Action

The possible toxicity of sodium caprate is associated to its mechanism of action as enhancer. There is a general agreement that C10 exerts its enhancing effect mainly via the paracellular route, inducing dilatations of the tight junctions [32,48]. Two research groups have mostly explored the mechanism implicated in the paracellular enhancing effect of sodium caprate: Lindmark *et al.* [7,30] and Tomita *et al.* [35,49]. To elucidate certain aspects of the mechanism of action of sodium caprate, the authors used different compounds that regulate intracellular events directly related to TJ permeability. These authors agree that C10 is able to modulate paracellular permeability by increasing intracellular calcium levels through the activation of phospholipase C in the plasma membrane, as represented in (Fig. 2). The increase in calcium levels is considered to induce the contraction of calmodulin-dependent actin microfilaments, resulting in increased paracellular permeability [50,51]. The findings of Sakai *et al.* [52] also suggest that one mechanism by which C10 could exert its effect involves structural alterations in the cytoskeletal actin filaments induced by changes in intracellular calcium levels. Recently, Watson *et al.* [18] investigated the mechanism of action of sodium caprate at functional level. They observed that the increase in paracellular permeability induced by C10 is not attributable to tight junction pore dilatation. As an alternative explanation, the authors postulate the possibility that this fatty acid acts by increasing the opening probability of restrictive pores, i.e., C10 could act by increasing the number of restrictive pores. This action would be mediated by alterations in junctional proteins. Lindmark *et al.* [7] have

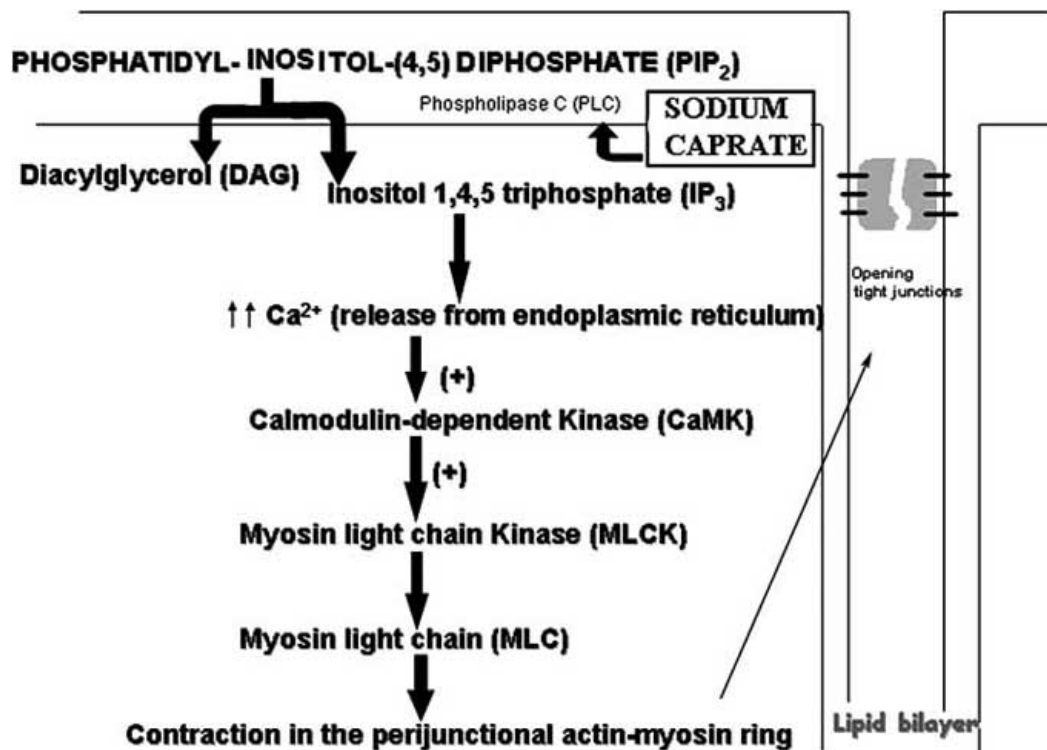


Fig. (2). Proposed mechanism of action of sodium caprate through the phospholipase-C- dependent pathway.

shown proteins ZO-1 and occludin to be involved in the morphological changes in TJ structure observed after C10 exposure in both cell culture and intestinal tissue.

### ***In Vivo* Studies with Sodium Caprate**

The effectiveness of any compound for enhancing intestinal permeation must be assessed by *in vivo* studies. The degree of bioavailability enhancement, as well as the effect on C<sub>max</sub> and the area under the curve (AUC), are the indicators most commonly used in studies of this kind.

The *in vivo* setting clearly constitutes a more complex and dynamic environment, which hampers assessment of the promoting effect. Furthermore, the formulation used to administer the drug and promoter (solution, capsules, microcapsules, enteric-coated capsules, suppositories) could influence the efficacy of an absorption enhancing excipient. Similarly, the route of administration used (peroral, intrajejunal, intracolonic, rectal) is directly related to the success of oral drug bioavailability enhancement [3]. For extensively information on the factors influencing the *in vivo*

performance of permeation enhancers, readers are referred to the reviews published by Aungst [3] and Ward *et al.* [4].

Table 1 summarizes the published *in vivo* studies describing the more relevant experimental factors conditioning the effect of sodium caprate upon drug bioavailability. As can be seen, most investigations have been performed in rats [31, 44, 53-56], though dogs [55], pigs [45], rabbits [57] and even humans [46, 58] have also been used. As a whole, it can be concluded that this medium chain fatty acid is capable of improving oral drug bioavailability. However, it must be stressed that only two studies have been carried out to date in humans, and no effect was reported in one of them [58].

The drugs involved in these investigations have been peptides, antibiotics and polar, high MW drugs such as antisense oligonucleotides or glycyrrhizin. When the drug and enhancer are administered as a solution, the effective C10 doses in the reported studies are comparable. Closer examination shows that all these doses are in the range of 0.1-0.5 mmol/kg. Curiously, when these amounts are

**Table 1. Summary of Studies Investigating *In Vivo* Effects of Sodium Caprate**

Drug	Species	Route (dosage form)	Dose	Enhancing effects	Reference
FOSFOMYCIN	Rats	Intrajejunal-Intracolonic (Solution)	0.5-1 % w/v	drug plasma levels Concentration-dependent effect specially in colon	[53]
ACYCLOVIR	Rats	Rectal (Suppositories)	1, 2, 4 and 6 %	4% of C10 F(%) by 119%	[54]
DMP <sup>728(a)</sup>	Rats and dogs	Oral ( hard capsules/microcapsules)	40.2 % of the powder formulation	AUC, C <sub>max</sub> , and F(%) in rats (2-fold) and in dogs (60%)	[55]
AMPICILLIN	Humans	Rectal (Suppositories)	25 mg C10 per suppository	BA <sup>(c)</sup> (1,8 fold) C <sub>max</sub> and AUC (2, 3 and 2,6 fold respectively)	[46]
D-DECAPEPTIDE	Rats	Intraileal (Solution)	0.05, 0.2 and 0.5 mmol/kg (1ml/kg)	F(%) dose-dependently until 5-fold	[31]
PHENOXIMETHYL PENICILLIN AND ANTIPIRINE	Humans	Rectal perfusion (Solution)	3.5 mg/ml 50-100 mM	Slight increase in drug plasma concentrations. No effect in the effective rectal permeability of the drugs (Peff)	[58]
ISIS 2503-ISIS 10 4838 <sup>(b)</sup>	Pigs	Intrajejunal (Solution)	25, 50 and 100 mg/kg (0.5 ml/kg)	AUC, C <sub>max</sub> and F(%). The enhancing effect is dose-independent	[45]
NORFLOXACIN	Rabbits	Oral (Hard capsules)	10 mg/kg or 50 mg/kg per capsule	t <sub>max</sub> , C <sub>max</sub> but AUC <sub>0-24</sub> does not change. Effect dose-independent	[57]
GLYCYRRHIZIN	Rats	Per oral (Solution)	0.2, 0.5, 1 and 2 % (w/v) (5 ml/kg)	F(%) (60-fold). Concentration dependent effect on AUC <sub>0-4h</sub> (the trend was toward a levelling off at 1% w/v)	[56]
ACAMPROSATE	Rats	Oral (Solution)	13, 16 and 50 mM	No effect in acamprostate BA <sup>(c)</sup>	[44]

(a) Platelet glycoprotein IIb/IIIa receptor antagonist.

(b) Antisense oligonucleotides.

(c) Bioavailability calculated from urinary recovery.

F(%) Absolute oral bioavailability.

transformed into concentrations, the values obtained are in some cases quite different and clearly higher (25-1000 mM) than those tested in the *in vitro* models. Regarding the concentration-dependent effect of C10, the results are not always coincident. Chao *et al.* [31] and Ishizawa *et al.* [53] reported clear dose-dependent enhancement in drug bioavailability and plasma levels, respectively. Sasaki *et al.* [56] showed the mean AUC<sub>0-4 h</sub> value measured after oral administration of glycyrrhizin and C10 to increase in a concentration-dependent manner, though the trend was seen to level off at 1% (w/v). In contrast, according to the results obtained by other authors [45,54,57], the enhancing effect of sodium caprate is dose-independent.

It is worth mentioning that two studies have compared the *in vivo* enhancing effect of several medium chain fatty acids [53, 56], concluding that the strength of this effect is in the following order: caprate > laurate > caprylate. Hence, sodium caprate seems to be the most potent promoter among the MCFAs tested. In these studies, it is important to consider the potential toxicity associated with the promoter. Not all reported *in vivo* studies have investigated the possible toxic effects of C10. The scarce results are, however, in concordance. This fatty acid, at the doses tested, was well tolerated by the intestinal mucosa, and no membrane damage was observed [31,45,53]. The data contributed by Lindmark *et al.* [46] indicate that even though C10, formulated as suppositories, caused nonspecific damage to the rectal mucosa, the fatty acid exerts a protective effect upon the latter.

Raouf *et al.* [45] also investigated the pharmacokinetics of C10 in pigs, showing it to be rapidly absorbed. Interestingly, they noticed that an enhancement in sodium caprate residence time in the intestine and the prolongation of C10 exposure to the intestinal membrane were associated with improved oral bioavailability of the tested compound. These results are in agreement with those obtained in rats and humans [8,58].

### Concluding Remarks

Sodium caprate can be considered a promising agent for use as an enhancing excipient in drug delivery. C10 effect is dependent on its permeability in the tissue upon which it is required to act. Further studies are needed to confirm its null or low toxicity, and to evaluate its enhancing efficacy for each particular drug substance.

## CHITOSAN AND ITS DERIVATIVES

### General Considerations

High MW polymers such as chitosan and its derivatives have gained considerable attention as permeation enhancers. Because of their high MW, these polymers are supposedly not absorbed from the gut, and systemic side effects are thus excluded. In addition, prolonged localization in the mucosa is ensured, which in turn would prolong the promoting effect.

Apart from their enhancing properties, chitosan and its derivatives are of great interest as excipients and drug carriers in the pharmaceutical field, due to their biodegradability, biocompatibility and recent FDA application [59-61].

To date, many publications have dealt with the absorption enhancing effects of chitosan and its derivatives in several epithelia. In this review, we will try to summarize the studies related to intestinal absorption enhancement, affording an update to a previous review [62].

### Chemical Structure and Physicochemical Characteristics

Chitin is a natural polysaccharide present in crustacean shells, insects, and fungi, as well as in some microorganisms. Apart from cellulose, it is the most common natural polymer. Chitin is a straight homopolymer composed of N-acetyl glycosamine units with a three dimensional -helical configuration [60] (Fig. 3A). The partial deacetylation of chitin yields a copolymer comprising glycosamine and N-acetyl glycosamine, linked by (1:4) glycosidic bonds, called chitosan (Fig. 3B). Chitosan is actually a denomination describing a series of polymers with different molecular weights (from 50 kDa to 2000 kDa) and degrees of deacetylation [63]. These two factors are very important for the physicochemical properties of chitosan, and thus exert a major impact on its promoting effects – as will be discussed later.

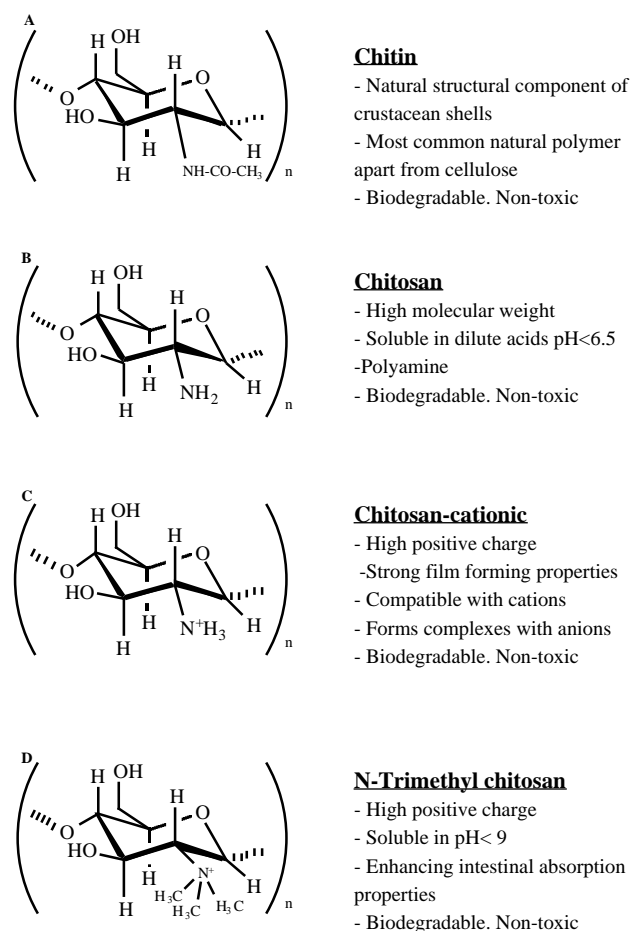
Chitosan is a weak base, being insoluble at alkaline and neutral pH values. In acidic medium, the amine groups are protonated and a positively charged soluble polysaccharide is found (Fig. 3C). The solubility of chitosan salts (hydrochloride, glutamate) depends on the degree of deacetylation involved [64]. The insolubility of chitosan in neutral and basic environments limits its absorption-enhancing properties at the pH of the intestine and colon. However, due to the primary group at the 2-position of each subunit, further chemical modifications are easily attainable [65]. Taking this possibility into account, different chitosan derivatives with improved solubilities can be obtained. For instance, substitution of the primary amine with methyl groups results in N-trimethyl chitosan chloride (TMC) derivatives (Fig. 3D), which display much greater aqueous solubility than chitosan at neutral and alkaline pH values [66]. It seems that substitution of the primary amine and the prevention of hydrogen bond formation between the amine and hydroxylic group are responsible for the improved solubility of quaternized chitosan [59].

Based on the mentioned structural properties of this polymer, a great number of chitosans and derivatives could easily be obtained and tested. To afford a global vision of the investigations dealing with these compounds as intestinal absorption enhancers, we consider it more appropriate to first analyze chitosan salts and then TMC polymers, which have been extensively investigated - particularly in recent years.

### Intestinal Enhancing Properties of Chitosan Salts

#### *In Vitro* Studies

The effect of chitosan on intestinal permeability across tight junctions was first reported by Artursson *et al.* [67]. These authors found chitosan glutamate to enhance <sup>14</sup>C-mannitol transport *in vitro* (Caco-2 cell monolayers). Posteriorly, it was confirmed that chitosan hydrochloride and chitosan glutamate, in a slightly acidic environment, increase the permeation of low molecular weight markers as well as



**Fig. (3).** Chemical structure of chitosan and its derivatives.

of large hydrophilic compounds (PEG-4000 and fluorescein dextrans) in intestinal epithelial cells [68,69]. These studies have compared the enhancing effect of both chitosan salts (pH 6.2), revealing greater effectiveness for chitosan glutamate. It was also found that both salts are insoluble at pH 7.4 and prove to be ineffective as permeation enhancers [69]. This pH-dependent effect has also been evidenced by other authors [70,71].

Schipper *et al.* [72] investigated the effect of chitosan structural characteristics (molecular weight and degree of deacetylation) on their absorption enhancing properties *in vitro* (Caco-2 cell monolayers), using chitosan hydrochloride salts at pH 5.5. It was found that the capacity of chitosan to improve mannitol transport is dependent on molecular weight and the degree of deacetylation; accordingly, while chitosans with a high degree of deacetylation were efficient as permeation enhancers at low and high MW, those with low degrees of deacetylation were efficient only at high molecular weights. Most subsequently published articles in this field coincide that > 80% deacetylation affords the greatest promoter effect on cells in culture [73].

Regarding the effect of chitosans salts on drug intestinal permeability *in vitro*, there is general agreement that these polymers are potent absorption enhancers for poorly absorbed drugs such as atenolol [74] and peptide drugs

[75,76]. Studies with busserelin, 9-deglycinamide 8-arginine vasopressin (DGAVP) and insulin have evidenced a strong increase in the transport of these drugs in the presence of chitosan glutamate and chitosan hydrochloride (acidic environment).

A crucial aspect related to the use of permeation enhancers is their toxicity. Since chitosans are polymers, it is assumed that their intrinsic absorption is minimal, and they are consequently not expected to show systemic adverse effects [77]. As to their local toxicity, Schipper *et al.* [72] investigated the influence of the structural properties of chitosans on toxicity, showing the degree of deacetylation to largely dictate toxicity. The authors observed that chitosans with a low degree of deacetylation (< 35%) displayed clear dose-dependent toxicity, whereas those with a high degree of deacetylation (35% and 49%) showed low toxicity. Molecular weight does not seem to influence the severity of side effects so strongly. Chitosan toxicity has been further evaluated by other researchers based on trypan blue exclusion studies and confocal laser scanning microscopy [68,76]. No deleterious effects upon the cells were demonstrated. Thus, the general opinion regarding chitosan salt toxicity *in vitro* appears to be that they offer a very safe toxicity profile [65].

As in the case of sodium caprate, the toxicity profile of chitosan is directly related to the mechanism of action involved. It is clear that this polymer increases paracellular permeability [62]. In an early study, the permeation-enhancing properties of chitosan were suggested to be a combination of mucoadhesion and action upon paracellular permeability [67]. Posteriorly, Shipper *et al.* [78] investigated (in Caco-2 cells) the mechanism underlying absorption enhancement at molecular level, using two chitosans with different chemical compositions and molecular weights. Even though the chitosans reportedly induced absorption enhancement with different kinetics and displayed different toxicities [72], their mechanisms of action were found to be very similar. The polymers were able to bind tightly to the epithelium and to induce redistribution of cytoskeletal F-actin and the TJ protein ZO-1, this being followed by enhanced transport via the paracellular pathway. The authors also concluded that the binding and promoting effects of chitosan are mediated by their positive charges. This was further confirmed by Dodane *et al.* [79] using chitosan hydrochloride with a degree of deacetylation of 80%. Involvement of tight junctions was visualized by confocal laser scanning microscopy using occludin and ZO-1 proteins. They also observed a slight perturbation of the plasma membrane, indicating an increased intracellular uptake. According to the authors, this observation suggests an action of chitosan upon intracellular uptake. A transient effect of chitosan on the cell barrier was proposed, since reversible effects on the permeability and structure of Caco-2 cell were detected. Recently, Smith *et al.* [73], using Western blotting of Caco-2 cells fractions, observed that ZO-1 protein translocates from the membrane to the cytoskeleton in response to treatment with chitosan. This tendency was also observed for occludin. The authors used chitosan glutamate with a degree of deacetylation of 85%. Globally, it can be concluded that chitosan is able to enhance the paracellular route of absorption by tight junction

disruption, inducing the translocation of TJ proteins from the membrane to the cytoskeleton.

Another important aspect in relation to the use of chitosan as absorption enhancer in drug delivery systems is the presence of a mucosal barrier throughout the gastrointestinal tract. It should be noted that even though Caco-2 cell monolayers represent a very good *in vitro* model of intestinal absorption, they lack a mucus layer covering the epithelium. To address this issue, Shipper *et al.* [74] investigated chitosan enhancing effects on mannitol permeability using a mucus producing cell line (HT29-H). It was shown that the presence of an intact mucus layer reduces the absorption-enhancing effect of chitosan hydrochloride. Mucus may, therefore, act as a diffusion barrier for chitosan polymers. To overcome this important limitation, the authors suggested increasing the effective concentration of the enhancer.

### ***In Vivo Studies***

*In vivo* studies of the enhancing effects of chitosan on intestinal absorption are scarce but concordant. Lue en *et al.* [80] reported that the intraduodenal application of busserelin and chitosan hydrochloride in a gel formulation at pH 6.7 significantly increased drug bioavailability in rats. Similarly, Thanou *et al.* [81], using chitosan hydrochloride 1.5% w/v at pH 5 and the pig as animal model, found the intraduodenal administration of octreotide acetate (a somatostatin analog) together with the polymer to produce a 3-fold increase in drug bioavailability compared to control. However, these same authors [70] previously reported that chitosan hydrochloride administered intraduodenally to rats at pH 7 did not significantly increase octreotide bioavailability. Hence, the chitosan pH-dependent effect observed *in vitro* was also confirmed *in vivo*. The explanation for this phenomenon is based on the fact that chitosan, being a weak base, loses its positive charge in neutral and basic media - thus proving to be ineffective as an absorption enhancer [60].

### **Intestinal Enhancing Properties of N-Trimethyl Chitosan Hydrochloride Derivatives (TMC)**

The TMC derivatives (Fig. 3D) have gained considerable interest because of their solubility properties in neutral and basic environments. Consequently, a great volume of data have been published on the intestinal enhancing properties of these polymers. It is difficult to draw clear conclusions from the results obtained, however, due to the multiple variables implicated in the designing of the experiments - such as degree of TMC quaternization, pH, concentration, and the compound or drug tested. We will first present the *in vitro* research, followed by the *in vivo* studies, which mainly have been conducted as comparative *in vitro-in vivo* studies.

### ***In Vitro Studies***

The different *in vitro* studies performed with TMC are summarized in (Table 2). The different variables that can influence the enhancing properties of these polymers have been included. TMC derivatives are especially effective in enhancing the transport of small hydrophilic compounds (e.g., mannitol) [68,71,82-84,86,87], though they also improve the transport of large molecules (drugs) such as busserelin, insulin, DGAVP and octreotide acetate [70,82,85].

Throughout the multiple studies performed (for more detailed information see Table 2), the researchers have abundantly shown the degree of quaternization to play an important role in the absorption enhancing properties of these polymers, especially in neutral and basic environments. This may be explained by the positive charge density on the TMC molecules, determined by the degree of quaternization. TMC polymers with a higher degree of quaternization have more positive charges available for interaction within the TJ to induce opening of the latter [86]. Thus, there is general agreement that the promoting effect of TMC derivatives increases with an increase in their degree of quaternization [70,71,83,84,86,87]. Discrepancies exist regarding the optimum degree of quaternization. Thus, maximum absorption enhancement of mannitol and busserelin was recorded with TMC 60 (degree of quaternization 60%) [70,84]. However, it has recently been reported that the best and maximum permeation-enhancing results are achieved with TMC 49 (degree of quaternization 48.8%)[86, 87].

The studies published in the literature agree on the low or scarce toxicity of TMC derivatives.

### ***In Vivo Studies***

The few *in vivo* studies reported to date are relatively recent and have been conducted using TMC derivatives with a high degree of quaternization (TMC 40 and TMC 60), and involving the same drugs tested *in vitro*, i.e., busserelin and octreotide acetate [70,81,85]. All these data reveal increased drug bioavailability when the drug is administered intraduodenally in rats and pigs, with TMC40 and TMC60 at neutral pH values. Moreover, a concentration-dependent effect of these polymers was observed [81, 85]. No toxicity studies have been presented to date.

### **Others Chitosan Derivatives**

Because of its positive charge, TMC is only suitable for improving the intestinal absorption of macromolecular therapeutic agents with neutral or basic properties. Hence, when chitosan salts and TMC were evaluated for their compatibility with low molecular weight heparin (LMWH), a highly anionic polysaccharide, strong aggregation was observed with subsequent fiber formation and precipitation. To overcome this problem, another chitosan derivative has been synthesized: mono-N-carboxymethyl chitosan (MCC) [88]. This polymer is perfectly soluble in aqueous environments at neutral and alkaline pH values, and is compatible with anionic and neutral compounds. MCC was evaluated as a potential absorption enhancer of LMWH *in vitro* (Caco-2 cell monolayers) and *in vivo* (rats) [88]. Both the *in vitro* and *in vivo* results indicated that MCC derivatives are capable of significantly increasing the intestinal absorption of LMWH. Moreover, these polymers were seen to present non-toxic characteristics. The authors concluded that carboxymethyl modifications of chitosan may be suitable absorption enhancers for the peroral delivery of anionic macromolecules.

In summary, it can be concluded that chitosan and its derivatives are promising excipients for use as enhancers in the peroral delivery of poorly absorbed drug. Furthermore, due to their particular properties, chitosans are currently used

**Table 2. Overview of *In Vitro* (Caco-2 Cell Monolayers) Studies with TMC Derivatives as Absorption Enhancers**

Degree of quaternization	Marker compound drug tested	pH concentrations	Enhancing effect	Toxicity	Ref.
12.28%	Mannitol(MW 182.2) FITC-dextran (MW 4400) Buserelin (MW 1300)	pH 6.7 1.5-2.5% (w/v)	Papp <sub>man</sub> <sup>(a)</sup> (32-60 fold) Transport of FITC-dextran (167-373 fold) and buserelin (28-73 fold) CLSM <sup>(b)</sup> shows that TMC hydrochloride opens TJ.	No deleterious effect to the cell (trypan blue exclusion technique)	[82]
12.28%	Buserelin DGAVP <sup>(c)</sup> Insulin	pH 4.4-6.2 1.5 and 2.5 % (w/v)	transport of all the tested drugs	No deleterious effect to cell monolayer (trypan blue exclusion technique)	[76]
12.28%	Mannitol PEG 4000	pH 6.2 0.25% (w/v)	transport of both markers	No deleterious effects to the cells	[68]
61.2% (TMC-H) 12.3% (TMC-L)	Mannitol	pH 6.2 pH 7.4 0.05-1.5 % (w/v)	At pH 6.2 both polymers caused in Papp <sub>man</sub> At pH 7.4 only TMC-H was able to Papp <sub>man</sub> (31-48 fold) even at low concentration (0.05% w/v)	Caco-2 cells remained undamaged and functionally intact (trypan blue exclusion technique)	[71]
19.9% TMC-H 12.6% TMC-L	Mannitol PEG 4000 (MW 4000)	1.5-2.5 % w/v	Papp <sub>man</sub> 51 fold (TMC-L) and 97 fold (TMC-H)	No deleterious effect to the cell (trypan blue exclusion studies)	[83]
40% TMC 40 60% TMC 60	Mannitol	pH 6.2, pH 7.4 0.05-1.0 % (w/v)	At pH 7.2 TMC 40 and TMC 60 Papp <sub>man</sub> in a higher degree than at pH 6.2. At pH 7.2 TMC 60 Papp <sub>man</sub> in a higher degree than TMC 40.	Toxicity was negligible (propidium iodide nuclear stain)	[84]
40% TMC 40 60% TMC 60	Buserelin	pH 7.21 % w/v	Both TMC's transport of buserelin being the effect of TMC 60 more prominent (60 fold vs 21 fold)	—	[70]
60%	Otreotide acetate	pH 7.4 0.25 -1.5% (w/v)	Papp <sub>man</sub> in a dose dependent manner (from 34 to 121 fold). Linear correlation was observed between Papp <sub>man</sub> values and TMC concentration.	Viability and integrity of cell monolayers was not altered at the end of experiment (trypan blue exclusion studies)	[85]
22% TMC 22 38% TMC 38 43% TMC 43 49% TMC 49	Mannitol	pH 7.4 0.0625-0.5 % w/v	Papp <sub>man</sub> with an increase in the degree of quaternization of TMC being TMC 49 the most effective at the concentration of 0.5 % (w/v)	—	[86 <sup>(d)</sup> ]
12-59%	Mannitol PEG 4000	pH 7.4	Papp of PEG 4000 and Papp <sub>man</sub> with an increase in the degree of quaternization reaching a maximum for TMC-49	—	[87]

(a) Papp<sub>man</sub>= apparent permeability coefficient of mannitol.

(b) CLSM= Confocal laser scanning microscopy.

(c) DGAVAP= 9 desglycinamide-8-arginine vasopressin.

(d) The *in vitro* technique used was everted intestinal sacs from rats.

in gastrointestinal delivery systems. For an in-depth review of this subject, see [89].

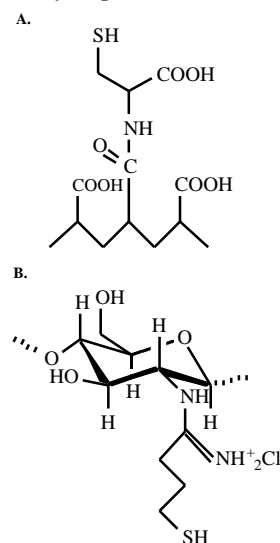
## OTHER ENHANCERS

The great interest in the peroral administration of poorly absorbing therapeutic agents has led to the development of innovative strategies. This is the case, for instance, of zonula occludens toxin (Zot), a 45-kDa protein elaborated by *Vibrio cholerae* that is able to reversibly regulate tight junction permeability [90]. This toxin interacts with a specific intestinal epithelial surface receptor, with subsequent activation of a complex intracellular cascade of events that regulate TJ permeability [91]. The potential of Zot to enhance the paracellular transport of marker compounds and drugs was first investigated by Fasano *et al.* These authors showed that Zot reversibly enhances rabbit intestinal permeability to insulin *in vitro* in the jejunum and ileum, though no substantial changes were detected in the colon [92]. Posteriorly, Cox *et al.* [93,94] demonstrated that Zot enhances transport across Caco-2 cell monolayers of low and high molecular weight markers. It was also shown that the *in vitro* permeabilities of drugs with low oral bioavailability such as paclitaxel, acyclovir, and cyclosporine and enamine anticonvulsants were increased with Zot. The enhancing effects of this toxin were compared with those of TMC 40 and TMC 60 [93]. According to the results obtained, it would appear that Zot possesses superior transport-enhancing properties when evaluated in the context of chitosan analogs. Furthermore, the enhancing properties of Zot were found to be reversible and non-toxic [90,94]. Recent studies have identified a smaller 12 kDa fragment of Zot, referred to as *G* [95] - this fragment being responsible for the intrinsic tight junction modulation activity. In 2003, Salama *et al.* [96] reported that this biologically active fragment (*G*) is able to increase mannitol permeability across Caco-2 cell monolayers. *G* was found to be non-cytotoxic at the concentration tested. These authors also examined the *in vivo* effect of *G* using the rat as animal model. When mannitol was administered intraduodenally with *G* only, no significant differences were observed in terms of the pharmacokinetic parameters. However, when the active fragment was used in the presence of protease inhibitors (PI), significant increases were obtained for Cmax and AUC of mannitol. In the opinion of the authors, these studies illustrate the potential usefulness of *G* in enhancing oral drug delivery.

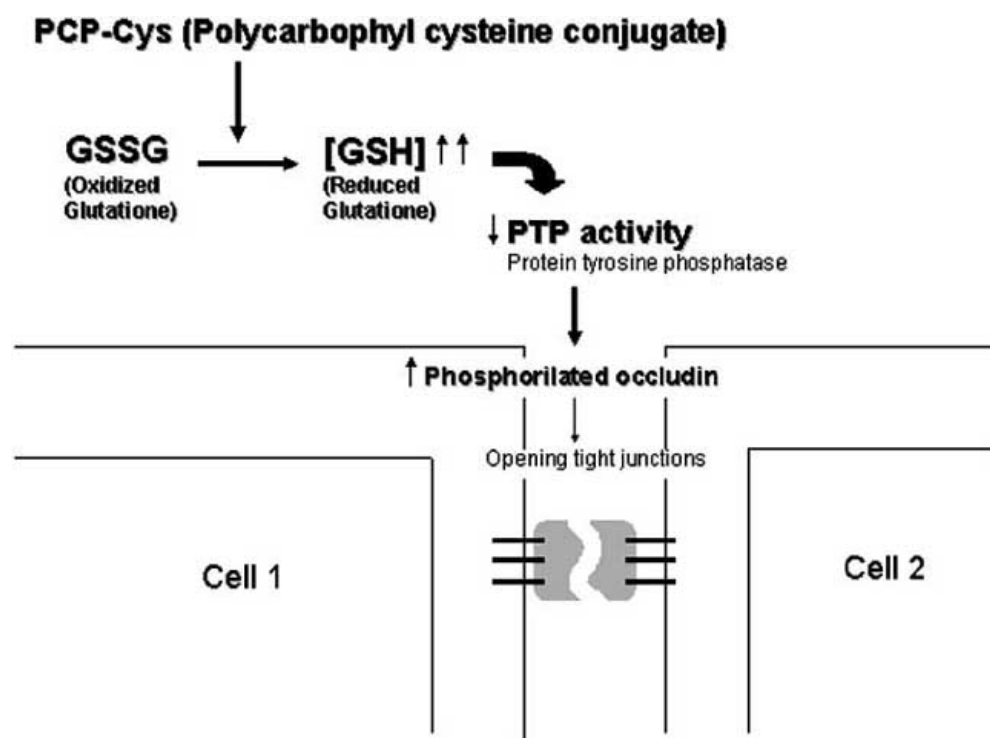
An alternative class of permeation enhancers is represented by thiolated polymers - also called thiomers. These are polymers in which the thiol groups are covalently bound. It has been shown that polycarbophyl polymers (PCP) display permeation enhancing effects [75]. This property, however, could be significantly improved as a result of the covalent attachment of cysteine (Cys) to this polymer (PCP-Cys) (Fig. 4A), as has recently been shown by Clausen *et al.* [97]. Accordingly, this thiolated polymer (PCP-Cys) is able to significantly increase the transport of marker compounds (sodium fluorescein) and peptide drugs (bacitracin-fluorescein isothiocyanate and insulin-fluorescein isothiocyanate) across the intestinal mucosa of guinea pigs (*in vitro* studies). The thiol groups, covalently attached to the polymer, seem to be responsible for the improved

permeation-enhancing properties of these conjugates. Furthermore, the improved mucoadhesive properties of these polymers provide a somewhat prolonged residence time which in some cases can lead to additional improvement in drug bioavailability. Based on all these features, the authors concluded that these thiolated polymers could constitute a promising excipient for the development of peptide drug delivery systems. Posteriorly, this same research group explored the mechanism of action of thiolated PCP-Cys in depth [98]. They showed that these compounds exert their permeation enhancing effects via glutathione. It seems that PCP-cys can transform oxidized glutathione (GSSG) to reduced glutathione (GSH), prolonging GSH concentration at the apical membrane. GSH is reportedly capable of inhibiting protein tyrosine phosphatase (PTP) activity by almost 100% [99], which leads to more phosphorylated occludin and to more open TJ (Fig. 5) [100].

Other thiolated polymers such as sodium carboxymethylcellulose-cysteine [101], chitosan-cysteine [102] and chitosan-4-thio-butylamidine (chitosan-TBA)(Fig. 4B)[103] have also displayed permeation-enhancing properties for hydrophilic compounds and drugs when evaluated using *in vitro* techniques. The addition of the permeation mediator GSH improves this effect. This association is referred to as a thiomers/GSH system [104]. Different thiomers/glutathione systems have been used depending on the hydrophilic macromolecular drugs tested. Hence, for salmon calcitonin, a peptide drug of net cationic charge, chitosan-TBA was used. In contrast, insulin and low molecular weight heparin are anionic drugs - thus being incompatible with chitosan. In these cases, an anionic thiolated polymer - poly(acrylic acid) - cysteine conjugate was used. When *in vivo* experiments were performed, a significantly improved pharmacological efficacy/ bioavailability was achieved by using these systems. These results are in good agreement with those obtained *in vitro*. The authors concluded that due to their high efficacy and minimal toxicological risks, the thiomers/GSH systems represent a promising new generation of oral permeation-enhancing delivery systems for hydrophilic macromolecules [104].



**Fig (4).** Chemical structure of polycarbophyl-cysteine conjugate (A) and chitosan-4-thio-butylamidine (B).



**Fig. (5).** Postulated effect of polycarbophyl-cysteine conjugate on intestinal paracellular permeability.

Another interesting group of recently discovered compounds that can also serve as paracellular permeation enhancers are the nitric oxide (NO) donors. In 1995, Salzman *et al.* [105] showed that sodium nitroprusside (SNP), an NO donor, induced a concentration-dependent increase in fluorescein sulfonic acid transport in Caco-2 cell monolayers. Similarly, Utoguchi *et al.* [106] reported that S-nitroso-*N*-acetyl-penicillamine (SNAP), another NO donor, was able to greatly enhance the rectal absorption of insulin - this effect being concentration-dependent. Posteriorly, Yamamoto *et al.* [107] also observed this absorption-enhancing effect upon 5(6)-carboxyfluorescein transport with other NO donors such as NO5 [3-(2-hydroxy-1-methylethyl)-2-nitrosohydrazino]-1-propanamine] and NO12 [*N*-ethyl-2-(1-ethyl-hydroxy-2-nitrosohydrazino)-ethanamine], using an *in vitro* Ussing-chamber method and the rat jejunum and colon. Regional differences in the promoting effect of NO12 were found (colon > jejunum). Their findings also demonstrated NO12 action to be mediated by nitric oxide, with partial inclusion of dilatation of tight junctions in the epithelium. As these agents exhibit low toxicity, the authors suggested that NO donors may be useful for enhancing the intestinal absorption of poorly absorbing drugs.

## CONCLUSIONS

There is a general agreement over the usefulness of paracellular permeation enhancers as a practical basis for developing poorly absorbable drugs such as peptides, peptides analogs or others hydrophilic macromolecules for administration via the oral route. Considerable effort both in the academic setting and in industry has focused on the

development of absorption enhancers that are both effective and safe. Among these compounds, sodium caprate, chitosan, and *N*-trimethyl-chitosan hydrochloride derivatives seem to be promising candidates. Most studies to date have been carried out using *in vitro* techniques - the results obtained *in vitro* and *in vivo* not always being in good agreement. It is hoped that more *in vivo* studies will be published soon. The safety of these intestinal permeability-modifying compounds is a crucial aspect in the development of drug delivery systems including enhancers. Cytotoxicity evaluations have been performed mainly *in vitro*, and it seems that the results obtained are not always predictive of *in vivo* toxicity. All such concerns have not impeded continued efforts to search for agents that can increase paracellular permeability via modulation of specific cellular process regulating tight junction physiology. This is the case of zonula occludens toxin, thiolated polymers or NO donors, which are currently investigated as paracellular permeation enhancers.

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