1. INTRODUCTION

1.1 Diabetes Mellitus

Diabetes is one of the costliest healthiness troubles in the human race; without a doubt costs maintain to spiral in the face of common increasing popularity of obesity. The implication of the epidemiological burden of diabetes lies in the convolution of this metabolic disease which may, if left untreated or not correctly treated; develop into complications, many of which are life-threatening and very costly to treat. In the developed world, diabetes is one of the main causes of cardiovascular disease including heart attacks and strokes, the most common cause of adult blindness in the nonelderly, the foremost cause of non-traumatic subtraction in adults, and diabetic nephropathy is the main driver of renal dialysis.

“Diabetes” - to siphon first worn 250 AD by Greek physician Aerates reflected clinical symptom of increased fluid excretion and wasting “Diabetes mellitus” - mellitus, Latin for honey Coined in 1674 by Thomas Willis, personal physician to King Charles II

- Failing in glucose hemostasis
- Prominent blood glucose levels
- Absent or scarce pancreatic insulin discharge
- May or may not have synchronized impairment in insulin action

Type 1- Selective B cell devastation severe or absolute insulin deficiency immune or idiopathic subtypes

Type 2- Resistance to insulin action combined with a relative deficiency in insulin secretion insulin produced in B cells but not adequate to overcome resistance

Type 3- other types of causes, ie, pancreatic disease, drug therapy

Type 4- Gestational diabetes first occurrence during pregnancy Insulin unseen from pancreatic Beta cells Glucose enters through GLUT4 Converted to G6P U ATP production U ATP inhibits K+ channel-depol. Ca+2 channel opens, [Ca+2]U Stim. insulin secretion (exocytosis)

1.2 Insulin

- Synthesized as proform
- Proteolyzed in Golgi (A and B chains, S-S linked)
- stored in vesicles (along with equimolar C-peptide)
- Binds transmembrane tyrosine kinase receptor (pM affinity)
• Basal, 30-90 pM
• Postpranal, 360-540 pM
• Affinity decreases with pH, ie acidosis will decrease efficacy of exogenous insulin in diabetics
• Affinity decreased by glucocorticoids
• Receptor requisite activates intrinsic tyrosine kinase activity consequential in recruitment of various effectors-IRS proteins major intention
  -Recruitment of GLUTs,
  -Endocytosis of insulin-IR complex, activation of Ras, PI3K,
  -Increased glycogen, protein, fat
  -Increased glucose uptake
  -Increased glucose utilization
  -Decreased formation of glucose from glycogen

1.2.1 For Type I Diabetes, insulin replacement is the only therapy
THERAPEUTIC GOAL: faithfully mimic normal insulin levels throughout day
Glucose levels: -Fasting: <140 mg/dL -2-hr postprandial: <175 mg/Dl HbA1c concentration <8%
In the US alone, treatment of patients with diabetes and diabetic complications is predictable to cost over 100 billion dollars each year. In fact, the International Diabetes Federation estimate direct costs of diabetes to be approximately 6% of the total health budget of cost-effectively developed countries. Accordingly, the need for new options to treat diabetes and purposely type-2 diabetes (T2D) at early stages of the disease is escalating at incredible momentum.

Advances in the field of biotechnology have brought a lot of novel and potent active compounds. However, the progress of these molecules as medicines is largely impaired by the fact that they are not administrable by the oral route. Indeed, the oral one is the most opportune route of administration for both patients and medical staff. However, administering peptide and protein drugs orally is a frightening dare due to their very short life in the gastric and intestinal fluids. In addition, they undergo from a poor absorption rate through the intestinal barrier. Among the unlike approaches developed, polymeric micro- and nanoparticles characterize an interesting approach. Certainly, they shield the encapsulate drug from the outside cruel conditions; they also may favor the uptake by intestinal cells.
Chapter 1

Introduction

Polymeric particles will separate the encapsulated drug from the external medium therefore protecting the peptide from the peptidases, allowing, then, their uptake by enterocytes. After absorption, polymeric particles will gradually mortify according to a kinetic profile depending on the nature of the polymer, thus providing a sustained and controlled release of the drug. Polymeric particles have been shown to cross the intestinal wall, although only in minute quantities.\(^{19}\) The size of the particles as well as the nature of the polymer is critical parameters involved in particle uptake by the GI tract. Therefore, this review will first introduce a brief overview of particle preparation methods and the physiology of particle absorption. Then, the studies using polymeric particles to get better oral insulin delivery will be reviewed.\(^{20}\)

Given orally, peptides and proteins are degraded by the enzymes from the gastric and intestinal juices rich in proteases such as trypsin or chymotrypsin.\(^{21}\) Therefore, they do not reach intact the site of absorption, namely the enterocytes. Furthermore, the brush border and the cytosol of the absorptive cells are full of peptidases that will degrade small peptides resulting from the hydrolysis of the proteins into amino acids that are readily resorbed in the blood.\(^{22}\) Thus, the first goal to develop an oral formulation for peptide and protein drugs is to reduce or even better to avoid enzyme degradation.\(^{23}\) Polymeric particles will isolate the encapsulated drug from the external medium therefore protecting the peptide from the peptidases, allowing, then, their uptake by enterocytes. After absorption, polymeric particles will slowly degrade according to a kinetic profile depending on the nature of the polymer, thus providing a sustained and controlled release of the drug.

Polymeric particles have been shown to cross the intestinal wall, although only in minute quantities. The size of the particles as well as the nature of the polymer are critical parameters involved in particle uptake by the GI tract. Therefore, this review will first introduce a brief overview of particle preparation methods and the physiology of particle absorption. Then, the studies using polymeric particles to improve oral insulin delivery will be reviewed.

Among controlled release formulations, polymeric nano and microparticles have shown interesting promise for protein delivery. Nanoparticulate hydrogels consisting of alginate, agar, agarose, chitosan, or synthetic polymers have been developed and tested over the past two decades. Nanoparticulate delivery systems have the potential to improve protein stability, increase the duration of the therapeutic effect and permit administration through nonparental routes.\(^{24}\)
Oral delivery is the preferred route for administration because it is non-invasive, avoids injections, and decreases risk of infection. It is also physiologically desirable, since the exogenous protein imitates the physiological pathway undergoing first hepatic bypass. The intestinal absorption of proteins has been reported and a combination of mechanisms described to explain how proteins cross the intestinal mucosa.

One approach to improve the gastrointestinal uptake of poorly absorbable drugs like insulin is to entrap the protein within colloidal nanoparticles, which provide degradative protection in the gastrointestinal tract and facilitate transport into systemic circulation. Special attention has been given to mucoadhesive particles which maintain contact with intestinal epithelium for extended periods, promoting penetration of active drug through and between cells due to the concentration gradient between nanoparticles and intestinal membrane. In fact, insulin was observed to be directly internalized by enterocytes in contact with intestine, and retention of drugs at their absorptive sites by mucoadhesive carriers is a synergic factor. Furthermore, uptake of nanoparticles by the M cells of the Payer’s patches was demonstrated, being absorbed transcellularly, serving as a major gateway for nanoparticle absorption as well as absorption through the much more numerous gut enterocytes. Endocytosis occurs through clathrin coated pits and vesicles, fluid phase endocytosis and phagocytosis. It is well accepted that hydrophobic, negatively charged, protein-loaded nanoparticles smaller that 1 nm potentially show the best absorption rate, although other factors may govern nanoparticle absorption. Polymers such as alginate and chitosan have been described as biocompatible, biodegradable and mucoadhesive, enabling numerous pharmaceutical and biomedical applications.
1.2.2 Types of Insulin

<table>
<thead>
<tr>
<th>Types of insulin</th>
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<th>Trade name</th>
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<th>Duration of Action (hrs)</th>
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<td>Aspart</td>
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1.2.3 Selection of Companies that Will Be Presented

A number of companies have claimed to be developing an oral insulin formulation. However, it is not easy to evaluate the level of activity of these companies. It appears as if some companies have vanished or are not interested in this topic currently (e.g., Autoimmune, Biosante, Coremed, Cortecs, Eligen, Nobex, and Protein Delivery). Most of the developments of these companies have failed in phase II clinical studies, showing insufficient metabolic control in patients with diabetes. The definition of “activity” used for the selection of companies presented subsequently in this review is that they have presented new data from clinical–experimental or clinical studies between 2006–2009 at scientific meetings. Five companies working on oral insulin fulfill these criteria: Emisphere, United States; Biocon, India; Diabetology, United Kingdom; Diasome, United States; and Oramed, Israel. According to the selection criteria used, the approaches followed by other companies that have presented no human data thus far (e.g., Access Pharma, United States; Apollo, Australia; and
Merrion, Ireland) will not be presented. Whereas a number of companies try to develop an oral insulin formulation, there is only one company developing. A more rigid definition focus on published studies only would have reduced even further the number of companies that can be presented in this review. Most of these companies are relatively small, with the exception of Biocon. The limited economical resources of these companies are most probably the best explanation for the small number of (good) clinical–experimental and clinical studies that have been performed with a given development (with wide differences between the companies). In our review of published studies, including those presented as abstracts/posters only, it appears as if most of the studies performed were not performed according to the standards of Good Clinical Practice (GCP). Additionally, many have used an unusual study design (e.g., no appropriate control group or highly selected groups of patients). In summary, the validity of many of these studies is at least dubious.

1.2.4 Advantages of Oral Insulin

Despite this very negative view, the attractiveness of this route of insulin administration is so high that research continues. As stated earlier, for patients with diabetes, it is quite attractive to swallow an insulin pill. The hope is that this convenience would lead to a better compliance of the patients toward the start and maintenance of an insulin therapy. The hope is that this increased compliance in turn leads to better metabolic control, reducing the risk of development of diabetes-related complications with all their consequences. However, it is not only an improvement in the quality of life that makes oral insulin attractive. If the insulin would be absorbed in the gut, this peptide would be (like all other amino acids and nutritional components) transferred directly toward the liver with the bloodstream draining the gut. At the liver level, the exogenously applied insulin would control hepatic glucose production to the same extent, as this is induced by endogenously secreted insulin in healthy subjects. This more “physiological insulin delivery” would be associated with reduced peripheral hyperinsulinemia (as is the case with SC insulin administration).

1.2.5 Oral Insulin: Emisphere

Since the late 1990s, this U.S.-based company has tried to develop an oral insulin formulation. However, it appears as if these activities were stopped per a statement on the company’s homepage. From 2001–2004, this company has performed a number of phase I / IIa clinical–experimental studies evaluating the time-action
profile of this oral insulin formulation, the dose-response relationship, and the impact of a meal on insulin absorption. In a proof of concept study, different doses of insulin in combination with an agent that interacts with insulin in a manner that promotes the uptake in the gut and meanwhile protects the insulin were administered orally as a single dose to 12 non-insulin dependent patients with type 2 diabetes and 4 control subjects. In all cases, a glucose-lowering effect was demonstrated, preceded by an increase in plasma insulin levels. Studying the metabolic effect of this oral insulin formulation with a glucose clamp approach, allowing a precise measurement of the blood-glucose-lowering effect, showed that swallowing a capsule with 300 U insulin together with 200 ml of water induced a clear increase in glucose infusion rates (GIR) to keep blood glucose at the target level within 30–60 min after intake by a patient with type 2 diabetes. This study documented for the first time the PD properties of an oral insulin formulation in comparison to SC-injected regular insulin. It also proved that therapeutically relevant amounts of insulin are absorbed rapidly after administration. The rapid increase and decrease in the metabolic effect induced should allow good coverage of prandial insulin requirements. At the same time, it should reduce the risk of developing late postprandial hypoglycemic events. It should be mentioned that the relative biopotency of this rapidly absorbed oral insulin formulation was 20% when taking into account the areas under the GIR in the first 60 min after administration; however, the relative biopotency was only 3% when using longer time intervals (0–6 h). Even if, for optimal control of postprandial excursions, the immediate effect is of great relevance, calculation of the relative biopotency over restricted time periods can be misleading and should be used with caution.34

In a randomized, controlled, double-blind, parallel group pilot study in 13 patients (7 treated, 6 controls) with type 2 diabetes well controlled with dietary treatment, the safety and efficacy of treatment with this oral insulin formulation was studied for two weeks. Each subject received either 300 IU insulin + 160 mg carrier or 200 mg carrier alone (administered as 2 tablets 10 min before main meals and before bedtime). In comparison to the control group, blood glucose levels of the patients treated with oral insulin were significantly lower after an oral glucose tolerance test in comparison to baseline but not in comparison to the control group. The oral insulin was well tolerated, i.e., no side effects or hypoglycemic events were observed.
In another study, an improved formulation of this oral insulin was studied in eight patients with type 2 diabetes.\textsuperscript{35} Administration of two tablets (each 150 IU insulin + 80 mg carrier) or placebo (200 mg carrier alone) in a subgroup of four patients, which consumed a mixed meal (441 kcal, 66% carbohydrates), induced a more rapid increase in insulinemia, which was accompanied by lower postprandial excursions. However, in view of the small sample size, it is not surprising that most differences did not reach statistical significance.\textsuperscript{36}

In 2006, Emisphere performed a 90-day double-blind phase II clinical study in India with 145 patients with type 2 diabetes on oral antidiabetes drugs (OADs; Metformin). The patients were randomized in four different groups and treated with three different insulin doses or placebo. No significant differences in metabolic control [hemoglobin A1c (HbA1c)] between the groups were observed despite treatment with up to 1000 U of oral insulin per day. The negative outcome of this study was explained with problems in conducting the study adequately. Unfortunately, the results of this study were never presented in a full publication. Most probably it was the negative outcome of this study that hampered cooperation with a big pharmaceutical company and stopped this development despite a lot of effort for a number of years.

\textbf{1.2.6 Oral Insulin: Biocon}

This large Indian-based pharmaceutical company has taken over the oral insulin technology developed by Nobex. Thus the formulation of their current oral insulin candidate (IN-105) is based on several years of development with oral insulin analogs, including HIM2. IN-105 is a human insulin molecule conjugated on position B29 with polyethylene glycol via an acyl chain.\textsuperscript{37} The current formulation for IN-105 is a second-generation tablet, which is declared to be simple to manufacture, uses readily available excipients, and has an attractive stability profile at ambient conditions.\textsuperscript{38} It appears as if Biocon is intensively working on this development.\textsuperscript{39}

IN-105 is declared to have the following characteristics:

- Improved half-life in the digestive tract and improved absorption,
- Lower immunogenicity as compared to insulin\textsuperscript{40}
- Lower mitogenicity as compared to insulin\textsuperscript{41}
- Retains a similar pharmacological activity as insulin, and
- Conserves safety profile and good clearance profile as compared to insulin.
1.2.7 Oral Insulin: Diabetology

This small U.K.-based company with an ambitious name has had an oral insulin formulation (CapsulinTM) in development for a number of years that is not a new chemical entity (in contrast to, for example, the Biocon development). This should enable a simpler approval procedure by the regulatory authorities. The dry powder mixture, which contains insulin, stabilizer, and solubilizer. “generally regarded as safe”/Pharmacopeia excipients), is packaged in an enteric coated capsule (with 150 U) that protects the insulin from gastric degradation. The capsule is declared to pass intact through the stomach to the small intestine. The coating shall dissolve in the jejunum in an area with neutral pH, and the capsule content is subsequently released. The excipients (an aromatic alcohol and a solubilization aid) are supposed to enhance insulin absorption through the intestinal mucosal layer. Diabetology has performed some early clinical–experimental proof of concept studies in healthy subjects and patients with type 1 diabetes and more recently a phase IIa randomized, open, crossover study in 16 patients with type 2 diabetes. One group of eight patients participated on two glucose clamp study days with 150 U Capsulin on one day and SC injection of 12 IU regular insulin on the other day. The other group of patients received 300 U Capsulin on one study day and also 12 IU regular insulin the other day. In the 10 days between the two clamps, patients were instructed to swallow one capsule in morning and one in the evening (300 U per day) 60 min prior to breakfast and evening meal (no placebo control). For the 6 h period of the glucose clamp study days, the glucose requirements to keep blood glucose constant for both doses of Capsulin (150 and 300 U) was ~50% that of the 12 IU SC regular insulin injected into the abdomen. Therefore, no dose-related effect was observed with Capsulin. From the time-action profiles, it appears as if 150 and 300 U Capsulin had comparable intersubject variability to SC regular insulin. The onset of the metabolic effect with Capsulin and regular insulin was slow, i.e., maximal GIR were observed after several hours. This might hamper prandial insulin coverage; however, this was not studied until now. In addition, after 6 h, a significant amount of metabolic effect (even if the absolute level was low with 1 mg/kg/min) was still present, which can induce late postprandial hypoglycemic events. Substitution of the oral agents by Capsulin for a 10-day period did not compromise fasting blood glucose levels; however, the level of metabolic control established was mediocre with average glucose levels around 9 mmol/liter.
1.2.8 Oral Insulin: Diasome

The approach followed by this small U.S.-based company is a novel insulin delivery system that can be used for oral and SC insulin delivery. The key components of this are hepatic-directed vesicles loaded with insulin (HDV-I) that were developed some years ago.\textsuperscript{51} These vesicles are composed of liposomes (<150 nm diameter) that contain insulin attached to a specific proprietary hepatocyte-targeting molecule (HTM). The HTM is proposed to selectively target the delivery of the encapsulated insulin to the hepatocytes similar to normal insulin physiology. This oral HDV-I should be stable at low pH and in blood.\textsuperscript{52} It should be small enough (20–50 nm) to cross membrane barriers and to avoid enzymatic degradation. In contrast to all other oral insulin formulations, HDV-I is declared to have a high biopotency, i.e., it is formulated as an oral gel capsule with 5 U insulin only.

More recent studies were performed in a large diabetes research center in patients with type 2 diabetes\textsuperscript{22} and type 1 diabetes.\textsuperscript{52} Shows the results of a small single-blind placebo-controlled trial in six patients with type 2 diabetes with current diagnosis of the disease (residual endogenous insulin secretion). Patients swallowed capsules with different insulin content while also taking their usual OADs 30–45 min prior to breakfast (60 g carbohydrates). While adding oral insulin to the treatment improves postprandial glycemic excursions in comparison to placebo, escalating doses of this oral insulin does not induce a further improved metabolic control. It might be that, with the lowest dose already, a full suppression of hepatic glucose production was established; however, it might also be that only a certain amount of insulin was absorbed despite an increase in dose.\textsuperscript{53} In the randomized, double-blind (for injectable insulin arms only, SC regular human insulin and SC HDV-I), open-label (oral HDV-I) study with 30 patients with type 1 diabetes, the metabolic control (average glycemia) achieved was worse with oral insulin in comparison to the two patient groups with SC insulin treatment.\textsuperscript{54}

Beginning in 2009, a large long-term study is being conducted in 40 U.S. centers with 230 patients (placebo controlled).\textsuperscript{55}

1.2.9 Oral Insulin: (Potential) Side Effects

With oral insulin, considerable amounts of insulin (and other excipients) have to be applied. The question is, can this insulin induce side effects? If there is an even distribution and degradation of most of the insulin taking place in the stomach, the concentration of insulin in the lower GI should be low. Nevertheless, it might be that
locally high insulin concentrations in the GI show up. The question is, can such high levels of a growth-promoting substance like insulin induce cancer development or enhance the development of existing tumors? It is known that there is a modestly increased risk of colorectal cancer in SC-insulin-treated patients with type 2 diabetes but not in patients with type 1 diabetes. Also, in animal studies with application of high doses of oral insulin over prolonged periods of time during insulin-tolerization studies, no carcinogenic effects have been observed. Even if insulin has no side effects, one has to acknowledge that the other substances (very different chemical compounds) added to improve uptake of insulin in the gut might have safety issues, especially if taken repeatedly over prolonged periods of time. The absorption enhancers added to oral insulin formulations such as chemical solubilizers (i.e., sodium lauryl sulfate) or even biological ones (i.e., bile salts) have the ability to extract and solubilize lipids (such as those of the cell membrane) and threaten the integrity of cell structures even on a short/medium term. In view of the history with inhaled insulin, all potential risks (even if there is only a theoretical risk) of oral insulin require careful consideration. One has to acknowledge the high costs for such investigations, as many patients must be involved and followed up on for long periods of time. Nevertheless, it might not only save the companies a lot of money in the long run, but also most importantly, avoid risks for patients.

Diabetes is one of the costliest health problems in the world; indeed costs continue to spiral in the face of widespread increasing prevalence of obesity. The significance of the epidemiological burden of diabetes lies in the complexity of this metabolic burden. Diseases which may, if left untreated or not appropriately treated, develop into complications, many of which are life-threatening and very costly to treat. In the developed world, diabetes is one of the main causes of cardiovascular disease including heart attacks and strokes, the most common cause of adult blindness in the nonelderly, the leading cause of non-traumatic amputation in adults, and diabetic nephropathy is the main driver of renal dialysis.
In the US alone, treatment of patients with diabetes and diabetic complications is estimated to cost over 100 billion dollars each year. In fact, the International Diabetes Federation estimates direct costs of diabetes to be approximately 6% of the total health budget of economically developed countries. Consequently, the need for new options to treat diabetes and specifically type-2 diabetes (T2D) at early stages of the disease is increasing at tremendous speed. The importance of good glycemic control to reduce the risk of vascular complications of hyperglycemia is well established. However, T2D is a progressive disease, and the need for increasing the intensity of treatment to maintain glycemic control is an indicator of that progression. A variety of new oral antidiabetic drugs (OAD) have emerged for the treatment of T2D—dipeptidyl peptidase-4 inhibitors, glucagon-like peptide 1 analogs, thiazolidinediones, glinides, selective sodium glucose cotransporter, inhibitors, and several other unique agents now in development.

Despite recent progress, the management of T2D with OAD alone appears to be suboptimal for many patients. Ultimately, most patients will require insulin therapy, although insulin is still all too often thought of as “last resort” or “endstage” therapy. This and other misperceptions frequently limit the early initiation of insulin therapy, even among patients to whom OAD are no longer adequate. Many patients with a high pretreatment hemoglobin A1C (HbA1c) are not adequately controlled by a single oral agent even at high dose suggesting that earlier, more aggressive treatment in primary care is required. Insulin is the only agent of “universal” value for treatment of diabetes mellitus (T1D and T2D). In reality, current classes of OAD are truly effective only in combination with insulin produced by cells (endogeneous insulin) or administered by injection or other delivery systems (exogeneous insulin), or combination of both endogeneous and exogeneous. Insulin can be used in therapy of diabetes without any other drugs but not vice versa.

1.2.10 Therapeutic value of Insulin
The need for insulin depends upon the balance between insulin secretion and insulin resistance. Mounting evidence suggests that insulin therapy not only produces symptomatic improvements, but also that insulin treatment can help correct the underlying pathogenetic mechanisms responsible for type 2 diabetes, namely, insulin resistance and impaired insulin secretion. In a number of studies, insulin therapy greatly improved insulin secretion, presumably by reducing hyperglycemia. There
is research evidence that insulin protects islets from apoptosis and that insulin may even increase cell regeneration. Interestingly, short term insulin therapy appears to result in long-term improvement in blood-glucose control, especially when administered in the earliest stages of diabetes. For example, in a recent study by Chen et al. it was shown that in newly diagnosed T2D with elevated fasting glucose levels, a 2–3-week course of intensive insulin therapy can successfully lay a foundation for prolonged good glycemic control. The ease with which normoglycemia is achieved on insulin may predict those patients who can later succeed in controlling glucose levels with attention to diet alone. Based on these and similar observations from other investigators, some diabetes experts have advocated initiating intensive insulin therapy early in the course of T2D, or immediately after a diet and exercise regimen fails, in an effort to preserve remaining cell function and improve long-term glycemic control. Finally, insulin also presents some advantages from a pharmacodynamic stand-point as compared with OAD. Unlike the other blood-glucose-lowering medications, insulin has an exceptionally wide therapeutic window, i.e., there is no known maximum dose of insulin beyond which a therapeutic effect will not be relevant. It is noteworthy that relatively large doses of insulin compared with those required to treat T1D, may be necessary to overcome the insulin resistance of T2D and lower HbA1C to the target level.

1.2.11 Why is new delivery forms of insulin needed?

The clinical course of T2D is characterized by defects in both insulin secretion and insulin action. The defect in insulin secretion seems to be progressive; newly diagnosed patients in the U.K. Prospective Diabetes Study had 50% of normal insulin secretion upon initial diagnosis, but this had declined to <25% of normal insulin secretion 6 years after diagnosis. The results of multiple investigations strongly suggest that good glycemic control in T2D often requires insulin supplementation therapy unfortunately; many patients with T2D who could benefit from insulin therapy do not receive it or do not receive it in a timely manner. Part of this gap appears to be attributable to reluctance to taking insulin among patients and resistance to prescribing insulin among health care providers. This resistance is based on a variety of factors, primarily beliefs and perceptions regarding diabetes and its treatment, the nature and consequences of insulin therapy, and how others would regard insulin therapy. For example, T2D patients tend to be older and more difficult
to train on testing their own blood-glucose levels and to self-administer injectable insulin.

1.2.12 Disadvantages of existing insulin products

Although insulin is the most effective therapy for reducing blood-glucose levels, the current injectable forms of insulin present several drawbacks. The need for multiple injections daily is a substantial disadvantage. This is due to a number of factors including needle phobia, non-physiological delivery to the wrong target tissues, poor pharmacodynamics and often non-ideal treatment initiation. In regards to adverse effects, the risk of hypoglycemic episodes and weight gain represent concerns for injection therapy with insulin. Moreover, the safety of long-acting insulin analogs has recently been questioned. A number of recent studies suggest that these long-acting synthetic chemical entities may produce an increased risk of breast cancer. As reviewed by Smith and Gale a number of retrospective studies submitted in 2008 and 2009 suggest that use of insulin glargine is, after adjustment for dose, associated with a possible increase in tumor risk in humans. Interpretation of this analysis proved controversial, but the implications are serious. In the case of glargin, the most commonly used long-acting insulin analog, the question has been raised as to whether this insulin analogue may have specific growth promoting potential, which could be expressed as tumor-promoting activity in patients.

Issues in development of new chemical entities for treatment Diabetes By many accounts, the pharmaceutical industry is experiencing a severe decline in research productivity. More and more capital is being invested in research and development, but the rate at which new drugs are introduced to the market is failing to keep pace. According to the FDA, new compounds entering Phase I development today have only an 8% chance of reaching the market versus a 14% chance 15 years ago. And the Phase III failure rate has risen to 50% versus 20% just ten years ago. This issue is particularly apparent in the field of diabetes.

Moreover, in 2008, FDA introduced a new guideline for clinical development of anti-diabetes agents. This new guideline stipulates a minimum number of test subjects to be enrolled and a minimum duration of the evaluation period on pivotal clinical trials. The reason for the high safety demands of novel anti-diabetes treatments involve

(i) A rapidly increasing size of the target population,
(ii) A decrease in the average age at diagnosis and
(iii) Life-long requirement for therapy of subjects once obtaining the diagnosis. Furthermore, in 2008, concern over reports that link diabetes drugs to cardiac problems. U.S. drug regulators today ask diabetes drug makers for more safety data. These requests for additional data could delay new product approvals by years and add millions of dollars to development and post-market commitment costs.

1.2.13 Advantage of Portal Insulin Delivery

The basic goal of tight control of blood-glucose using intensive insulin therapy is to mimic insulin secretion by the normal pancreas. Today, this involves designing a regimen that includes long-acting or intermediate-acting insulin preparations that approximates basal insulin secretion (i.e., the small amount of insulin the pancreas secretes continuously) and a short-acting insulin preparation that is administered before mealtimes and mimics the extra insulin the pancreas secretes to handle the postprandial rise in blood-glucose levels. The advent of insulin pumps is one more recent approach to fine-tuning delivery in order to gain greater control.

There have been a number of pharmaceutical approaches to developing a non-invasive delivery route for insulin, most of them topical (e.g. patches) or inhalation. These share the same weakness as injectable insulin: the delivered protein passes first into the peripheral circulation and only thereafter, in much diluted form, into the liver. Thus topically & subcutaneously administered insulin result in fat and muscle being exposed to higher insulin levels than the liver. This is in contrast to endogeneous insulin which is delivered from the pancreas into the portal system and the liver.

Oral delivery would share this advantage. The emergence of new non-invasive delivery technologies that allows administration of insulin through the portal vein instill hope for therapeutic breakthrough. For example, reports on portal delivery of insulin from islet grafts in the portal vein demonstrate that portal delivery of insulin is important in maintenance of normal whole-body insulin sensitivity. Moreover, the advantages of portal insulin delivery are intensively discussed in the publications dedicated to development of oral insulin delivery systems. Oral insulin administration better mimics endogeneous production and is more convenient for the patient than conventional subcutaneous insulin administration. Given orally and delivered to the portal vein normal vein/arterial insulin distribution would be restored. In addition, portal delivery of insulin may present advantages in terms of lower risk for carcinogenesis. Since insulin after absorption undergoes a substantial
first pass clearance, only a small fraction of the administered drug will reach the extrahepatic circulation. After oral delivery, the absorbed insulin will predominantly exert its effect in the liver where it also is metabolized. Consequently, upon oral delivery of insulin, a lower continuous extrahepatic insulin tone is predicted to occur which may be advantageous in the light of insulin’s mitogenic properties.75

1.2.14 Industry efforts in the field of oral Insulin
The various challenges associated with the production of a reliable insulin formulation for peroral delivery includes
(i) Maintaining storage stability of the active protein,
(ii) Avoiding enzymatic degradation in the gastrointestinal tract and
(iii) Overcoming poor spontaneous insulin permeability through the gastrointestinal system.

Despite the multiple technical challenges several companies have announced initiatives in this direction, including Apollo Life Sciences, Biocon, Diabetology Ltd., Emisphere, Oramed and Orin Pharmaceuticals AG.

One may speculate that the administration of human recombinant insulin without chemical modifications or co-formulation with any new chemical entity may provide advantages in terms of development risks.76 For example, three biotechnology companies Diabetology Ltd., Oramed and Orin Pharmaceuticals have indicated progress in this respect and have in fact reached the stage of Phase II clinical trials of oral insulin preparations. Diabetology Ltd. has developed a delivery system which significantly increases the absorption of peptides, proteins and other macromolecules across the intestinal wall when delivered orally without any chemical modification of the active compounds.77 The delivery system is based on a simple mixture of components consisting of excipients with absorption-enhancing activities. Results were recently announced from 10-day repeat-dosing of CapsulinTM oral insulin in patients with T2D. The study showed that CapsulinTM was able to improve glycemic control throughout the day, including mealtimes, with reduced glucose fluctuations and postprandial glucose rises. These observations were associated with significant improvements in HbA1C, weight and triglycerides between pre-study screen and study close.78 At the same time CapsulinTM was found to be safe and well tolerated with no serious adverse events and no hypoglycemia occurring. Another company, Oramed, has announced the completion of a Phase IIa stage clinical trial of ORMD 0801, which is a capsule formulation of insulin that
incorporates adjuvants. These adjuvants are intended to protect the active protein while in transit through the harsh chemical environment of the gastrointestinal tract and promote its transport across the intestinal mucosa. ORMD 0801 was well tolerated by all patients and in two thirds of the subjects analyzed, statistically significant reductions in glucose as well as C-peptide were observed. Finally, Orin Pharmaceuticals AG is preparing a Phase II clinical trial in T2D for its oral insulin preparation. The company has developed a proprietary formulation consisting of dextran matrix for oral delivery of insulin. Being included in the dextran matrix, insulin is protected against proteolytic degradation in the gastric ventricle. Subsequently, the dextran matrix is degraded by endogenous enzymes in the mucosa of the small intestine resulting in targeted delivery at a site where insulin is spontaneously absorbed at an optimal rate. Early clinical testing in T2D patients has demonstrated absorption at clinically useful levels.

Treatment of early stage T2D is going to require an unprecedented demonstration of safety for the patient. In the future, subjects will start treatment at earlier stages (and ages) perhaps without any other ailments. Moreover, subjects with T2D are likely to live longer and need to maintain the therapy for extended periods of time, perhaps for decades. Consequently, insulin, which is a naturally occurring hormone, will present advantages as compared to new chemical entities.

In summary, a number of industry programs are currently evaluating non-invasive delivery approaches that more closely mimics the natural exposure and action of insulin as well as being more convenient and safe to the patient.

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to achieve promptly and then maintain the desired drug concentration. So that purpose the oral route is the most preferred route of administration of drugs because of low cost of therapy, ease of administration, patient compliance and flexibility in formulation, etc. In the past few decades, numerous oral drug delivery systems have been developed to act as drug reservoirs from which the active substance can be released over a specific period of time at a predetermined and controlled rate.

The oral route of drug administration is most important method of administrating drugs for systemic effect. The parenteral route is not routinely used or not possible to self administration of medication. The topical route of administration has only
recently been employed to deliver drugs to the body for systemic effect. It is probable that at least 90% of all drugs used to produce systemic effect are administered by the oral route.\textsuperscript{80} Drugs that are easily absorbed from the Gastrointestinal (GI) tract and having a short half-life are eliminated quickly from the blood circulation. To avoid these problems oral controlled drug delivery systems have been developed as they releases the drug slowly into the GIT and maintain a constant drug concentration in the serum for longer period of time.

1.3 Controlled Drug Delivery System

Treatments of acute and chronic diseases have been accomplished by delivery of drugs to patients using various pharmaceutical dosage forms. These dosage forms are known to provide a prompt release of drug. But recently several technical advancements have been done and resulted in new techniques for drug delivery. These techniques are capable of controlling the rate of drug release.\textsuperscript{81} The term-controlled release has a meaning that goes beyond scope of sustained release. The release of drug ingredients from a controlled release drug delivery advances at a rate profile that is not only predictable kinetically, but also reproducible from one unit to another.\textsuperscript{82} Blood levels within the therapeutic range for an extended period of time. To achieve the same, a drug may be administered repetitively using a fixed dosing interval. This causes several potential problems as like saw tooth kinetics characterized by large peaks and troughs in the drug concentration-time curve, frequent dosing for drugs with short elimination half-life, and above all the patient noncompliance.\textsuperscript{83} Controlled release (CR) DDS attempt to sustain drug blood concentration at relatively constant and effective levels in the body by spatial placement or temporal delivery. Thus CRDDS offer various advantages viz. reduce blood level fluctuations, minimize drug accumulation, employ less total drug, improve patient compliance, and minimize local and systemic side effects.\textsuperscript{84} During the last decade there has been interest in developing site-specific formulations for targeting the drug to the colon. The colon is a site where both local and systemic drug delivery can take place.\textsuperscript{85} Colon-specific drug delivery is intended to improve the efficacy and reduce side effects by exerting high drug concentrations locally at the disease site. Because of the distal location of the colon in the gastrointestinal (GI) tract, an ideal colon-specific drug delivery
system should prevent drug release in the stomach and small intestine, and effect an abrupt onset of drug release upon entry into the colon. Lower doses may be adequate and, if so, systemic side effects may be reduced. Site-specific means of drug delivery to colon could also allow oral administration of peptide and protein drugs, which normally become inactivated in the upper parts of the gastrointestinal tract. Vaccines, insulin and growth hormone are examples of such candidates. However, the permeability of the epithelium of the colon to peptide and protein drugs is fairly poor, and bioavailability is usually very low. The incidence of asthmatic attacks is, for example, greatest during the early hours of the morning. Because dosage forms remain longer in the large intestine than in the small intestine, colon-specific formulations could be used to prolong drug delivery.

1.4 Anatomy and Physiology of Colon

In GIT large intestine starts from the ileocecal junction to the anus having a length of about 1.5 m (adults) and is divided into three parts, viz. colon, rectum and anal canal. The colon consists of caecum, ascending colon, transverse colon, descending colon and sigmoid colon. Colon is made up of four layers, serosa, muscularis externa, submucosa and mucosa. The epithelium consists of a single layer of cells, which lines the crypts and covers the surface of the mucosa. Three major cell types found in the epithelium are the columnar absorptive cells, goblet (mucous) cells and entero endocrine cells. Adjacent columnar absorptive cells are attached to one another near apical margins by a junctional complex. Mucus production in the colon is a function of goblet cells and the proportion of goblet cells increases in the elderly.

The colon and the rectum have an anatomic blood supply. The arterial blood supply to the proximal colon is from the superior mesenteric artery and the inferior mesenteric artery supplies the distal colon. The venous drainage is via the superior (proximal colon) and inferior (distal colon) veins. The arterioles and capillary branches pass to the epithelial surface between the crypts and form an extensive network of capillary plexi. Apart from the stomach, the GIT has normal bacterial flora, which inhibits the growth of other organisms. Some species of the normal microflora produce short chain fatty acids or antibiotics such as 'colistin', and which also prevents the growth of pathogens by competing with
them for nutrients. The mucus lining of GIT forms a barrier against bacterial invasion of the gut wall. The major function of the colon is the consolidation of intestinal content into faeces by the absorption of water and electrolytes and to store the faeces until excretion. In addition it also provides suitable environment for the growth of colonic microorganisms.\textsuperscript{92} The diagram of large intestine is shown in Figure 1 and the properties of human GIT are presented in Table 2.

\textbf{Figure 1 Diagram of the large intestine}\textsuperscript{93}
Table 2: Properties of human GIT and metabolic & enzymatic reactions by human GIT microflora.  

<table>
<thead>
<tr>
<th>Properties of GIT</th>
<th>Measured values</th>
<th>Microbial metabolic and enzymatic reactions in human GIT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surface area</strong></td>
<td>2-10^6 cm^2</td>
<td>Hydrolysis of glucuronides, hydrolysis of glycosides, hydrolysis of –CO-NH-compounds (amides, glycine conjugates and N-acetyl compounds), hydrolysis of esters, hydrolysis of sulfamates, dehydroxylation, demethylation, dealkylation (O-demethylation, N-demethylation and other), dehalogenation, Heterocyclic ring fusion, reduction of azo groups, reduction of aldehydes, reduction of ketones, reduction of alcohols, reduction of N-oxides, reduction of arsenic acids, aromatization, nitrosamine formation.</td>
</tr>
<tr>
<td><strong>Length</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total GIT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>500-700 cm</td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td>20-30 cm</td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td>150-250 cm</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>200-350 cm</td>
<td></td>
</tr>
<tr>
<td>Colon Rectum</td>
<td>90-150 cm</td>
<td></td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>1-3.5</td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>5-7</td>
<td></td>
</tr>
<tr>
<td>Jejunum Ileum</td>
<td>6-7</td>
<td></td>
</tr>
<tr>
<td>Colon Rectum</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

1.5 Colonic Absorption

As absorption capacity of colon is very high which is attributed to the colon transit time, which can be as long as 20-35 hr, hence it is ideally suited for absorption. The absorption is influenced by the transport of water, electrolytes and ammonia across the mucus, and it is more in the proximal colon than the distal colon.  

Drug molecules pass from the apical to basolateral surface of epithelial cells by- 

Passing through colonocytes (trans cellular transport), or Passing between adjacent colonocytes (para cellular transport) Small amphipathic drugs may pass this barrier through transcellular transport. However, transit through the cell cytoplasm may result in its extensive enzymatic extraction and degradation. Paracellular transport may be the most promising means of general drug absorption in colon. Since the membrane fluidity of proximal colonocytes is higher than distal colonocytes, drugs can easily pass via a passive absorption process in the proximal colon. Additionally, carrier mediated uptake of the drug in the colon is not extensive and usually related to the metabolic events of the resident bacteria. Receptor mediated endocytosis and pinocytosis could, however lead to transcellular transport of drug.
1.5.1 Barriers to Colonic Drug Absorption
Drug absorption through the colon can be limited by number of barriers. In the lumen itself, specific and non-specific drug binding can occur through the interaction of drug with dietary components and products released from bacteria residing in the colon. As the drug stays within the lumen it will be passed from the proximal to distal colon by muscularis activity. During this passage, the bacterial content increases dramatically, which could further compromise the drug bioavailability. The mucus barrier at the epithelial surface can present a formidable physical barrier to uptake as a result of specific and non-specific drug binding. Mucus-drug incompatibility can be compounded if the delivered drug stimulates the mucus secreting goblet cells because the transit through mucus is diffusion limited, the greater the thickness of this barrier, longer the time required for an individual molecule to reach the epithelial surface. The unstirred water layer (the space between mucus layer and epithelial cells) presents another barrier to colonic absorption, particularly for lipophilic drugs. A pH gradient may also exist across the unstirred water layer. This lower pH at the colonocyte surface may dramatically alter drug solubility and since drug transport within the unstirred layer is driven by chemical potential, altered drug solubility can affect absorption. Probably the most significant barrier to epithelial transport of drugs in the colon occurs at the level of the epithelium. Here, the lipid bilayers of the individual colonocytes and the occluding junctional complex (OJC) between these cells provide a physical barrier to drug absorption.

1.5.2 Common methodologies for studying colonic absorption
Absorption of drug molecule from the colon is the result of a series of complex events. In-vitro approaches, using isolated colonic epithelial cells or colonic tissue, have been used to study the role of physical and enzymatic barriers in colonic drug absorption. Relatively pure cell population, isolated from colonic crypt and surface regions, are useful in assessing the potential for metabolic modification and/or trans membrane uptake of drug moiety. Instead of using primary colonocyte isolates, most laboratories have used a 'colonic carcinoma cell line' for in-vitro monolayer studies with T-84, caco-2 and HT-29. Individually none of these carcinoma cell lines simulate the heterogeneous nature of colonic tissue. Another in-vitro method using everted sac, traces drug transport from the external mucosal surface to the internal serosal surface. This
method has been used to study the physical pore size for paracellular transport of drug molecules in the colon produced by a series of permeation enhancers. In situ, loops of colon flushed slowly with a solution through the lumen have also been used to evaluate drug uptake by monitoring venous blood and lymph, which drain the segments of the colon. In-vitro methods for the assessment of colonic drug absorption have been set up in a variety of animal model including rat, dog and pig.\textsuperscript{102} Colonoscopy is provided to be a relatively simple and well-tolerated method for assessing the absorption of drug from formulation in the man. A potentially powerful model, a xenograft of human foetal bowel grafted and matured in nude mice is described. Using this model, both in-vitro and in-vivo colonic drug uptake could be assessed. Pharmacokinetic data can be used to evaluate the absorption indirectly while direct methods include colonoscopy. Gamma scintiography is now a days the preferred method to study GI transit behavior.\textsuperscript{103}

1.5.3 Factors Influencing colonic drug absorption

Before a drug is absorbed by the colonic epithelium, it must reach the absorption site. Thus if given orally, it must first reach the colon. Factors, which affect transit time through the upper GIT and colonic motor pattern, that influence residence and contact time will determine the site of drug absorption. The residence time in different parts of the colon may influence drug absorption because of the many physiological differences in the motility, luminal environment's absorptive capacity and other characteristics of the proximal and distal colon.\textsuperscript{104} Diet and the relation of drug ingestion to mealtime may be very important. The drug must then move from the bulk phase to the epithelial surface. Factors such as colonic motor patterns, viscosity, and binding or entrapment will determine the rate of mixing and hence, movements to the mucosal surface. The molecule must then pass across the mucus and unstimred layer to the membrane where it can pass through either the lipid cell membrane or water filled pores.\textsuperscript{105} The thickness of the mucus layer may determine motility. The lipid solubility and size of the drug will determine ease of transport across the epithelium. The rate of absorption of weak bases and acids will be determined by the pH of absorptive site and partition coefficient. The pH of the microclimate at the mucosal surface may determine the dissociation of the drug and hence its absorption rate. The factors determining
mucosal blood flow are not well understood but may be important. Finally, the metabolism of the drug by intestinal bacteria and the changes in the luminal environment caused by fermentation has a major influence. The diet, especially the ingestion of dietary fiber and other bacterial substrates that are fermentable or that hold water in the colon may influence several of the steps before a drug is absorbed.\textsuperscript{106}

1.5.4 Enhancers of colonic drug absorption

The colonic epithelial permeability of most potential drug molecules, particularly peptides and proteins, is insufficient to achieve a therapeutic effect.\textsuperscript{107}

Hence, chemical enhancers like chelating agents have been used to enhance colonic permeability. Low concentration of sodium caprate, and sodium salicylate can enhance transcellular uptake of poorly permeable compounds through colonic mucosa.\textsuperscript{108}

Absorption enhancement by surfactants, fatty acids and mixed micelles may be due to improved solution solubility and/or stability of the drug being transported. Upon transport enhancement, low molecular weight drugs are directed twice as often to a transcellular pathway than the paracellular route. Haemorrhage, intestinal obstruction, immunosuppression, burn trauma, non-thermal trauma, sepsis, radiation, injury, endotoxosis and clostridium and clostridium difficile toxin-A, all exhibit increased colonic permeability. Increased colon permeability associated with a disease state may be useful in the treatment where improvement of the condition may reduce the mucosal permeability and naturally reduce the transport of a therapeutic molecule.\textsuperscript{109}

1.6 Factors Governing Colonic Drug Delivery

There are many factors that influence the drug delivery to colon. They include:

1.6.1 Transit through GIT

In order to reach colon in an intact form, the drug delivery systems should surpass the barriers in the stomach and small intestine. Normally, the small intestinal transit is not influenced by the physical state, size of the dosage form and presence of food in the stomach. The mean transit time of the dosage form is about 3-4 hrs to reach the ileocecal junction.\textsuperscript{110} During this period the dosage form is exposed to enzymes present in small intestine. Compared to the other region of GIT,
movement of material through the colon is slow. The colonic transit time of a capsule in adult is 20-35 hrs. Improved residence time with subsequent longer transit time and the contact of dosage form with microflora in colon govern the release and absorption of drug from dosage form.\textsuperscript{111}

1.6.2 Gastric emptying

Once the dosage form enters the stomach, the primary concern is how long it will remain there before being discharged into the duodenum. Emptying generally completes in 5-10 min up to 2 hrs depending on phase of the stomach at the time of drug administration. It is preferable for a colonic delivery system to spend little time in the stomach. Such system may release the drug at a distant locus from the colon.\textsuperscript{112}

1.6.3 Stomach and Intestinal pH

The pH of GIT must be considered when enteric coatings (bioerodible polymers) are used to deliver drugs to colon. Since, in such systems, GIT pH gradient is used to trigger drug release.\textsuperscript{113}

1.6.4 Colonic Microflora

The concentration of gut microflora rises considerably in the terminal ileum to reach extraordinarily high levels in the colon. The gut bacteria are capable of catalyzing a wide range of metabolic events. Many colon-specific drug delivery systems rely on enzymes unique to gut microflora to release active agents in the colon. However, only two or three enzyme systems namely azoreductases and glycosidases (including glucuronidase) have been explored in this area.\textsuperscript{114} A large number of polysaccharides is actively hydrolyzed by gut microflora leading to the possibility of using naturally occurring biopolymer as drug carriers. There is certainly a room for innovative approaches to carry and release drugs in the colon based on the metabolic capabilities of the colon microflora. The second class of enzymes used to trigger the release of drugs in the colon is glycosidases (including glucuronidases). The main bacterial groups responsible for $\beta$-glycosidases activity are lactobacilli, bacteroides and bifidobacteria.\textsuperscript{115}
1.7 Approaches for Colonic Drug Delivery

Various approaches that can be used for the development of colon targeted drug delivery systems are summarized in Table 3.

Table 3: Different approaches used for colonic drug delivery

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Approach</th>
<th>Basic Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Chemical Approaches</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Azo conjugates</td>
<td>Conjugation via an azo bond conjugation with cyclodextrin conjugation with</td>
</tr>
<tr>
<td></td>
<td>Cyclodextrin conjugates</td>
<td>glycoside conjugation with glucuronide conjugation with dextran conjugation</td>
</tr>
<tr>
<td></td>
<td>Glycosidic conjugates</td>
<td>with polypeptide conjugation with polymer</td>
</tr>
<tr>
<td></td>
<td>Glucuronide conjugates</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dextran conjugates</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Polypeptide conjugates</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Polymeric conjugates</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Pharmaceutical Approaches</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH dependent systems</td>
<td>Formulation coated with enteric polymers release the drug when pH moves towards</td>
</tr>
<tr>
<td></td>
<td>Time released systems</td>
<td>alkaline side.</td>
</tr>
<tr>
<td></td>
<td>Pressure dependent systems</td>
<td>Based on the concept of delaying the release of drug after a lag time of 3-5</td>
</tr>
<tr>
<td></td>
<td>Microbially triggered</td>
<td>hours that is equivalent to small intestine transit time.</td>
</tr>
<tr>
<td></td>
<td>systems Polysaccharides</td>
<td>Based on the strong peristaltic waves that lead to a temporary increase in</td>
</tr>
<tr>
<td></td>
<td>Osmotic controlled delivery</td>
<td>luminal pressure in the colon.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Drugs are released following degradation of the polymer due to the action of</td>
</tr>
<tr>
<td></td>
<td></td>
<td>colonic bacteria.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Based on the utilization of gelable properties of chitosan at acid condition</td>
</tr>
<tr>
<td></td>
<td></td>
<td>to produce osmotic pressure and its colon specific biodegradation to form in-situ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>delivery pores for drug release.</td>
</tr>
</tbody>
</table>
1.8 Colon-Specific Drug Delivery Systems

Rectal administration offers the shortest route to target drugs to colon. However, reaching the proximal part of the colon via rectal administration is difficult. Rectal administration can also be uncomfortable for the patient, and compliance may be less than optimal.\textsuperscript{117}

There are several ways in which drugs can be targeted on the colon when they are administered by mouth.\textsuperscript{118} In time-dependent formulations the drug concerned is released during the period of gastrointestinal transit time. Release from formulations that contain pH-dependent polymers takes place on the basis that pH is higher in the terminal ileum and colon than in the upper parts of the gastrointestinal tract. The colon is also home to large numbers of bacteria of many kinds. Prodrugs and dosage forms from which drug release is triggered by the action of colonic bacterial enzymes have therefore been devised.\textsuperscript{119}

1.8.1 Drug release based on variation of pH and time

In the stomach pH ranges between 1 and 2 in the stomach through 6.6 in the proximal small intestine to a peak of about 7.5 in the distal small intestine. The right, mid, and left colon have pH values of approximately 6.4, 6.6 and 7.0 respectively. The pH of the colon is often lower than the pH of the small intestine, which is as high as 8 or 9.\textsuperscript{120}

Use of pH-dependent polymers is based on these differences in pH levels. The polymers described as pH-dependent in colon specific drug delivery are insoluble at low pH levels but become increasingly soluble as pH rises.\textsuperscript{121} One of the simplest approaches for designing pH dependent multiparticulate colon specific delivery system is to formulate enteric coated granules. When coated granules, microparticles, or nanoparticles are filled into a gelatin capsule or compressed together with conventional excipients in the form of tablets, the formulation is regarded as a multiparticulate dosage forms. Various pH dependent polymers include cellulose acetate phthalate (CAP), poly vinyl acetate phthalate, hydroxypropyl methyl cellulose phthalate, methacrylic acid copolymers, Eudragit L100 and Eudragit S100, which dissolve at pH 6.0 and 7.0 respectively.\textsuperscript{122}
Table 4: Enteric Polymers Utilized in Development of Modified-Release Formulations for Colonic Delivery.\(^{123}\)

<table>
<thead>
<tr>
<th>Enteric polymers</th>
<th>pH for Dissolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyvinyl acetate phthalate (PVAP) (Coateric)</td>
<td>5.0</td>
</tr>
<tr>
<td>Cellulose acetate trimellitate (CAT)</td>
<td>5.5</td>
</tr>
<tr>
<td>Hydroxypropyl methylcellulose phthalate (HPMCP) HP-50</td>
<td>&gt;5.0</td>
</tr>
<tr>
<td>HP-55 and HP-55</td>
<td>&gt;5.5</td>
</tr>
<tr>
<td>Hydroxypropylmethylcellulose acetate succinate (HPMCAS) LF Grade MF Grade HF Grade</td>
<td>&gt;5.5</td>
</tr>
<tr>
<td>Methacrylic acid copolymer, Type C (Eudragit L100-55)</td>
<td>5.5</td>
</tr>
<tr>
<td>Methacrylic acid copolymer dispersion (Eudragit L30D-55)</td>
<td></td>
</tr>
<tr>
<td>Methacrylic acid copolymer, Type A (Eudragit L-100 and Eudragit L12,5)</td>
<td>6.0</td>
</tr>
<tr>
<td>Cellulose acetate phthalate (CAP) (Aquateric)</td>
<td>6.0</td>
</tr>
<tr>
<td>Methacrylic acid copolymer, Type B (Eudragit S-100 and Eudragit S12,5)</td>
<td>6.0</td>
</tr>
<tr>
<td>Eudragit FS30D</td>
<td>7.0</td>
</tr>
<tr>
<td>Shellac (MarCoat 125 &amp; 125N)</td>
<td>7.0</td>
</tr>
</tbody>
</table>

There are various problems with this approach. Minor variation in pH between the small intestine and colon makes the pH dependent system less specific in terms of targeted release in colon, time dependent formulation predominantly depends on the transit time of delivery system in the GIT.\(^{124}\) Disadvantage of enteric coated systems is that a substantial amount of drug may be released in the small intestine before the delivery system arrives in the colon. Moreover, pH in the gastrointestinal tract varies between and within individuals.\(^{125}\) It is affected by diet and disease.\(^{126}\) For example, during acute stage of inflammatory bowel disease colonic pH has been found to be significantly lower than normal.\(^{127}\) In ulcerative colitis pH values between 2.3 and 4.7 have been measured in the proximal parts of the colon.\(^{128}\) Although a pH dependent polymer can protect a formulation in the stomach and proximal small intestine, it may start to dissolve even in the lower small intestine, and the site-specificity of formulations can be poor.\(^{129}\)
1.8.2 Drug release based on the colonic Microflora

The colonic bacteria are predominately anaerobic in nature and secrete enzymes that are capable of metabolizing substrates such as carbohydrates and proteins that escape the digestion in the upper GI tract. Over 400 distinct bacterial species have been found, 20% to 30% of which are of the genus bacteroides. Most bacteria inhabit the proximal areas of the large intestine, where energy sources are enormous. Carbohydrates arriving from the small intestine form the main source of nourishment for bacteria in the colon. The carbohydrates are split into short-chain fatty acids, carbon dioxide and other products by the enzymes glycosidase and polysaccharidase. Protease activity in the colon can result in cleavage of proteins and peptides. In the proximal colon the pH is lower than the pH at the end of the small bowel because of the presence of short-chain fatty acids and other fermentation products. Diet can affect colonic pH. The upper region of the GI tract has a very small number of bacteria and predominantly consists of Gram-positive facultative bacteria. The concentration of bacteria in the human colon is around 1000 CFU/ml.

The presence of colonic microflora has formed a basis for development of colon-specific drug delivery systems. Interest has focused primarily on azo reduction and hydrolysis of glycoside bonds. However, the colonic microflora varies substantially between and within individuals, depending on diet, age and disease. Such variations need to be taken into account in developing colon-specific formulations based on the presence of colonic microflora. There is also significant proteolytic activity in the colon, although this is 20 to 60 times less than in the small bowel. Even when proteolytic activity is relatively low, a drug may remain much longer in the colon than in the small intestine, resulting in longer exposure to proteolytic activity.

1.8.3 Prodrug approach

Pro-drugs are conjugates of drugs with carrier molecules mostly of inert nature. The microbial enzymes in the colon are responsible for the cleavage of the drug–carrier bond. A variety of pro-drugs have been synthesized, mainly azo compounds, glycosides, esters and amides. Pro-drugs meant for colonic delivery must not be cleaved by digestive enzymes of the upper GI tract and should not be susceptible to chemical hydrolysis. Moreover, pro-drug absorption in the small intestine should be negligible. Because of these
requirements, the hydrophilicity, the molecular weight and the charge of the carrier molecules have to be regarded as critical parameters.\textsuperscript{135} The azo pro-drug sulfasalazine,\textsuperscript{136} consisting of the drug mesalazine and the carrier molecule sulfapyridine, was the first pro-drug available for the treatment of inflammatory bowel disease. Due to side-effects caused by the pharmacologically active sulfapyridine carrier, other carrier molecules such as sulfanilic acid, pamino benzoic acid and its amino acid derivatives (benzalazine; ipsalazide; balsalazide, colazide) and mesalazine itself (olsalazine, dipentum) have been used in its place. A disadvantage of the use of pro-drugs is the need for suitable functional groups such as amino-, hydroxy- or carboxy groups in both drug and carrier molecules. Sometimes spacer molecules are necessary to link the drug to the carrier molecule, which often leads to complicated drug release kinetics. Moreover, the pro-drug approach is not very versatile approach as for the approval of any newly synthesized pro-drug, toxicity study is required by regulatory agencies. Furthermore, prodrugs are considered as new chemical entities and need a lot of evaluation before being used as drug carriers.\textsuperscript{137}

**1.8.4 Polysaccharides based delivery system**

Many natural polysaccharides such as chondroitin sulphate, pectin, dextran, guar gum etc. have been investigated for their potential in designing colon specific drug delivery. These are broken down by the colonic microflora to simple saccharides. Most of the polysaccharide based delivery systems protect the bioactive from the hostile conditions of the upper GIT. Hydrolysis of the glycosidic linkages on arrival in the colon triggers the release of the entrapped bioactive. The main saccharolytic species responsible for this biodegradation are Bacteroides, Bifidobacterium, Eubacterium, Peptococcus, Peptostreptococcus, Ruminococcus, Propionibacterium, and Clostridium.\textsuperscript{138} Characteristics of various polysaccharides suitable for colon specific drug delivery are presented in Table 5.
Table 5: Characteristics of various polysaccharides for colon specific drug delivery. 139

<table>
<thead>
<tr>
<th>Polysaccharide</th>
<th>Main chains</th>
<th>Source</th>
<th>Comments</th>
<th>Microbes responsible for Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylose</td>
<td>α-1,4 D-glucose</td>
<td>Storage polysaccharide in plant</td>
<td>One of the constituents of starch; used as excipients in tablets formulation</td>
<td>Bacteroids Bifidobacterium</td>
</tr>
<tr>
<td>Arabinogalactan</td>
<td>β-1,4 and β-1,3 D-galactose</td>
<td>Plant cell walls</td>
<td>Neutral pectin; hemicellulose; used as thickening agent in food industry</td>
<td>Bifidobacterium</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>Galactose residues which are sulfated and alternatively linked in α-1,3 and α-1,4 linkage</td>
<td>Sea weed extract</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Chitosan</td>
<td>Deacetylated P-1,4 N-acety 1-D glucosamine</td>
<td>Shell of marine invertebrates</td>
<td>Deacetylated chitin; used in medicine as tablet component and absorption enhancing agent</td>
<td>Bacteroids</td>
</tr>
<tr>
<td>Cyclodextrin</td>
<td>α-1,4 D-glucose</td>
<td>----</td>
<td>High stability against amylase, drug solubilizing agent and absorption enhancer</td>
<td>Bacteroids</td>
</tr>
<tr>
<td>Dextran</td>
<td>α-1,6 D-glucose</td>
<td>Bacterial</td>
<td>Used in medicine as plasma expander</td>
<td>Bacteroids</td>
</tr>
</tbody>
</table>
The human colon contains a large number of complex bacteria essentially absent from the stomach or the small intestine and the colonic microflora can produce a large number of degrading enzymes, so a microbially controlled delivery system is the most appealing among the approaches described. Microflora activated systems formulated by using non-starch polysaccharides are highly promising because these polysaccharide remains undigested in the stomach or the small intestine and can only be degraded by the vast anaerobic microflora of the colon.\textsuperscript{140} Furthermore, this strategy exploiting the abrupt increase of the bacteria population accomplishes greater site specificity for initial drug release. The polysaccharides are inexpensive, naturally occurring and easily available.\textsuperscript{141}

### 1.9 Single Unit Versus Multiparticulate Dosage Forms

Single unit colon targeted drug delivery system may suffer from the disadvantage of unintentional disintegration of the formulation due to manufacturing defects or unusual gastric physiology that may lead to drastically compromised systemic drug bioavailability or local therapeutic action in the colon.\textsuperscript{142} Recently, much emphasis is being laid on the development of multiparticulate dosage forms in comparison to single unit systems because of their potential benefits like increased bioavailability, reduced risk of systemic toxicity, reduced risk of local irritation and predictable gastric emptying.\textsuperscript{143} Among these, multiple-unit systems proved to be better than single dosage forms due to their more predictable and reproducible gastrointestinal transit time and less local irritation and/or adverse effects.\textsuperscript{144} The use of multiparticulate drug delivery systems in preference to single unit dosage forms for colon targeting purposes dates back to 1985 when Hardy and co-workers showed that multiparticulate systems enabled the drug to reach the colon quickly and were retained in the ascending colon for a relatively long period of time. Because of their smaller particle size as compared to single unit dosage forms these systems are capable of passing through the GI tract easily, leading to less inter- and intra subject variability.\textsuperscript{145} Moreover, multiparticulate systems tend to be more uniformly dispersed in the GI tract and also ensure more uniform drug absorption.\textsuperscript{146}
Generic Name
Insulin

Chemical Structure\textsuperscript{147}

![Chemical Structure Image](image)

State
Solid

Drug Type
Approved

Indications
Insulin is a protein hormone secreted by the b-cells of the islets of Langerhans in the pancreas. It is secreted in response to elevated blood glucose and amino acid levels, and promotes the efficient storage and utilization of these fuel molecules by controlling the transport of metabolites and ions across the cell membrane. Insulin promotes the entry of glucose, fatty acids and amino acids into cells and enhances glycogen, protein and lipid synthesis.\textsuperscript{148} Insulin is made up of two polypeptide chains namely, chain-A (21 amino acids) and chain-B (30 amino acids), which are held together by two disulfide bonds (see the structure given below). Insulin deficiency causes a disease called diabetes mellitus and, hence, administration of exogenous insulin is used to control this disease.\textsuperscript{149} The various preparations of insulin available in the market include bovine insulin (from cattle), porcine insulin (from pigs), a mixture of both, or recombinant human insulin. Since insulin has a very short life in the plasma, insulin products that last longer are also available. In these products, insulin is complexed with zinc, globin, protamine or a combination of any of these.\textsuperscript{150}