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## Diabetes mellitus: A journey of insulin

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### ABSTRACT

Diabetes mellitus is a chronic ailment that impairs the production of or response to insulin, a hormone that helps to convert food into energy. Its complications are responsible for excess morbidity and mortality, loss of independence and reduce quality of life. Among the major cause of disablement and early death are ischemic heart disease, retinopathy, nephropathy, peripheral vascular disease and neuropathy. Insulin replacement therapy has been used in the clinical management of diabetes mellitus for more than 85 years. As subcutaneous injection is a painful episode so various approaches like transdermal, pulmonary, intranasal, colon targeted delivery, oral delivery is tried as an alternative way. Among them oral delivery is the challenging one because insulin cannot administered orally due to rapid enzymatic digestion in stomach. For oral delivery various technology, formulation and various modification approaches are going on. It is high time to invent an acceptable non-invasive insulin delivery for the diabetes to improve patient compliance and decrease the morbidity.

**Key Words:** Insulin, Approaches, Obstacles, Route of administration, Non-invasive.

### INTRODUCTION

Diabetes mellitus is a common disease and its complications are responsible for excess morbidity and mortality, loss of independence, and reduced quality of life (Giriraj *et al.*, 2003; Ahmed *et al.*, 2006). Diabetes mellitus is a serious pathologic condition that is responsible for major healthcare problems worldwide and costing billions of dollars annually. The prevalence of diabetes continues to increase steadily as more people live longer and grow heavier. Type-1 diabetes comprises those forms of diabetes that are primarily due to insulin deficiency. Type-2 diabetes comprises those forms that result from a primary defect in insulin resistance (often associated with obesity), coupled with a relative insulin deficiency. Eventually, progressive loss of insulin producing  $\beta$ -cell of pancreas function in type-2 diabetes creates an absolute insulin deficiency and complicated by glucose (glucosamine) and lipid mediated toxicities.

The American Diabetes Association recently

recommended an etiological classification of diabetes (2003). Type I diabetes normally occurs in childhood, has relatively acute onset, and requires insulin for survival. Insulin is a hormone made by the pancreas. With each meal insulin is released to help the body use or store the glucose (sugar) it gets from food. People with type I diabetes make no insulin. People with type II diabetes make some insulin, but not enough. For many people with type 2 diabetes the insulin has a harder time working.

### THE HISTORY OF INSULIN

Nearly 100 years have passed since Von Mering and Minkowski first demonstrated that pancreatectomized dogs exhibited signs and symptoms characteristic of diabetes mellitus (Banting *et al.*, 1922; Dhawan *et al.*, 2009). The brief history of different approaches regarding insulin is tabulated in table 1.

### ROUTE OF ADMINISTRATION OF INSULIN

Subcutaneous route is the present mode of administration of insulin. Other non-invasive mode of administration is under investigation though

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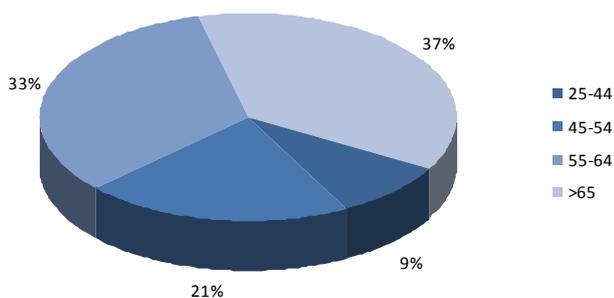
**Table 1: Different events about insulin over the last decades.**

Year	Events	Reference
1921	Fredrick G. Banting, Charles H. Best, J.J.R. Macleod and James B. Collip discovered insulin, a peptide which lowers blood sugar.	Bliss <i>et al.</i> , 1982
1922	Bovine insulin was first administered to human.	Bliss <i>et al.</i> , 1982
1936	Development of protamine, slow release insulin.	Jersild <i>et al.</i> , 1956
1944	Standard syringe was released.	Maheux
1950	Isophane NPH (neutral protamine Hagedorn) insulin.	Jersild <i>et al.</i> , 1956
1951	Amorphous 'lente' insulins (IZS) – semilente, lente and ultra-lente – were developed.	Jersild <i>et al.</i> , 1956
1956	First antidiabetic oral drug – sulfonamide was invented.	Spencer <i>et al.</i> , 1956; Ho <i>et al.</i> , 1991; Balsells <i>et al.</i> , 1997
1963-1966	Human insulin was chemically synthesized.	Meienhofer <i>et al.</i> , 1963; Kung <i>et al.</i> , 1966; Katsoyannis <i>et al.</i> , 1966
1970	Insulin pump was first used.	Maheux
1974	Highly purified animal insulin (less than 1 pmol/L of protein impurities) was introduced.	Teuscher <i>et al.</i> , 1974
1975	Fully synthetic insulin (CGP 12 831) was synthesized.	Teuscher <i>et al.</i> , 1979
1978	Insulin production started from E. Coli.	Keen <i>et al.</i> , 1980
1980	First recombinant DNA 'human' insulin.	Keen <i>et al.</i> , 1980
1982	FDA approved Humulin R (rapid) and Humulin N (NPH).	Maheux
1986	Insulin pen was first introduced.	Maheux
2006	Pfizer invented inhaled insulin - Exubera®.	Imam, 2011

pulmonary delivery show similar efficacy to injected insulin.

### Subcutaneous route

The present mode of insulin administration is by the subcutaneous route by which insulin is presented to the body in a non-physiological manner. Insulin injected subcutaneously at least twice a day is having many inherent disadvantages include local pain, inconvenience of multiple injections, and occasional hypoglycemia as a result of overdose, itching, allergy, hyperinsulinemia, and insulin lipodystrophy around the injection site. Lastly,



**Figure 1: Distribution of diabetic patients according to age group.**

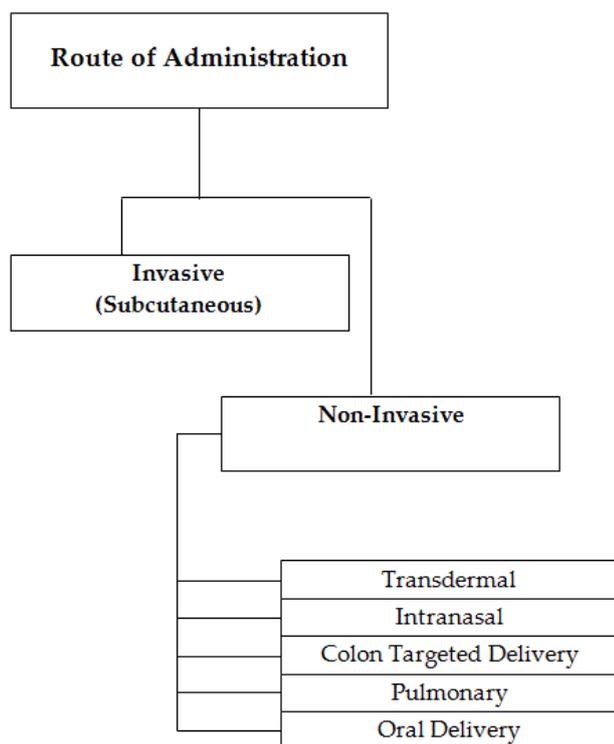
clinical trials have shown that even on injectable insulin treatment, a significant percentage of patients fail to attain lasting glycemic control due to non-compliance (Pamnani *et al.*, 2008).

### Approaches for Non-invasive Insulin Delivery

Despite the newer insulin formulations and strategies that allow the clinician to mimic normal endogenous insulin secretory profiles, intensive insulin therapy has not achieved widespread acceptance because of barriers to its use from both patients and physicians. Specifically, there may be concerns from the patient about fear, inconvenience, pain or the anxiety of insulin injections (Graff *et al.*, 1998; Maisey *et al.*, 1999). Furthermore, the patient may feel as if they are advancing to insulin therapy because they have been noncompliant with their treatment regimen (Hunt *et al.*, 1997).

### Transdermal approach

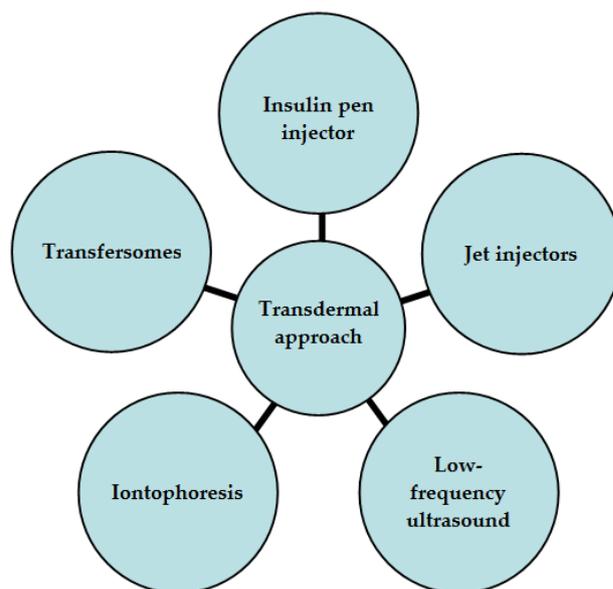
There are several methods for transdermal delivery of insulin. Pulsatile insulin uses microjets to pulse insulin into the patient, mimicking the physiological secretions of insulin by the pancreas (Arora *et al.*, 2007).



**Figure 2: Route of administration of Insulin** (Cefalu *et al.*, 2004; Dhawan *et al.*, 2009; Kinesh *et al.*, 2010).

### ***Intranasal Approach***

Delivery of insulin using an intranasal approach was first suggested over 65 years ago, but it was not until the 1980s that this approach was seriously evaluated (Major *et al.*, 1995; Saudek *et al.*, 1997). Feasibility has been demonstrated, as intranasal insulin (60 or 120U) given pre-meal to 17 patients with type 2 diabetes and compared with placebo resulted in reductions in postprandial glucose at both 60 and 120 minutes (Coates *et al.*, 1995). However, the major limitation of this approach is poor bioavailability across the mucous membranes. This was demonstrated in studies by Hilsted *et al.* (1995) as they reported doses of intranasal insulin that were approximately 20 times higher than those needed with subcutaneous injection. To overcome the problem of limited bioavailability, absorption-enhancing compounds such as bile salts and polyethylene ether derivatives have been evaluated for nasal insulin and have resulted in increased absorption and effective biological activity. It appears, however, that the chance of nasal irritation has increased with these changes (Gizurason *et al.*, 1991; Jacobs *et al.*, 1993). The feasibility of intranasal insulin has been demonstrated, but limitations do



**Figure 4: Different types of transdermal approaches** (Golden *et al.*, 1988, Mitragotri *et al.*, 1995; Saudek *et al.*, 1997; Stieber *et al.*, 1998; Kanikkannan *et al.*, 1999; Bohannon *et al.*, 1999;; Rosenstock *et al.*, 2001; Dixit *et al.*, 2007).

exist and further studies are needed to establish long-term safety and efficacy.

### ***Colon-targeted delivery systems***

Proteolytic enzymes in the stomach degrade insulin, but in intestines peptidase activity is low and drainage into lymph is maximized. Researchers are exploring colon-specific delivery for insulin. To achieve colon specific delivery of insulin, Hideyuki prepared azopolymer-coated pellets containing



**Figure 5: Intranasal route for insulin delivery.**

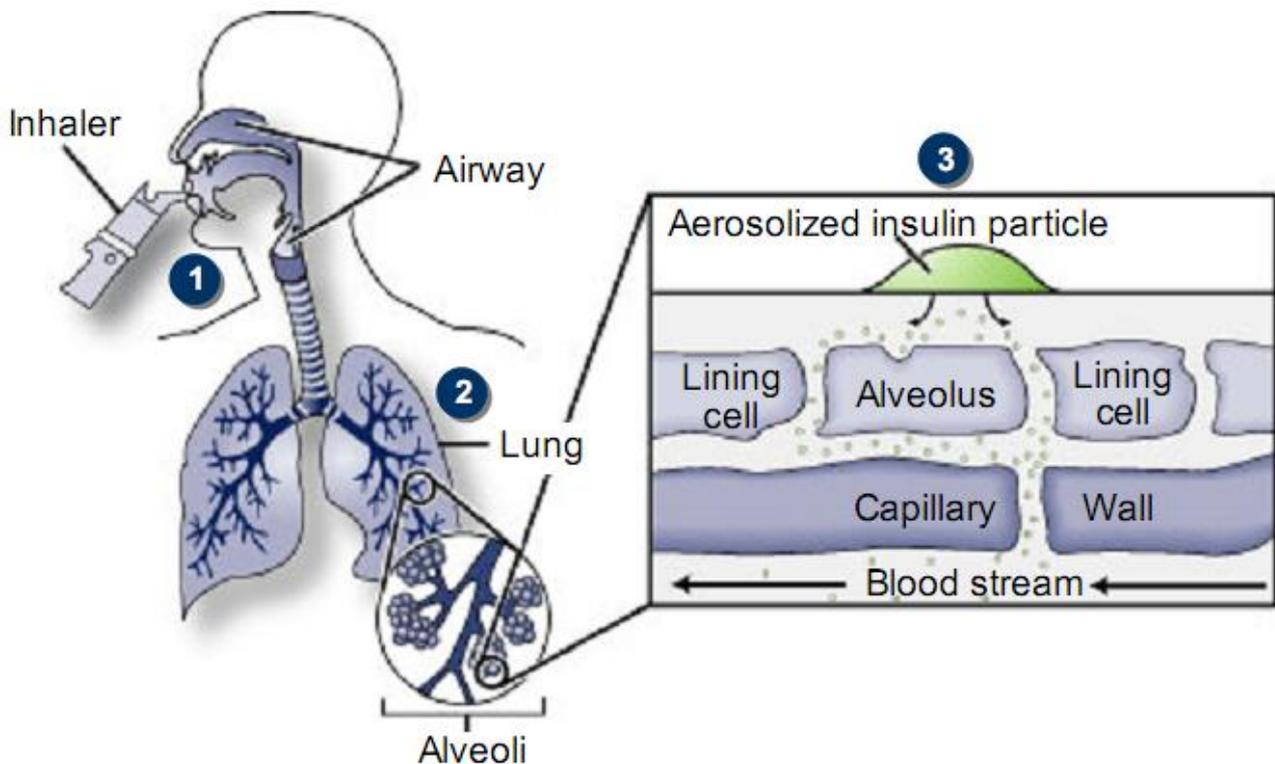


Figure 6: Inhalation of Insulin (Maheux).

fluorescein isothiocyanate dextran (FD-4) (Tozaki *et al.*, 2001). *In vitro* drug-release experiments were carried out according to *Japanese Pharmacopoeia XII* (rotating basket method). The release of FD-4 from the pellets in phosphate buffered saline was very small. However, the release of FD-4 was markedly increased in the presence of rat ceecal contents. The pharmacodynamic studies of the azopolymer-coated pellets containing these peptides with camostat mesilate (protease inhibitor) were carried out by measuring the hypoglycemic effects. A slight decrease in plasma-glucose levels was observed following the oral administration of these pellets containing 12.5 IU of insulin compared with the same dose of insulin solution. The authors concluded that azopolymer-coated pellets with protease inhibitor might be useful carriers for the colon-specific delivery of insulin.

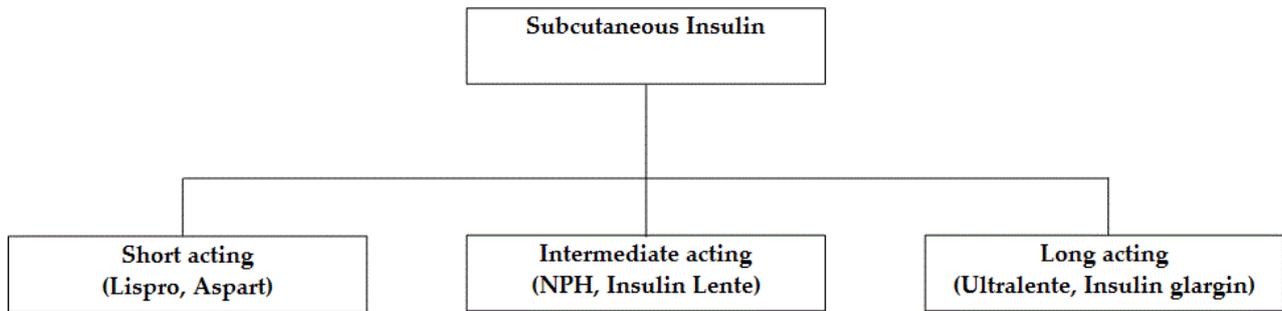
Yakugaku Zasshi developed two types of microcapsular devices containing new acrylate-based nanogels with a specific solute-permeability for the delayed- or thermosensitive-release of peptide drugs (Ichikawa *et al.*, 2007). A nanogel-particle of acrylic terpolymer, ethyl acrylate-methyl methacrylate-2-hydroxy-ethyl methacrylate, was

newly synthesized by emulsion polymerization to construct delayed-release microcapsules. The insulin-loaded lactose particles were spray coated with the acrylic terpolymers. These microcapsules showed a pH-in-dependent delayed-release profile.

#### **Pulmonary approach**

Finally, insulin delivered through the oral cavity can also be considered to have its uptake in the pulmonary bed. However, the idea of pulmonary delivery of insulin is not a new idea, as the first report of inhaled insulin was noted in 1925 (Über *et al.*, 1925) truly remarkable given that this was reported very shortly after the first clinical use of insulin. (Banting *et al.*, 1922) The high permeability of the lung's large surface area makes it an ideal route for the administration of insulin. The lung has hundreds of millions of alveoli that are richly vascularised and where drug absorption takes place. In addition, the surface area is quite large, as the alveolar capillaries provide a total surface area of 50-140m<sup>2</sup> for absorption (Heinemann *et al.*, 2002).

Insulin delivered in this way is believed to be transported across the alveolar cells by transcytosis, but this has yet to be conclusively proven. Once



**Figure 3: Classification of insulin according to their activity period** (Galloway *et al.*, 1993; Howey *et al.*, 1994; Campbell *et al.*, 1996; Home *et al.*, 1998; Browne *et al.*, 2000; Lopre *et al.*, 2000; Lantus *et al.*, 2001; White *et al.*, 2003; Nielsen *et al.*, 2005; Calara *et al.*, 2005; DeFronzo *et al.*, 2005; Sarabu *et al.*, 2005).

deposited in the alveoli, the inhaled insulin molecules are taken up into vesicles, transported across the epithelial cells and then released into the interstitial fluid between the epithelium and the alveolar capillary endothelium. The insulin is once again taken up into vesicles, transported across the capillary endothelium and released into the bloodstream, where it exerts a rapid systemic effect (Wigley *et al.*, 1971; Pilcher *et al.*, 1987; Klonoff *et al.*, 1999). This process is extremely rapid and pulmonary uptake results in a very rapid peak in insulin levels, that is, after 15-20 minutes (Enzmann *et al.*, 1984; Adams *et al.*, 1992). Therefore, the physiological and anatomic barriers that limit successful implementation of other routes of non-invasive insulin delivery do not appear to be a major concern when one considers the feasibility of pulmonary delivery of insulin, based in large part on the favorable anatomy of the lung.

Inhaled insulin claimed to have similar efficacy to injected insulin, both in terms of controlling glucose levels and blood half-life. Currently, inhaled insulin is short acting and is typically taken before meals;

an injection of long-acting insulin at night is often still required (Nice *et al.*, 2006). When patients were switched from injected to inhaled insulin, no significant difference was observed in Hb<sub>A1c</sub> levels over three months. Accurate dosing was a particular problem, although patients showed no significant weight gain or pulmonary function decline over the length of the trial, when compared to the baseline (Cefalu *et al.*, 2001).

The Exubera® insulin formulation is a spray-dried, amorphous insulin powder containing 60% insulin in a buffered, sugar based matrix. In January 2006, Pfizer and its partner Nektar Therapeutics received marketing approval for Exubera®, their insulin DPI. It was available from September 2006 to October 2007 in the United States. It is licensed for use by both type 1 and type 2 diabetes patients. As of October 18, 2007, Pfizer has announced that it will no longer manufacture or market Exubera®, because it failed to gain acceptance of patients and physicians (Imam, 2011).

**Table 2: Characteristics of modern insulin formulation** (Illinois Extension, 2003; Cefalu *et al.*, 2004; Modi *et al.*, 2007).

Insulin formulation	Mechanism of action	On set of action	T <sub>max</sub>	Duration of action
Lispro/Aspart®	Dissociates into monomeric formulation leading to rapid absorption	5-15 min	1-2 hr	4-6 hr
Human regular®	Unknown	30-60 min	2-4 hr	6-10 hr
NPH/Lente®	Produces insulin through hormone conversion	1-2 hr	4-8 hr	10-20 hr
Human ultralente®	Unknown	2-4 hr	Unpredictable	16-20 hr
Glargine®	Unknown	1-2 hr	Flat	Up to 24 hr

**Table 3: Different approaches of oral insulin delivery.**

Characteristics	Approaches	Reference
<b>Depending on barrier</b>	Enzyme Inhibitors	Fujji <i>et al.</i> , 1985; Saffran <i>et al.</i> , 1986; ziv <i>et al.</i> , 1987; Yamamoto <i>et al.</i> , 1994; Patki <i>et al.</i> , 1996; Chang <i>et al.</i> , 1999 Agarwal <i>et al.</i> , 2001; Shah <i>et al.</i> , 2002; Liu <i>et al.</i> , 2003 Tuesca <i>et al.</i> , 2006
	Penetration Enhancers	Koosapur <i>et al.</i> , 1999; Schiling <i>et al.</i> , 1999; Soltero <i>et al.</i> , 2001, Thanou <i>et al.</i> , 2001; Eaimtarakam <i>et al.</i> , 2002; Plate <i>et al.</i> , 2002; Gowthamarajan <i>et al.</i> , 2003; Salamat-Miller <i>et al.</i> , 2005; Toriska <i>et al.</i> , 2005; Rieux <i>et al.</i> , 2006; Lin <i>et al.</i> , 2007
	Dosage form stability	Pearlman <i>et al.</i> , 1991; Agarwal <i>et al.</i> , 2001
<b>Technology dependent</b>	Liposomes	Choudhari <i>et al.</i> , 1994
	Coated liposomes	Cantenys <i>et al.</i> , 1983; Ramadas <i>et al.</i> , 2000; Ye <i>et al.</i> , 2000; Wu <i>et al.</i> , 2004; Zhang <i>et al.</i> , 2005
	Hydrogel	Yupeng <i>et al.</i> , 2001; Dorkoosh <i>et al.</i> , 2002; Nakamura <i>et al.</i> , 2004; Kavimandan <i>et al.</i> , 2006
	Nanoparticle	Damage <i>et al.</i> , 1997; Attivi <i>et al.</i> , 2005; Sarmiento <i>et al.</i> , 2006; Tiyaboonchai <i>et al.</i> , 2006; Damage <i>et al.</i> , 2007; Chalasani <i>et al.</i> , 2007; Lin <i>et al.</i> , 2007; Cui <i>et al.</i> , 2007; Simon <i>et al.</i> , 2007
	Erythrocytes	Al-Achi <i>et al.</i> , 1998
	Nanocubicles	Chung <i>et al.</i> , 2004
	Microparticles	Lowman <i>et al.</i> , 1999; Tozaki <i>et al.</i> , 2001; Peppas <i>et al.</i> , 2004; Qi <i>et al.</i> , 2004; Senthil <i>et al.</i> , 2004; kim <i>et al.</i> , 2005; Reis <i>et al.</i> , 2007; Ubaidulla <i>et al.</i> , 2007
Mucoadhesive system	Whitehead <i>et al.</i> , 2004	
<b>Formulation dependent</b>	Tablet	Krauland <i>et al.</i> , 2004
	Microemulsions	Cho <i>et al.</i> , 1989; Ritschel <i>et al.</i> , 1992; Torisaka <i>et al.</i> , 2005
	Oral insulin pills	Kinesh <i>et al.</i> , 2010
	Oral spray	Kinesh <i>et al.</i> , 2010

## ORAL DELIVERY: THE CHALLENGE

### Obstacles of Oral insulin Delivery

Generally, peptides and proteins such as insulin cannot be administered via the oral route due to rapid enzymatic degradation in the stomach, inactivation and digestion by proteolytic enzymes in the intestinal lumen, and poor permeability across intestinal epithelium because of its high molecular weight and lack of lipophilicity (Nakamura *et al.*, 2004; Jain *et al.*, 2005; Sajeesh *et al.*, 2006). The oral bioavailability of most peptides and proteins therefore is less than 1%. The challenge here is to improve the bioavailability to anywhere between 30-50% (Lee *et al.*, 1991).

### Enzymatic Barrier

The harsh environment of the gastrointestinal tract (GIT) causes insulin to undergo degradation. This is because digestive processes are designed to breakdown proteins and peptides without any discrimination (Tuesca *et al.*, 2006). Insulin therefore

undergoes enzymatic degradation by pepsin and pancreatic proteolytic enzymes such as trypsin and  $\alpha$ -chymotrypsin (Patki *et al.*, 1996; Agarwal *et al.*, 2001). Overall, insulin is subjected to acid catalyzed degradation in the stomach, luminal degradation in the intestine and intracellular degradation. The cytosolic enzyme that degrades insulin is insulin-degrading enzyme (IDE) (Chang *et al.*, 1999). Insulin is however not subject to proteolytic breakdown by brush border enzymes (Agarwal *et al.*, 2001). Insulin can be presented for absorption only if the enzyme attack is either reduced or defeated.

### Intestinal Transport of Insulin

Another major barrier to the absorption of hydrophilic macromolecules like insulin is that they cannot diffuse across epithelial cells through lipid bilayer cell membranes to the blood stream (Lin *et al.*, 2007). In other words, insulin has low permeability through the intestinal mucosa (Torisaka *et al.*, 2005). There is no evidence of active

**Table 4: Market status of non-invasive products.**

Brand Name	Dosage form	Company name	Launching year / Status
Exubera®	Powder Inhaler	Pfizer	2006
Oral-lyn®	Oral spray	Generex Biotechnology	2007
Nasulin®	Nasal spray	CPEX Pharmaceuticals	2010
Transgene®	Nanoparticles	Biotek	Human trial ongoing
In-105®	Tablet	Biocon	Phase-II trial ongoing
ORMD-0801®	Capsule	Oramed Pharmaceuticals	Phase II b complete

transport for insulin (Schilling *et al.*, 1999). It has been found however that insulin delivery to the midjejunum protects insulin from gastric and pancreatic enzymes and release from the dosage form is enhanced by intestinal microflora (Koosapur *et al.*, 1999; Schilling *et al.*, 1999). Various strategies have been tried out to enhance the absorption of insulin in the intestinal mucosa and in some cases; they have proven successful in overcoming this barrier.

#### Dosage form Stability

The activity of proteins depends on the three dimensional molecular structure. During dosage form development, proteins might be subject to physical and chemical degradation. Physical degradation involves modification of the native structure to a higher order structure while chemical degradation involving bond cleavage results in the formation of a new product (Agarwal *et al.*, 2001). Proteins must be characterized for change in conformation, size, shape, surface properties, and bioactivity upon formulation processing. Changes in conformation, size, and shape can be observed by spectrophotometric techniques, X-ray diffraction, differential scanning calorimetry, light scattering, electrophoresis, and gel filtration (Pearlman *et al.*, 1991).

#### Approaches for oral insulin delivery

Most peptides are not bioavailable from the GIT after oral administration (Kinesh *et al.*, 2010, Cho *et al.*, 1989). Therefore, successful oral insulin delivery involves overcoming the enzymatic and physical barriers (Tuesca *et al.*, 2006) and taking steps to conserve bioactivity during formulation processing (Agarwal *et al.*, 2001). In developing oral protein delivery systems with high bioavailability, three practical approaches might be most helpful (Morishita *et al.*, 2006):

1. Modification of physicochemical properties such as lipophilicity and enzyme susceptibility.
2. Addition of novel function to macromolecules.
3. Use of improved carrier systems.

### CONCLUSION

Insulin is the only treatment for type-2 diabetes patient. From 1922 to till date various attempt have been made to achieve different route of administration of insulin. After subcutaneous route, inhalation route has comparable bioavailability but inhaler insulin is rejected by patient and physician. Now-a-days a lot of research subjected for oral delivery of insulin. Liposome, microemulsion, hydrogels, nanoparticles etc. have been investigated and prepared for the oral delivery of insulin.

### REFERENCES

- Agarwal, V., Khan, M.A. (2001). Current status of the oral delivery of insulin. *Pharm. Tech.* 25(10): 76-90.
- Agarwal, V., Reddy, I.K., Khan, M.A. (2001). Polyethylacrylate based microparticulate of insulin for oral delivery: preparation and in vitro dissolution stability in the presence of enzyme inhibitors. *Int. J Pharm.*; 225 (1-2), 31-39. [http://dx.doi.org/10.1016/S0378-5173\(01\)00740-2](http://dx.doi.org/10.1016/S0378-5173(01)00740-2)
- Ahmed, I., Goldstein, B. (2006) *Diabetes Mellitus, Clin. Dermatol.* 24 (4): 237-246. [DOI]
- Al-Achi, A., Greenwood, R. (1998). "Erythrocytes as Oral Delivery Systems for Human Insulin," *Drug Dev Ind Pharm* 24 (1): 67-72. <http://dx.doi.org/10.3109/03639049809082354> PMID:15605599
- American Diabetes Association, Implications of the United Kingdom Prospective Diabetes Study. *Diabetes Care* 26 (suppl 1), S28 -S32, (2003). <http://dx.doi.org/10.2337/diacare.26.2007.S28>
- Arbit, E., and Miriam K. (2009). Oral Insulin: The Rationale for This Approach and Current Developments. *Journal of Diabetes Science and Technology* 3: 562-567.

- Arora, A, Hakim, I., Baxter, J. (2007). Needle-free delivery of macromolecules across the skin by nanoliter-volume pulsed microjets. *Proc. Natl. Acad. Sci. U.S.A.* 104 (11): 4255-4260. <http://dx.doi.org/10.1073/pnas.0700182104> PMID:17360511 PMCID:1838589
- Attivi, D. (2005). "Formulation of Insulin-Loaded Polymeric Nanoparticles using Response Surface Methodology," *Drug Dev. Ind. Pharm.* 31 (2), 179-189. <http://dx.doi.org/10.1081/DDC-200047802> PMID:15773285
- Balsells, M., Corcoy, R., Mauricio, D., Morales, J., Garcia-Patterson, A., Carreras, G., Puig-Domingo, M., de Leiva, A. (1997). Insulin antibody response to a short course of human insulin therapy in women with gestational diabetes. *Diabetes Care*; 20:1, 1172-1175. <http://dx.doi.org/10.2337/diacare.20.7.1172> PMID:9203458
- Banting, F.G. (1922). "Pancreatic Extracts," *J. Lab. Clin. Med.* 7, 464-472.
- Banting, F.G., Best, C.H. (1922). The internal secretion of the pancreas. *J Lab Clin Med*; 7: 465-480.
- Bliss, M. (1982). *The Discovery of Insulin*. Chicago, University of Chicago Press, p 155.
- Bohannon, N.J.V. (1999). Insulin delivery using pen devices: simple-to-use tools may help young and old alike. *Postgrad Med* 106: 57-68. <http://dx.doi.org/10.3810/pgm.1999.10.15.751>
- Browne, D.L., Avery, L., Turner, B.C. (2000). What do patients with diabetes know about their tablets? *Diabet.Med.* 17, 528-531. <http://dx.doi.org/10.1046/j.1464-5491.2000.00327.x> PMID:10972583
- Calara, F., Taylor, K., Han, J., Zabala, E., Carr, E.M., Wintle, M., Fineman, M. (2005). A randomized, open-label, crossover study examining the effect of injection site on bioavailability of exenatide (synthetic exendin-4). *Clin. Ther.* 27(2), 210-215. <http://dx.doi.org/10.1016/j.clinthera.2005.02.008> PMID:15811484
- Campbell, R.K., Campbell, L.K., White, J.R. (1996). Insulin lispro: its role in the treatment of diabetes mellitus. *Ann. Pharmacother.* 30, 1263-71. PMID:8913409
- Cefalu, W., Skyler, J., Kourides, I., Landschulz, W., Balagtas, C., Cheng, S., Gelfand, R. (2001). "Inhaled human insulin treatment in patients with type 2 diabetes mellitus". *Ann Intern Med* 134 (3): 203-7. PMID:11177333
- Cefalu, W.T. (2004). Evolving Strategies for insulin delivery and therapy, *drugs* 64 (11), 1149-1161.
- Chalasanani, K.B. (2007). A Novel Vitamin B12-Nanosphere Conjugate Carrier System for Peroral Delivery of Insulin, *J Control Release* 117 (3), 421-429 Epub 2006.
- Cho, Y.W., Flynn, M. (1989). Oral delivery of insulin. *Lancet*; 1518-1519. [http://dx.doi.org/10.1016/S0140-6736\(89\)92954-1](http://dx.doi.org/10.1016/S0140-6736(89)92954-1)
- Chung, H. (2004). "Self-Assembled 'Nanocubicle' as a Carrier for Peroral Insulin Delivery," *Diabetologia* 45 (3), 448-451. <http://dx.doi.org/10.1007/s00125-001-0751-z> PMID:11914752
- Coates, P.A., Ismail, I.S., Luzio, S.D. (1995). Intranasal insulin: the effects of three dose regimens on postprandial glycaemic profiles in type II diabetic subjects. *Diabet Med*; 12: 235-239. PMID:7758260 [DOI]
- Cui, F.D. (2007). Preparation of Insulin-Loaded PLGA-Hp55 Nanoparticles for Oral Delivery, *J. Pharm Sci.* 96 (2), 421-427. <http://dx.doi.org/10.1002/jps.20750> PMID:17051590
- Damge, C. (1997). Poly (alkyl cyanoacrylate) Nanospheres for Oral Administration of Insulin, *J Pharm Sci.* 86 1403-1409. <http://dx.doi.org/10.1021/jps970124i> PMID:9423155
- Damge, C., Maincent, P., Ubrich, N. (2007). Oral Delivery of Insulin Associated to Polymeric Nanoparticles in Diabetic Rats, *J Control Release* 117 (2), 163-170, Epub 2006. <http://dx.doi.org/10.1016/j.jconrel.2006.10.023> PMID:17141909
- DeFronzo, R.A., Bergenstal, R.M., Cefalu, W.T. (2005). Efficacy of inhaled insulin in patients with type 2 diabetes not controlled with diet and exercise. *Diabetes Care* 28, 1922-1928. <http://dx.doi.org/10.2337/diacare.28.8.1922> PMID:16043733
- DeFronzo, R.A., Ratner, R.E., Han, J., Kim, D.D., Fineman, M.S., Baron, A.D. (2005). Effects of exenatide(exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes. *Diabetes Care* 28(5), 1092-1100. <http://dx.doi.org/10.2337/diacare.28.5.1092> PMID:15855572
- Dhawan, S., Chopra, S. (2009). Novel approaches for oral delivery. *Pharmaceutical Technology* 33(7).
- Dixit, N., Bali, V., Baboota, S., Ahuja, A., Ali, J. (2007). Iontophoresis - an approach for controlled drug delivery: a review. *Current drug delivery* 4 (1): 1-10. <http://dx.doi.org/10.2174/156720107779314802> PMID:17269912
- Dorkoosh, F.A. (2002). Peroral Delivery Systems based on Superporous Hydrogel Polymers: Release Characteristics for the Peptide Drugs Buserelin, Octreotide and Insulin, *Eur J Pharm Sci* 15 (5), 433-439. [http://dx.doi.org/10.1016/S0928-0987\(02\)00028-3](http://dx.doi.org/10.1016/S0928-0987(02)00028-3)
- Eaimtrakarn, S., Ramaprasad, Y.V., Ohno, T., Konishi, T., Yoshikawa, Y., Shibata, N. (2002). Absorption-enhancing effect of labrasol on the intestinal absorption of insulin in rats. *J. Drug Target.* 10 (3), 255-260. <http://dx.doi.org/10.1080/10611860290022688> PMID:12075827
- Elliott, R.B., Edgar, B.W., Pilcher, C.C. (1987). Parenteral absorption of insulin from the lung in diabetic children. *Aust Paediatr J* 23: 293-297. PMID:3326575
- Fujii, S., Yokoyama, T., Ikegaya, K., Sato, F., Yokoo, N. (1985). Promoting effect of the new chymotrypsin inhibitor fk-448 on the intestinal absorption of insulin in rats and dogs. *J.*

- Pharm. Pharmacol. 37 (8), 545-549.  
<http://dx.doi.org/10.1111/j.2042-7158.1985.tb03064.x>  
 PMid:2864414
- Galloway, J.A. (1993). New directions in drug development: mixtures, analogues, and modeling. *Diabetes Care* 16(3), S16-S23.
- Gänsslen, M., Über (1925) Inhalation von Insulin [abstract]. *Klin Wochenschr* 4: 71.  
<http://dx.doi.org/10.1007/BF01748135>
- Gedulin, B.R., Nikoulina, S.E., Smith, P.A., Gedulin, G., Nielsen, L.L., Baron, A.D., Parkes, D.G., Young, A.A. (2005). Exenatide (exendin-4) improves insulin sensitivity and  $\beta$ -cell mass in insulin-resistant obese fa/fa Zucker rats independent of glycemia and body weight. *Endocrinology* 146(4), 2069-76.  
<http://dx.doi.org/10.1210/en.2004-1349> PMid:15618356
- Giriraj K.G., Giriraj K.T. (2003). Oral Insulin-Fact or Fiction. *Resonance* 38-43.
- Gizurarson, S., Bechgaard, E. (1991). Intranasal administration of insulin to humans. *Diabetes Res Clin Pract* 12: 71-84. [\[DOI\]](#)
- Golden, M.P., Haymond, M., Hinnen, D.A. (1988). Position statement on jet injectors. *Diabetes Care* 11: 600-1.  
 PMid:3060330
- Gowthamarajan, K., Kulkarni, G.T. (2003). Oral Insulin - Fact or Fiction? Possibilities of achieving oral delivery for insulin. *Resonance*, 38-46.
- Graff, M.R., McClanahan, M.A. (1998). Assessment by patients with diabetes mellitus of two insulin pen delivery systems versus a vial and syringe. *Clin Ther* 20: 486-96.  
[http://dx.doi.org/10.1016/S0149-2918\(98\)80058-1](http://dx.doi.org/10.1016/S0149-2918(98)80058-1)
- Heinemann, L. (2002). Alternative delivery routes: inhaled insulin. *Diabetes Nutr Metab Dec*; 15 (6): 417-22.
- Heinemann, L., Heise, T., Wahl, L.C. (1996). Prandial glycaemia after a carbohydrate-rich meal in type I diabetic patients: using the rapid acting insulin analogue [Lys(B28), Pro(B29)] human insulin. *Diabet Med*; 13: 625-9.
- Heinemann, L., Pftzner, A., Heise, T. (2001). Alternative routes of administration as an approach to improve insulin therapy: update on dermal, oral, nasal and pulmonary insulin delivery. *Curr Pharm Des Sep*; 7 (14): 1327-51.
- Hilsted J., Madsbad S., Hvidberg A. (1995). Intranasal insulin therapy: the clinical realities. *Diabetologia*; 38: 680-4.  
<http://dx.doi.org/10.1007/BF00401839> PMid:7672489
- Ho, L.T., Lam, H.C., Wu, M.S., Kwok, C.F., Jap, T.S., Tang, K.T., Wang, L.M., Liu, Y.F. (1991). A twelve month doubleblind randomized study of the efficacy and immunogenicity of human and porcine insulins in non-insulin-dependent diabetics. *Zhonghua Yi Xue Za Zhi (Taipei)*; 47:313-319.
- Home, P.D., Lindholm, A., Hylleberg, B. (1998). The UK Insulin Aspart Study Group. Improved glycemc control with insulin aspart. *Diabetes Care*; 21: 1904-9.  
<http://dx.doi.org/10.2337/diacare.21.11.1904> PMid:9802741
- Howey, D.C., Bowsher, R.R., Brunelle, R.L., Woodworth, J.R. (1994). [Lys(B28), Pro(B29)]-human insulin, Arapidly absorbed analogue of human insulin. *Diabetes* 43, 396-402.  
<http://dx.doi.org/10.2337/diabetes.43.3.396> PMid:8314011
- Hunt, L.M., Valenzuela, M.A., Pugh, J.A. (1997). NIDDM patients' fears and hopes about insulin therapy. *Diabetes Care*; 20: 292-8. <http://dx.doi.org/10.2337/diacare.20.3.292> PMid:9051375
- I.L., Sytov, G.A. (2002). Mucoadhesive polymers with immobilized proteinase inhibitors for oral administration of protein drugs. *Biomaterials*; 23 (7), 1673-1677.
- Ichikawa, H., Fukumori, Y. (2007). "Design of Nanohydrogel-Incorporated Microcapsules for Appropriate Controlled-Release of Peptide Drugs," *Yakugaku Zasshi* 127 (5), 813-823. <http://dx.doi.org/10.1248/yakushi.127.813> PMid:17473523
- Imam, M.Z. (2011). *Pharmaceutical Dosage Forms*, publisher Altaf Medical Book Center, Dhaka, Bangladesh pp393.
- Jacobs, M., Schreuder, R.H., Jap-A-Joe, K. (1993). The pharmacodynamics and activity of intranasally administered insulin in healthy male volunteers. *Diabetes*; 42: 1649-55. <http://dx.doi.org/10.2337/diabetes.42.11.1649> PMid:8405708
- Jain, D., Panda, A.K., Majumdar, D.K. (2005). Eudragit S100 entrapped insulin microspheres for oral delivery. *AAPS PharmSciTech*, 6 (1): 1-27.  
<http://dx.doi.org/10.1208/pt060116> PMid:16353953  
 PMCid:2750417
- Jersild, M. (1956). Insulin zinc suspension; four years' experience. *Lancet*; 271:1009-1013.  
[http://dx.doi.org/10.1016/S0140-6736\(56\)90266-5](http://dx.doi.org/10.1016/S0140-6736(56)90266-5)
- Kanikkannan, N., Singh, J., Ramarao, P. (1999). Transdermal iontophoretic delivery of bovine insulin and monomeric human insulin analogue. *J Control Release*; 59: 99-105.  
[http://dx.doi.org/10.1016/S0168-3659\(98\)00184-9](http://dx.doi.org/10.1016/S0168-3659(98)00184-9)
- Katsoyannis, P.G., Tometsko, A., Zalut, C. (1966). Insulin peptides XII. Human insulin generation by combination of synthetic A and B chains. *J Am Chem Soc* 88:186.  
<http://dx.doi.org/10.1021/ja00953a048>
- Kavimandan, N.J. (2006). Synthesis and Characterization of Insulin-Transferrin Conjugates, *Bioconjug Chem.* 17 (6), 1376-1384. <http://dx.doi.org/10.1021/bc050344k> PMid:17105214 PMCid:2547411
- Keen, H., Glynn, A., Pickup, J.C. (1980). Human insulin produced by recombinant DNA technology: safety and hypoglycaemic potency in healthy men. *Lancet*; ii: 398-401.  
[http://dx.doi.org/10.1016/S0140-6736\(80\)90443-2](http://dx.doi.org/10.1016/S0140-6736(80)90443-2)
- Kinesh, V.P., Neelam, D.P. (2010). Novel approaches for oral delivery of Insulin and current status of oral insulin

- products. *International Journal of pharmaceutical Sciences and Nanotechnology*; 3(3):1057-1064.
- Klonoff, D.C. (1999). Inhaled insulin. *Diabetes Technol Ther* 1: 307-13. <http://dx.doi.org/10.1089/152091599317224>
- Kooshapur, H., Chaideh, M. (1999). Intestinal transport of human insulin in rat. *Med. J. Islamic Academy of Sciences*; 12:1, 5-11.
- Kraeling, M.E., Ritschel, W.A. (1992). Development of a Colonic Release Capsule Dosage Form and the Absorption of Insulin, *Methods Find Exp Clin Pharmacol* 14 (3), 199-209. PMID:1378164
- Krauland, A.H., Guggi D., and Bernkop-Schnurch A., (2004). Oral Insulin Delivery: The Potential of Thiolated Chitosan-Insulin Tablets on Nondiabetic Rats, *J Control Release* 95 (3): 547-55. <http://dx.doi.org/10.1016/j.jconrel.2003.12.017> PMID:15023465
- Kung, Y.T., Da, Y.C., Huang, W.T. (1966). Total synthesis of crystalline insulin. *Sci Sin*; 15:544. PMID:5960685
- Lantus [package insert]. Kansas City (MO): Aventis Pharmaceuticals, 2001.
- Laube, B.L., Georgopoulos, A., and Adams, III, G.K. (1992). Proving the efficacy of insulin delivered through the lungs as an aerosol. *J. Biopharm. Sci.* 3:163-169.
- Lee, V.H. (1991). Oral route of peptide and protein drug delivery In *Peptide and Protein Drug Delivery*, Chapter 16, Marcel Dekker Inc., New York, 691-738.
- Lepore, M., Pampanelli, S., Fanelli, C. (2000). Pharmacokinetics and pharmacodynamics of subcutaneous injection of long-acting human insulin analog glargine, NPH insulin, and ultralente human insulin and continuous subcutaneous infusion of insulin lispro. *Diabetes*; 49 (12): 2142-8. <http://dx.doi.org/10.2337/diabetes.49.12.2142> PMID:11118018
- Li, C.L., Deng, Y.J. (2004). Oil-based formulations for oral delivery of insulin. *J. Pharm. Pharmacol.* 56 (9): 1101-1107. <http://dx.doi.org/10.1211/0022357044175> PMID:15324478
- Lin, Y.H (2007). Preparation and Characterization of Nanoparticles Shelled with Chitosan for Oral Insulin Delivery, *Biomacromolecules* 8 (1): 146-152. <http://dx.doi.org/10.1021/bm0607776> PMID:17206800
- Maheux, P., The evolving story of insulin delivery, Inhaled insulin [Presentation given at Pfizer Ltd].
- Major, R.H. (1935). The intranasal application of insulin. *J Lab Clin Med*; 21: 278-80.
- Meienhofer, J., Schnabel, E., Bremer, H. (1963). Synthese der Insulinketten und ihre Kombination zu insulactiven Präparaten. *Z Naturforsch*; 18:1120. PMID:14117584
- Mitragotri, S., Blankschtein, D., Langer, R. (1995). Ultrasound-mediated transdermal protein delivery. *Science*; 269: 850-3. <http://dx.doi.org/10.1126/science.7638603> PMID:7638603
- Morishita, M, Peppas, NA. (2006). Is the oral route possible for peptide and protein drug delivery? *Drug Discovery Today*. 11: 19-20. <http://dx.doi.org/10.1016/j.drudis.2006.08.005> PMID:16997140
- Nakamura, K., Murray, R.J., Joseph J.I., Peppas N.A., Morishita M., Lowman A.M. (2004). Oral insulin delivery using P(MAA-g-EG) hydrogels: Effects of network morphology on insulin delivery characteristics. *J. Cont. Release*, 95:589-599. <http://dx.doi.org/10.1016/j.jconrel.2003.12.022> PMID:15023469
- Nauck, M.A., Meier, J.J. (2005). Glucagon-like peptide 1 and its derivatives in the treatment of diabetes. *Regul. Pept.* 128(2), 135-48. <http://dx.doi.org/10.1016/j.regpep.2004.07.014> PMID:15780433
- NICE (2006). Diabetes (type 1 and 2), Inhaled Insulin - Appraisal Consultation Document (second). Retrieved 2006-07-26.
- Nielsen, L.L. (2005). Incretin mimetics and DPP-IV inhibitors for the treatment of type 2 diabetes. *D.D.T*, 10,703-710.
- Pamnani, D. (2008). Reality Check on Oral Insulin, *Pharma Express*, 16-31.
- Patki, V.P., Jagasia, S.H. (1996). Progress made in noninvasive insulin delivery. *Ind. J. Pharmacol.* 28: 143-151.
- Pearlman, R., Nguyen, T.H. (1991). Analysis of Protein Drugs, in *Peptide and Protein Drug Delivery*, V.H.L. Lee, Marcel Dekker, New York, Plate, N.A., Valuev.
- Rieux, A., Fievez, V., Garinot, M., Schneider, Y.J., Preat, V. (2006). Nanoparticles as potential oral delivery systems of proteins and vaccines: A mechanistic approach. *J. Cont. Release*; 116: 1-27. <http://dx.doi.org/10.1016/j.jconrel.2006.08.013> PMID:17050027
- Rosenstock, J. (2001). Insulin therapy: optimizing control in type 1 and type 2 diabetes. *Clin Cornerstone*; 4 (2): 50-64. [http://dx.doi.org/10.1016/S1098-3597\(01\)90029-8](http://dx.doi.org/10.1016/S1098-3597(01)90029-8)
- Saffran, M., Kumar, G.S., Savariar, C., Burnham, J.C., Williams, F., Neckers, D.C. (1986). A new approach to the oral administration of insulin and other peptide drugs. *Science*; 233(4768):1081-1084. <http://dx.doi.org/10.1126/science.3526553> PMID:3526553
- Sajeesh, S., Sharma, C.P. (2006). Cyclodextrin-insulin complex encapsulated polymethacrylic acid based nanoparticles for oral insulin delivery. *Int. J. Pharm.* 325(1- 2):147-154.
- Salamat-Miller, N, Johnston, TP. (2005). Current strategies used to enhance the paracellular transport of therapeutic polypeptides across the intestinal epithelium. *Int. J. Pharm.* 294: 201-216 <http://dx.doi.org/10.1016/j.ijpharm.2005.01.022> PMID:15814245

- Sarabu, R., Grimsby, J. (2005). Targeting glucokinase activation for the treatment of type 2 diabetes—a status review. *Curr. Opin. Drug. Discov. Devel.* 8, 631-637  
PMid:16159025
- Sarmiento, B. (2006). Development and Characterization of New Insulin Containing Polysaccharide Nanoparticles, *Colloids Surf B. Biointerfaces* 53 (2), 193-202.  
<http://dx.doi.org/10.1016/j.colsurfb.2006.09.012>  
PMid:17049214
- Saudek, C.D. (1997). Novel forms of insulin delivery. *Endocrinol Metab Clin North Am*; 26: 599-610  
[http://dx.doi.org/10.1016/S0889-8529\(05\)70269-3](http://dx.doi.org/10.1016/S0889-8529(05)70269-3)
- Sayani, A.P., Chien, Y.W. (1996). Systemic delivery of peptides and proteins across absorptive mucosae. *Crit Rev Ther Drug Carrier Syst*; 13: 85-184. PMid:8853960
- Schilling, R.J., Mitra, A.K. (1999). Intestinal mucosal transport of insulin. *Int. J. Pharm.* 62: 53-64.  
[http://dx.doi.org/10.1016/0378-5173\(90\)90030-8](http://dx.doi.org/10.1016/0378-5173(90)90030-8)
- Schlüter, K.J., Köhler, D., Enzmann, F. (1984) Pulmonary administration of human insulin in volunteers and type 1 diabetics [abstract]. *Diabetes*; 75A: 298.
- Shah, R.B., Ahsan, F., Khan, M.A. (2002). Oral delivery of proteins: Progress and prognostication. *Crit. Rev. Ther. Drug Carrier Syst.* 19(2): 135-169.  
<http://dx.doi.org/10.1615/CritRevTherDrugCarrierSyst.v19.i2.20> PMid:12197608
- Simon, M. (2007). Nanosized Insulin-Complexes based on BioDegradable Amine-Modified Graft Polyesters Poly(vinyl-3-(diethylamino)-propylcarbamate-co-(vinyl acetate)-co-(vinyl alcohol)]-graft-poly(l-lactic acid): Protection Against Enzymatic Degradation, Interaction with Caco-2 Cell Monolayers, Peptide Transport and Cytotoxicity, *Eur. J. Pharm. Biopharm.* 66 (2), 165-172.  
<http://dx.doi.org/10.1016/j.ejpb.2006.10.009> PMid:17150341
- Soltero, R., Ekwuribe, N. (2001). The oral delivery of protein and peptide drugs- A report. *Innovat. Pharmaceut. Technol.* 1:106-110.
- Spencer, A.G., Morgans M.E. (1956). Lente insulin; four years' experience. *Lancet*; 271: 1013-1017.  
[http://dx.doi.org/10.1016/S0140-6736\(56\)90267-7](http://dx.doi.org/10.1016/S0140-6736(56)90267-7)
- Teuscher, A. (1974). Treatment of insulin lipoatrophy with monocomponent insulin. *Diabetologia*; 10: 211-214.  
<http://dx.doi.org/10.1007/BF00423037> PMid:4845721
- Teuscher, A. (1979). The biological effect of purely synthetic human insulin in patients with diabetes mellitus. *Schweiz Med Wochenschr*; 109: 743-747. PMid:451498
- Thanou, M., Verhoef, J.C., Junginger H.E. (2001). Chitosan and its derivatives as intestinal absorption enhancers. *Adv. Drug. Delivery.* 50: S91-S101.  
[http://dx.doi.org/10.1016/S0169-409X\(01\)00180-6](http://dx.doi.org/10.1016/S0169-409X(01)00180-6)
- Tiyaboonchai, W. (2003). Insulin Containing Polyethylenimine-Dextran Sulfate Nanoparticles," *Int. J. Pharm.* 255 (1-2): 139-151. [http://dx.doi.org/10.1016/S0378-5173\(03\)00055-3](http://dx.doi.org/10.1016/S0378-5173(03)00055-3)
- Toorisaka, E, Hashida M, Kamiya N, Ono H, Kokazu Y, Goto M. (2005). An enteric-coated dry emulsion formulation for oral insulin delivery. *J. Cont. Release*; 107: 91-96.  
<http://dx.doi.org/10.1016/j.jconrel.2005.05.022>  
PMid:16039746
- Tozaki, H. (2001) Enhanced Absorption of Insulin and (Asu(1,7))eel-calcitonin Using Novel Azopolymer-Coated Pellets for Colon-Specific Drug Delivery," *J. Pharm. Sci.* 90 (1), 89-97. [\[DOI\]](#)
- Troels Keldmann Nasal Delivery outlook Direct-haler A/S available at:  
<http://www.directhaler.com/search/directhaler-h118.htm>  
[Accessed on 26 December 2011].
- Tuesca, A., Lowman, A. (2006). The oral delivery of insulin using protein conjugates in complexation hydrogels. Poster presentation, Biomaterials and Drug Delivery Laboratory, Drexel University.
- White, Jr J.R., Campbell, R.K., Hirsch, I.B. (2003) Novel insulins and strict glycemic control: analogues approximate normal insulin secretory response. *Postgrad Med*; 113 (6): 30-36.  
<http://dx.doi.org/10.3810/pgm.2003.06.1426> PMid:12838802
- Whitehead, K., Shen, Z., Mitragotri, S. (2004). Oral Delivery of Macromolecules Using Intestinal Patches: Applications for Insulin Delivery, *J. Control. Release* 98 (1), 37-45.  
<http://dx.doi.org/10.1016/j.jconrel.2004.04.013>  
PMid:15245887
- Wigley, F.W., Londono, J.H., Wood, S.H. (1971). Insulin across respiratory mucosa by aerosol delivery. *Diabetes*; 20: 552-556. PMid:5565002
- Yamamoto, A., Taniguchi, T., Rikyuu, K., Tsuji, T., Fujita, T., Murakami, M., Mranishi, S. (1994). Effects of various protease inhibitors on the intestinal absorption and degradation of insulin in rats. *Pharm. Res.* 11 (10): 1496-1500. <http://dx.doi.org/10.1023/A:1018968611962>  
PMid:7855059
- Zambanini, A., Newson, R.B., Maisey, M. (1999). Injection related anxiety in insulin-treated diabetes. *Diabetes Res Clin Pract*; 46: 239-246. [\[DOI\]](#)
- Ziv, E., Lior, O., Kidron, M. (1987). Absorption of protein via the intestinal walls: A quantitative model. *Biochem. Pharmacol.* 39(7):1035-1039. [\[DOI\]](#)