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Insulin Oral Delivery: A Review on Challenges and Potential Approaches

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ABSTRACT

Numbers of protein-based pharmaceuticals are increasing in marketplace. Despite rapid progress in the large-scale manufacture of therapeutic proteins, majority of protein products currently on the market are for parenteral administration. Therefore, these proteins are needed to be reformulated within delivery systems that can be value-added for optimal molecular activity. Oral administration being the most convenient and patient compliant route, enzymatic and penetration barriers are some of the major challenges. Hence, the microparticulate delivery systems provides the alternative to the parenteral delivey by loading the drug into pellets, microparticles, lipospheres and macroemulsions. Microspheres are one of the widely researched candidates as carrier systems as it offers minimum dose size, controlled drug release, reduction in systemic side effects, nullifying the possibility of dose dumping and reduced frequency of administration and therefore, increased patient compliance. Rationale of oral insulin delivery include as invasive parenteral (injected) insulin suffers from poor patient compliance due to needle phobia, pain, skin bulges, allergic reactions, common infections, and stress generated from the difficult long-term regimen of insulin therapy, orally administered insulin is absorbed directly from the intestine and then transported to the liver via portal circulation, where it inhibits hepatic glucose production. However, Ensuring adequate bioavailability of oral insulin, preserving its bioactivity, and maximizing the desired effects in the body are the most essential criteria for successful insulin delivery

Keywords: Therapeutic proteins, insulin, oral delivery, microspheres, biodegradable polymers

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INTRODUCTION

In recent years, the number of protein-based pharmaceuticals reaching the marketplace has increased exponentially. The clinical application of these drugs is limited by a lack of desirable attributes for adequate absorption or distribution. It therefore becomes critical to formulate these drugs into safe, stable and efficacious delivery systems. Protein and peptide drug delivery has been an area of intensive research because of their efficacy in several disease conditions. However, despite rapid progress in the large-scale manufacture of therapeutic proteins, the convenient and effective delivery of these drugs to the body remains a major challenge. Most protein products currently on the market are for parenteral administration; however, half-lives are short and they would benefit from the development of controlled release parenterals. Currently, peptide and protein drugs are marketed almost exclusively for parenteral administration. This is because these drugs face formidable enzymatic and penetration barriers when administered orally. A limitation of the parenteral route for delivery of peptides and proteins is the extremely short half-lives of these drugs – in the order of a few minutes. This demands repeated administration, which is inconvenient to the patient.

Research, development and sales of drug-delivery systems are increasing at a rapid pace throughout the world. This worldwide trend will intensify in the next decade as cuts in public health expenses demand lower costs and higher efficacy. To meet this demand, many efficient drugs currently in use will be reformulated within delivery systems that can be value-added for optimal molecular activity. A sustained, constant drug level at the therapeutic optimum is needed in the blood in many pathological conditions. Therefore, the preparation of controlled and targeted drug delivery systems is one of the most important tasks of pharmaceutical technology.[1] Most of these therapeutic peptides are still administered by the parenteral route because of their poor bioavailability when delivered via other routes. Peptide drugs are usually indicated for chronic conditions, and the use of injections daily during long-term treatment has obvious drawbacks.

Therapeutic Proteins Delivery

Colloidal drug delivery systems such as micro- and nanoparticulate delivery systems are proper for the above-mentioned purposes. The value of these delivery systems as orally administered controlled-release dosage forms has been evident for years. The microparticulate delivery systems include mainly pellets, microparticles, lipospheres and macroemulsions. The nanoparticulate delivery systems include mainly lipid or polymeric nanoparticles, microemulsions, liposomes, cochleates, and nonionic surfactant vesicles (niosomes). Drug can be embedded within a polymeric/proteinic coat or matrix network in either a solid aggregated state or a molecular dispersion, resulting in the formulation of microcapsules or microspheres, respectively. The aqueous solubility, which becomes for many drugs the main drawback during formulation either in a liquid form or in controlled release systems, has been overcome by microencapsulation techniques.

Biodegradable and biocompatible polymer materials as drug carriers have been investigated in the recent 15 years in large number of studies in various drug delivery systems. In microparticles, the drug diffusion can be easily controlled through the matrix structure, and sensitive materials (drugs, peptides, hormones, vaccines, pDNA) can be protected against the external environment. The advantage is that the drug release can be controlled; microparticles have a long duration of action, and dosage frequency and adverse effects can therefore be reduced.

The past decade saw increased interest in the formulation and delivery of biological drugs for a range of diseases. Unlike conventional drugs, the clinical development of these types of drugs has not been possible without some sort of sophisticated pharmaceutical technology. Among the most important therapeutic proteins and peptides being explored is insulin.

Insulin was isolated from bovine pancreas in 1922 by Frederick Banting and Charles Best, who received the 1923 Nobel Prize for Medicine with John McLeod. Conventional insulin treatment is basically a replacement therapy, in which exogenous insulin is administered subcutaneously to mimic, as closely as possible, the insulin secretion of the healthy pancreas. The subcutaneous route has been the mainstay of insulin delivery until now. Although these parenteral routes are satisfactory in terms of efficacy in the great majority of cases, they can result in peripheral hyperinsulinemia, the stimulation of smooth muscle cell proliferation, and the incorporation of glucose into the lipid of arterial walls, and they might therefore be the causative factor in diabetic micro-and macroangiopathy.[2] Moreover, the burden of daily injections,

physiological stress, pain, inconvenience, cost, risks, infection, inability to handle insulin, and the localized deposition of insulin, leading to local hypertrophy and fat deposition at the injection sites remain problems.[3] The synthesis of insulin by recombinant DNA technology represented an important scientific milestone and made large quantities of the protein available at an affordable price, a factor that led insulin to become one of the most popular proteins to be studied for non-parenteral delivery. Consequently, the results of research into several aspects of the delivery of the insulin are available. In recent years, there has been a great deal of interest in the exploitation of non-invasive routes for insulin delivery, and their development by the pharmaceutical industry, including oral, nasal, buccal, pulmonary, transdermal, rectal, and ocular drug delivery systems.[4, 5, 6] The objective is to provide an update on the most promising advances in non-invasively delivery systems for insulin that may overcome the barriers to its absorption.

The oral delivery of insulin remains an elusive goal to which many investigators aspire. However, there are several obstacles that limit the oral bioavailability of peptides and proteins. One of the major obstacles is the harsh environment of the gastrointestinal (GI) tract, which by its very nature, is intended to break down proteins and peptides into their constituent amino acids. In addition to enzymatic barriers, there are also physical barriers which make the oral delivery of a peptide or protein difficult.[7] The lining of the GI tract is composed of a thick wall of epithelial cells covered by a layer of polysaccharides and mucus, which can inhibit the oral delivery of a peptide such as insulin via the hepatic portal vein to the liver. Several approaches have been taken to improve the oral bioavailability of therapeutic proteins such as insulin. These strategies include the use of protease inhibitors, permeation enhancers, enteric coatings, and microsphere encapsulation. [8, 9, 10, 11, 12, 13, 14, 15] Of these techniques, microsphere encapsulation is the only oral drug delivery vehicle that has the potential to surpass both the enzymatic and physical barriers of the GI tract.

Most oral delivery strategies for insulin based on particulate carriers have been developed to circumvent the barriers to oral peptide delivery. They efficiently protect protein and peptide drugs against enzymatic degradation in the harsh environment of the GIT, provide high transfer of drugs across the epithelial mucosa, control the release rate, and target drug delivery to specific intestinal sites. Pathogens and microparticles smaller than 10 µm enter the gut-associated lymphoid tissues (GALT), which include Peyer's patches, the appendix, and small solitary lymphoid nodules. Peyer's patches are follicles of lymphoid tissue covered with a specialized epithelium containing M cells.[16] The potential modes of entry of submicron particles from the intestine include via M cells and enterocytes, and by paracellular routes. Histological evaluation of tissue sections has demonstrated that 100 nm particles diffuse throughout the submucosal layers, whereas larger particles (≥10 µm) are predominantly localized in the epithelial lining of the tissue. Lysosomal degradation is normally associated with the endocytotic uptake of microparticles, but because this can interfere with the antigen-sampling role of the M cells, Peyer's patches are deficient in lysosomes. These favorable characteristics of the GALT have stimulated research into targeting Peyer's patches for peptide and protein delivery.[17]

However, difficulties have been encountered that must be overcome to achieve success in carrier delivery systems: the low incorporation efficiency of hydrophilic drugs; the precise control of drug release; the avoidance of particle aggregation; and the possible accumulation of non-degradable particles in tissues. Even with degradable particles, the use of unreasonably high quantities of carrier can lead to harmful carrier toxicity.[18]

The desire to deliver protein and peptide biopharmaceuticals conveniently and effectively has led to the intense investigation of targeted delivery systems. Despite various challenges, progress toward the convenient non-invasive delivery of proteins and peptides has been made through specific routes of administration. The delivery of proteins and peptides to specific sites of action has been used to lower the total dose delivered and to concentrate the therapeutic dose at specific sites of pharmacological action.[19] Absorption is not uniform throughout the GIT, and site-specific absorption occurs because of differences in the composition and thickness of the mucus layer, pH, surface area, and enzyme activity.[20] Drug delivery to the colon, for instance, has several attractive features, including a prolonged residence time, reduced enzymatic activity, increased tissue responsiveness to absorption enhancers, and natural absorptive characteristics.[21] Oral administration offers a potential portal to the superficial layers of the GIT (local delivery) and to the blood and lymphatic systems (systemic delivery). However, the harsh hydrolytic environment of the GIT and the epithelial barriers to absorption pose major challenges to the success of this mode of drug delivery for peptide and protein drugs.

Insulin administration in a colon-targeted delivery system has been developed extensively over the past few years.[22] The colon-targeted delivery of insulin with sodium glycocholate was more effective in increasing hypoglycemic effects after oral administration. The combination of sodium glycocholate and poly (ethylene oxide) tended to prolong the absorption of insulin after oral administration using the colon-targeted delivery system.[23] The release of a peptide in a specific region of the GIT, where uptake into the lymph system is maximized or where enzyme activity is low, has been used to increase the absorption of drugs after oral administration.[24] Surface charge and particle size are the main factors that control the uptake of particulates by Peyer's patches.[25] Proteins such as lectins and transferrin have also been suggested as transport carriers in the gastrointestinal absorption of polypeptides. The total percentage of the administered dose taken up through the lymphoid tissue was statistically much greater than that absorbed through non-lymphoid tissue. It was estimated that 60% of the uptake in the small intestine occurred through the Peyer's patches, even though the patches comprise a small percentage of the total surface area of the small intestinal tissue. A significant amount of total uptake was also shown to occur in the large intestine, particularly in the lymphoid sections of this tissue.

It was demonstrated that lectin modified solid lipid nanoparticles containing insulin orally administered to rats resulted in relative bioavailabilities of between 4.99% and 7.11%.[9] Another group successfully maintained plasma glucose levels at pre-diabetic levels for 11 h after the oral administration of chitosan and insulin nanoparticles to diabetic rats.[5] In addition, microparticles composed of poly (methacrylic acid) and poly (ethylene glycol) and containing insulin that were orally administered to type 1 diabetic rats resulted in suppressed postprandial blood glucose levels.[8]

Microparticles

Microparticles are usually formed by the controlled precipitation of polymers and can be divided to the groups of: (i) microcapsule (spherical geometry with a continuous core region surrounded by a continuous shell; reservoir systems); (ii) microsphere (spherical matrix with dispersed or dissolved entrapped drug; matrix systems); and (iii) irregular geometry with many small droplets or particles of core material.

Microparticles have many advantages: (i) delayed or sustained release; (ii) prevention of side effects related to the presence of the drug in the stomach; (iii) protection of the drug from degradation in the acidic environment of the stomach; (iv) reduction in frequency of administration and avoidance of peak and valley effects in blood level; (v) biocompatibility; (vi) easy preparation; (vii) relative stability; and in special cases (viii) to obtain controlled or targeted release.[26],[27] Microparticles are widely discussed in the literature; this is why the literature review part of this thesis mainly focuses on microspheres prepared by the w/o/w emulsion-solvent evaporation method.

In recent years, interest in controlled and sustained release of drugs has increased as the pharmaceutical companies seek improved methods of delivering therapeutic dosages of medicines. Benefits to patients would be enhanced if dosages could be sustained for extended periods. Tunable, sustained delivery platforms are therefore necessary to achieve optimal and functional delivery of drugs. Microspheres are one of the widely researched candidates in such delivery platforms.

Microspheres are defined as spherical microscopic particles that range from 1 to 1000 µm in diameter.[28] They are homogeneous structures made up of a continuous phase of one or more miscible polymers in which particulate drug is dispersed throughout the matrix unlike microcapsules, which have an inner core surrounded by a distinct outer shell. A wide range of core materials have been encapsulated in microspheres, including adhesives, agrochemicals, live cells, active enzymes, flavours, fragrances, pharmaceuticals, and ink.[29] Other than drug encapsulation, microspheres have been used as fillers and bulking agents and even for embolization therapy.[30]

Microencapsulation of proteins into microspheres may have more limitation compared with peptides. This is because proteins have a more complex structure, characterized by a well-defined tertiary structure that may be affected by the harsh manufacturing conditions of the conventional emulsification process used for microsphere preparation.

The idea of controlled release from polymers dates back to the 1960s through the employment of silicone rubber and polyethylene polymer microcapsules as delivery systems. However, the requirement of

eventual surgical removal because of no degradability of these systems limited their applicability and urged the need to prepare systems which would be eventually eliminated from the body. Many new delivery systems like liposomes, hydrogels, etc. were designed thereafter and investigated but none of them emerged as perfect system due to issues with immunogenicity, stability, site and rate of administration, dosage, and control over release rates, pharmacokinetics and pharmacodynamics. In terms of release kinetics, delivery of most drugs, whether by oral administration or through injection, follows what is known as "first-order kinetics" characterized by initial high blood levels of the drugs, followed by exponential fall in blood concentration. This is problematic because once blood concentrations fall below certain levels, no therapeutic effect will be achieved. Furthermore, some drugs are toxic at high blood level concentrations. It is difficult to achieve a balance between effective levels and toxic levels when blood concentrations fall off so rapidly.

Ideal delivery of drugs would follow "zero-order kinetics", wherein blood levels of drugs would remain constant throughout the delivery period. Consequently, scientists have been searching for methods to deliver drugs with zero-order kinetics. An unmet challenge has been to select the best controlled release technology. Microspheres, with many advantages described below, are on the forefront of this selection. Advantages of microspheres as controlled drug delivery devices are: a decrease in single dosage size, a continuous drug release, decrease in systemic side effects, and reduced possibility of dose dumping, reduced frequency of administration, and, therefore, increased patient compliance. Non-invasive placement and localized release of desired amount of therapeutic agents, circumvents the deleterious side effects of systemic administration. This enables administration of larger, effective dosages. Furthermore, the size and size distribution of microspheres can be controlled to achieve a better predicted response. Moreover, microspheres can be manufactured with biodegradable materials or stimuli responsive materials which eliminate the need for device recovery. Because of their ability to act as a device for controlled release drug delivery, microspheres have been used to encapsulate many types of drugs, including small molecules, proteins, and nucleic acids and are easily administered through a syringe needle. They can provide sustained drug release for long periods of time. On the other hand, disadvantages of microspheres may include difficulty of large scale manufacturing, high cost of manufacturing, and inactivation of drug during fabrication and poor control of drug release rates. Nutropin Depot, which comprised of Genentech's recombinant human growth hormone (rhGH) encapsulated within poly (D, L-Lactide-co-glycolide) (PLG), was pulled from the market because of high cost of production and manufacturing. Microspheres as fillers and bulking agents tend to migrate away from the injection site, which can cause a loss of therapeutic effects and may also cause embolism, which can eventually lead to organ damage.

MATERIALS FOR MICROSPHERE FABRICATION

Natural Polymers

Natural polymers are of interest because of their biocompatibility, relative abundance and commercial availability, and ease of processing. Natural polymers include alginate, proteins, collagens (gelatin), fibrins, albumin, gluten, elastin, fibroin, hyaluronic acid, cellulose, starch, chitosan (chitin), pectin (pectinic acid), galactan, curdlan, gellan, levan, emulsan, dextran, pullulan, heparin, silk, chondroitin 6-sulfate, polyhydroxyalkanoates, Daucus carota, etc.

Synthetic Polymers

Synthetic polymers are largely divided into two categories: biodegradable and nonbiodegradable. Biodegradable synthetic polymers include poly (α-hydroxyester)s, polyanhydrides, and polyorthoesters.[31] Among these, the poly (α-hydroxyester)s such as polylactide (PLA), polyglycolide (PGA), and its copolymers are most extensively used. These polymers, which are biocompatible, biodegradable, and bioresorbable, are approved by the Food and Drug Administration (FDA) and are also advantageous due to their controlled degradation behavior. Some non-biodegradable polymers include polyvinylalcohol (PVA), polyhydroxyethymethacrylate (PHEMA), and poly (N-isopropylacrylamide) (PNIPAAm). Synthetic polymers have an advantage of large scale availability and tunable mechanical and chemical properties.

Methods of Preparation of Microspheres

For preparation of microspheres, certain requirements must be met while choosing an appropriate encapsulation process.[32] These are: (i) The biological activity and chemical stability of the incorporated drugs should be maintained as much as possible during the process of encapsulation. (ii) The encapsulation efficiency and the yield of the microparticles should be high enough for mass production. (iii) The size range of these microparticles produced should be reasonable (<250 um) so that they can be administered using the syringe needle via the parenteral pathway. (iv) The drug release profile should be reproducible without significant initial burst release. (v) The employed process should produce stable, non-aggregating microparticles, thus making it easy to prepare uniform suspension of microparticles.

There are various techniques available for microencapsulation of drugs including the emulsion-solvent evaporation/extraction methods (single emulsion and double emulsion methods including solid-oil-water, water-oil-oil and solid-oil-oil methods), spray drying and spray freeze drying, ultrasonic atomization, electrospraying, microfluidic methods, pore closing method, thermoreversible gel method, microfabrication method and *in situ* polymerization. Each method has its own advantages and disadvantages. The choice of particular technique depends on attributes of the polymer and the drug, the site of drug action, and the duration of therapy.[33, 34, 35] However, emulsion solvent removal is the oldest and most widely used method to accomplish encapsulation. Unlike spray drying method where temperature-sensitive compounds are degraded and control of the particle size is difficult, the emulsion solvent removal method does not require elevated temperatures. The emulsion solvent removal method does not require phase separation inducing agents, unlike phase separation and coacervation methods where residual solvents and coacervating agents are typically found in the microspheres. The emulsion solvent removal method thus allows for the creation of microparticles that have a more optimized release of the encapsulated material.

Single Emulsion Method

The single emulsion method is primarily used for encapsulating hydrophobic drugs through an oil-in-water (o/w) emulsification process. The premise for this method is the emulsification of a polymeric solution in an aqueous continuous phase. The polymer is dissolved in a water-immiscible, volatile organic solvent like dichloromethane (DCM) and the drug is also dissolved or suspended in polymer “oil” solution. The resulting mixture is then emulsified in large volume of water containing a surfactant like polyvinyl alcohol (PVA). The organic solvent in the emulsion is removed by either evaporation or extraction in large amount of water, resulting in the formation of compact microparticles.

Many methods have been utilized to achieve the dispersion of oil phase in continuous aqueous phase. The most common method is the use of a propeller style blade attached to variable speed motor. As the speed of the motor is increased, the size of the dispersed droplets decreases because of the high shear induced by the propeller. Homogenization is also used to prepare emulsions. A homogenizer equipped with a rotor and stator type blade is attached to a variable high-speed motor. Since high shear is used to produce the emulsion, the resultant product has size much smaller than the emulsions produced by conventional agitation. Other methods used include a microfluidizer, sonicator, and potentiometric dispersion.[36]

The rate of solvent removal, the concentration and type of surfactant used, type and volume of organic solvent, polymer molecular weight and emulsification stirring speed are reported to affect the physiochemical characteristics, encapsulation efficiency, and release of drugs from microspheres.[37, 38, 39, 40] This method, however, is only applicable for hydrophobic drugs because hydrophilic drugs may diffuse out or partition from the dispersed oil phase into the aqueous phase, leading to poor encapsulation efficiencies.[41, 42]

Double Emulsion Method

Double emulsion or water-in-oil-in-water (w/o/w) methods are largely used for encapsulating water-soluble drugs. This method has advantages of relative simplicity, convenience in controlling process parameters, and only requiring inexpensive instrumentation.[43] In w/o/w method, aqueous drug solution is dispersed in a polymer dissolved organic solution, e.g., PLGA in dichloromethane (DCM) or ethyl acetate (EtAc) to form a primary w/o emulsion. Then this primary emulsion is further dispersed in large volume of water containing an emulsifier, such as poly (vinyl alcohol) (PVA), to form a w/o/w double emulsion. Hardened

microspheres are formed by removing organic solvent from the polymer phase by solvent extraction or solvent evaporation.

The properties of w/o/w microparticles (such as loading capacity, encapsulation efficiency, release kinetics, and particle size) can be difficult to control and depend on protein (type and concentration), polymer (composition, MW, and concentration), volume ratio between drug and polymer solutions, emulsification method (time and intensity), and surfactant (type and concentration).[44]

Although double emulsion method is considered as the most convenient for water soluble proteins in terms of protein stability and encapsulation efficiency, there are two main disadvantages of this method. First, it requires use of hydrophobic and halogenated solvents (i.e. dichloromethane) that are toxic and not easily removed completely.[45] Second, inadequate protein release is often observed that is characterized by an initial burst release phase followed by slow or incomplete release that does not match the polymer degradation or removal rate.[46] Additionally, acidic degradation products, such as lactic and glycolic acid are often produced during microsphere degradation and biomacromolecule release.[47]

Parameters for Characterization of Microspheres

Characterization of microspheres involves examination of several parameters such as microsphere size and size-distribution, drug loading amount (or capacity), encapsulation efficiency, type of release profile, sphere porosity, and intactness of drug encapsulated.

Below the major parameters are discussed.

Particle Size and Surface Morphology

Microsphere size and surface morphology, usually determined by Scanning Electron Microscopy (SEM), can be affected by the polymer concentration, temperature, viscosity, stirring rate and the amount of emulsifier employed.

In the w/o/w method, increasing the polymer concentration often results in increased sphere size.[48] Increase in the processing temperature results in the increase in the mean particle size with a broad size distribution.[49] Stirring rate and emulsifier type and concentration have also been shown to affect the microsphere size. As the stirring rate increased, the particle size was seen to decrease as increased stirring results in formation of finer emulsions.[50, 51, 52] In general, microspheres made by mechanical stirring or vortex mixing are larger than those made by sonication.[53,54] The presence of poly-vinyl alcohol (PVA) as an emulsifier in the external aqueous bath stabilizes double emulsion droplets against coalescence, leading to smaller microspheres.[37, 39]

The volume of aqueous, organic and polymer phases also affect the particle size. Because the increased volume of the internal phase generates greater resistance to mechanical breakdown during emulsification, the particle size increases accordingly.[55]

In o/w method, the emulsification power in generating secondary emulsion has a profound influence on the particle size and protein encapsulation efficiency. The higher the emulsification intensity, smaller particles with higher encapsulation efficiency is obtained.[56]

Encapsulation Efficiency

The Encapsulation Efficiency (EE) of microspheres is defined as:

$$\text{Encapsulation Efficiency (\%)} = \frac{\text{Weight of encapsulated protein}}{\text{Weight of total protein used}} \times 100$$

The multiple emulsion (w/o/w) method has been used to prepare microspheres with high EE. In general, it has been found that inclusion of a drug protecting agent and the balance of osmotic pressure between internal and external aqueous phases are the key to obtaining high encapsulation efficiency. Poor

encapsulation efficiency becomes a limiting factor for scale-up production of microparticulate formulations. Many factors affect the encapsulation efficiency. In w/o/w method, increasing the concentration of polymer in the organic phase has been shown to increase the encapsulation efficiency.[57] In the case of proteins, a study on encapsulation of two bovine serum albumins with different solubility showed that the higher water solubility of bovine serum albumin resulted in lower encapsulation efficiency due to the higher tendency to diffuse into the external aqueous phase during microsphere formation. The higher drug loading generates a higher concentration gradient between emulsion droplets and the continuous aqueous phase, resulting in the higher protein flux to the external water phase and lower encapsulation efficiency[58]. Incorporation of surfactant usually reduces the protein encapsulation efficiency. The osmotic pressure between the inner and outer water phases also affects the encapsulation efficiency. When salt is added into the external phase, a dense polymer film is generated around the microspheres to balance the osmotic pressure generated in the inner aqueous phase, and an increase in encapsulation efficiency occurs.

In o/w method, the higher drug loading results in lower encapsulation efficiency similar to w/o/w methods, due to more loss of the drug into the continuous phase.[59] Encapsulation efficiency, along with particle size, was also seen to be affected by the emulsification power used in generating the secondary emulsion. Higher emulsification intensity leads to smaller particles with higher encapsulation efficiency.

Factors Affecting Microspheres Properties

A range of production parameters influence the physicochemical parameters of the resulting microspheres. Critical formulation parameters for the W1/O/W2 preparation process are:

Mechanical stirring

When W1/O emulsion is prepared by vortex-mixing, the obtained microspheres are large, however, when by sonication is applied, a microfine and homogeneous emulsion is formed. The encapsulation efficiency was reported to increase with increasing mixing rate, whereas other authors found no relationship between these parameters.

Viscosity

The more viscous the polymer solution is, the more difficult it is to break it down into smaller droplets, which leads to larger microparticles. A highly viscous phase and low mixing intensity can be useful in the preparation of microparticles containing sensitive drugs. Increase in the W1/O viscosity is related to an increase in the encapsulation efficiency, but W1-phase with higher viscosity will permit the water pass into this phase resulting in swelling and releasing their content into the W2-phase.

Osmotic gradient

The W1 phase usually contains stabilizers (protein, surfactant). The semipermeable surfactant membrane allows some concentration difference, but once the maximum limit is reached (around 10% w/w), transfer of the water droplets through the oil phase will occur. When the W2-concentration is nil, water can penetrate into the W1-droplets, resulting increased PS and viscosity of W2 phase. When the W2-concentration is twice the W1-concentration, internal water will migrate (W1 to W2) resulting smaller droplets.

Volume of the phases

The volume of the *W1-phase* affects the solidification time, as it decreases, an increase in entrapment and a small decrease in particle size can be observed. Low *oil phase* volume yields a viscous and concentrated polymer solution, so it is more difficult for the oil phase to be broken into smaller droplets, which results in increased particle size and porous matrix. The increase in the *W2-phase* volume leads to an increase in both the particle size and entrapment which is related to the reduced mixing or dispersion efficiency during the second emulsification step due to the larger volume.

Type of organic solvent-cosolvent

The integrity of the forming microsphere wall is controlled by the rate of extraction of the organic solvent to the W2 phase and by the rate of its evaporation from the W2 phase. The rate of solvent extraction is limited by the water-solubility of the organic solvent used, while the evaporation rate depends on its boiling point.

When polar cosolvent is used in the organic polymer solution and is emulsified into the aqueous medium, at the water-organic interface, cosolvents with low affinity for the polymer are the first to diffuse out from the W1/O emulsion droplet (depending on their physicochemical properties) until it attains equilibrium with the W2-phase. Addition of a polar cosolvent and therefore fast partitioning and extraction can decrease the interfacial tension between the organic and aqueous phases, and form a dense wall, which can prevent the confluence of the aqueous phases, and ensure a low particle size and a dense microsphere structure with high encapsulation efficiency. Addition of a cosolvent can increase the porosity, leading to drug loss and therefore lower encapsulation efficiency.

Polar cosolvents may act in two opposite ways: (i) increasing the polymer precipitation rate and (ii) at the same time decreasing entrapment, due to the confluence of the aqueous phases; thus, there can be a sensitive balance between these effects.

Temperature

Below room temperature the diffusion and evaporation rate of solvents become slow. Above 30□C, it is easier for the droplets to collide with each other and they may coalesce together at the same time with solidification, since the viscosity of the oil medium is lower at higher temperature. When the solidifying microspheres are exposed to $T > Tg$ of polymer, it will change to its rubbery state which is more flexible and fluent, so the polymer can move through the matrix and fill gaps and coat the existing drug crystals, as in situ micro-coating.

Stabilizers

Addition of buffers (TRIS or PBS) *to the W1-phase* could promote an influx of water from the W2-phase due to a difference in osmotic pressure. The addition of salts *to the W2-phase* results in formation of a dense and homogenous polymer matrix, although they could reduce the solubility of organic solvents in water, resulting the precipitation of polymer.

Cumulative Drug Release and Release Profile

The knowledge of the BCS characteristics of a drug can also be utilized by the formulator to develop a more optimized dosage form based on fundamental mechanistic, rather than empirical information. The *in vitro* dissolution rates of the microparticles can be measured at defined rpm in 37±1□C buffer solution/deionised water mixture of defined pH according to the USP Drug Release Test 2 criteria. Dissolution in the GI tract takes place under heterogeneous conditions; this is one of the reasons why different *buffer solutions* (citrate, acetate, phosphate or other) are used, although most of them do not correspond to the physiological situation in the human GI-tract. The use of *surfactants* in the dissolution systems has physiological significance also as natural surfactants like bile salts (wetting, micellar solubilization, and/or deflocculation). The types of oral polymeric controlled release systems according to the drug release are: (i) diffusion-controlled systems (reservoir device-microcapsules; and matrix device microspheres); (ii) dissolution-controlled systems; (iii) erosion-controlled systems; (iv) swelling-controlled systems and hydrogels; (v) chemically controlled systems; (vi) constant or zero-order release; and (vii) other delivery systems.

Drug diffusion can occur: (i) through polymer matrix; (ii) through water-filled pores/cavities; or (iii) through both, in parallel and/or sequence.

The factors affecting the drug release rate revolve around the structure of matrix where the drug is contained and the chemical properties associated with both polymer and drug. Slower release can be achieved

by using a polymer with slow degradation kinetics, but polymer degradation is not the only mechanism for release. The drug release can also be diffusion-controlled if the drug can travel through the pores formed during microsphere hardening. Thus, the release profile is highly dependent on the pore status of microsphere.[60] Other factors that affect the release profiles include the polymer molecular weight, drug distribution, polymer blending, crystallinity, size distribution of the microspheres and the effect of the drug itself on polymeric microsphere.

Drug Release Mechanisms from Microspheres

The drug release kinetics is affected by the type of polymer used in microsphere fabrication and the way in which the polymer degrades. Depending on the rate of hydrolysis of their functional groups, polymers can be broadly classified into two types: bulk eroding and surface eroding. Microspheres formed from bulk-eroding polymers like PLGA, degrade throughout the microsphere matrix and the resulting monomers, oligomers and the drug diffuse out of the sphere into the surrounding medium. Such microspheres show either a biphasic or triphasic release profile characterized by an initial burst release. Burst release is thought to occur as a result of the drug located at or near the surface of sphere or in pores/voids connected to the surface. After the burst phase, a network of pores is formed in the microsphere, as the drug, monomers and oligomers diffuse out of the sphere into the surrounding medium. Subsequently, this network of water-filled pores increases in size until the sphere falls apart. The third phase of release may be seen if the drug that is remaining in the sphere that reaches this critical state, is released completely as a result of the collapse. In contrast, surface eroding polymers like polyanhydrides are composed of relatively hydrophobic monomers linked by labile bonds that can degrade quickly into oligomers and monomers at the polymer/water interface via hydrolysis. Thus, penetration of water into polymer bulk is resisted. Drug is released at the microsphere surface as the polymer breaks down around it and also typically to a lesser extent by diffusion through the polymer phase. In such microspheres, largest rate of drug release is expected at the beginning of degradation and as time goes, since the surface area of sphere decreases, the release rate is expected to decrease.

Thus, the release of encapsulated drugs can be controlled by polymer degradation under physiological conditions. The factors influencing this type of drug release mechanism are found to be varying copolymer ratio, crystallinity, polymer molecular weight, microsphere size, type and amount of excipient used and the interaction of drug itself with the polymeric microsphere.[61, 62]

Oral Colon Delivery of Insulin: Rationale

In the area of oral delivery, a growing attention has been focused over the past few decades on the design and manufacturing of advanced formulations intended for release of bioactive compounds to selected regions of the gastrointestinal (GI) tract. By controlling the site of drug liberation throughout the gut, it would be possible to limit the tolerability issues associated with treatments that mainly affect specific GI districts, enhance the bioavailability of drugs that show regional differences in their stability and/or permeability profiles or, alternatively, improve the therapeutic outcome in the management of widespread local pathologies (e.g. phlogosis, ulcers, microbial infections, motility disorders). In particular, colon delivery appears to be related to a range of either potential or fulfilled interesting applications [63]. Indeed, besides its long-lasting exploitation for the topical treatment of intestinal pathologic conditions, such as primarily IBD, it is extensively investigated as a means of achieving therapeutic levels of systemically-acting drugs in the general circulation. This specifically relates to the pursuit of an improved oral bioavailability for degradable and poorly permeable macromolecules of high current relevance and therapeutic value. Among them, insulin is perhaps the most prominent example.

While insulin has been available for ninety years, it is not yet able to combat the diabetes pandemic that has developed in this century. Considering a predicted doubling in diabetes-related deaths between 2005 and 2030 [64] and the fact that the economic burden of diabetes represents approximately 6% of the total health budget of developed countries,[65] it is surprising that insulin, the most effective diabetes treatment, has not gained widespread use. While insulin can be used alone in the therapy of diabetes without any oral antidiabetic drug, the reverse is not true.[66] Insulin therapy is commonly delayed despite the dire consequences, partly due to the inconvenience and complications associated with insulin administration by injection. Thus, in the last decade, the focus has shifted from the development of insulin alternatives to the development of alternative delivery methods.

Invasive parenteral (injected) insulin suffers from poor patient compliance due to needle phobia, pain, skin bulges, allergic reactions, common infections, and stress generated from the difficult long-term regimen of insulin therapy. [67, 68] Moreover, many patients still experience hypoglycemic episodes despite easier glucose monitoring options. Parenteral insulin is also associated with nonphysiological delivery to the wrong target tissues, poor pharmacodynamics, non-ideal initiation and weight gain. [69] Developments in biotechnology have led to the development of alternatives to parenteral delivery, although these developments have not been rapid enough to meet the pressing demand.

The goal of an alternative delivery route is to reach the bloodstream by noninvasive means, which is inaccessible for a protein drug due to the multiple physicochemical barriers, including those arising in the innate immune system. Scientists are trying to evade these barriers efficiently through ocular, vaginal, rectal, oral (buccal, gastro-intestinal, and sublingual), nasal, and other routes.[70, 71] The barriers to reaching the bloodstream are either physical, such as poor absorption at barrier surfaces, or chemical, such as pH inactivation and enzymatic degradation. Lassmann-Vague and Raccah reviewed the obstacles present for different delivery routes. Delivery of insulin via the ocular route was tested in animal models in combination with different absorption enhancers, with particular attention given to toxicity as polymers were added to overcome low absorption. Vaginal and rectal routes have also been investigated, but the absorption rate and bioavailability are poor due to the thick mucosal layers in these tissues. The use of absorption enhancers (bile salts, chelating agents, surfactants, cyclodextrins, and dihydrofusidate) does not help as they may cause local reactions with severe complications. Nasal delivery has also been evaluated because of the easy access, high vascularity and large absorption area associated with this route. Unfortunately, highly active mucociliary clearance in the nose hindered prolonged drug action resulting in poor bioavailability. Buccal and sublingual insulin administration provide better results due to the low levels of proteolytic enzyme activity, the high vascularization of the tissue, the large surface area for absorption and the ease of administration. However, the multiple layers of oral epithelial cells represent a significant barrier to drug penetration, which, coupled with the continuous flow of saliva, leads to poor efficacy.

Taking all of this into account, the oral route is considered to be the most feasible and convenient method of drug administration to improve compliance among diabetic patients. In addition to the large surface available for absorption, orally administered insulin can mimic the physiological fate of insulin in the body, providing better glucose homeostasis. [72, 73] Orally administered insulin is absorbed directly from the intestine and then transported to the liver via portal circulation, where it inhibits hepatic glucose production. Unlike other delivery routes, the gut is the natural route of nutrient absorption into the circulation. The fact that the gut presents the largest absorption surface of all routes provides better efficacy. However, the oral delivery of peptide drugs is hindered by the structural instability of proteins and peptide drugs in the harsh environment of the gut, i.e., the highly acidic environment in the stomach and the presence of proteolytic enzymes.[74] Both natural and synthetic polymers have been applied to the design of delivery vehicles capable of overcoming absorption barriers in the form of hydrogels, beads, microspheres, nanoparticles, and other formulations. Natural polymers such as agar, agarose, alginate, and chitosan and synthetic polymers including poly(lactic acid), poly(lactic-co-glycolic acid), poly(phosphoesters), and poly(C-caprolactone) have demonstrated efficacy as protein carriers.[75, 76, 77] Polymeric carrier systems for insulin delivery must be biodegradable, nontoxic and biocompatible, non-immunogenic, easy to synthesize and characterize, and preferably water soluble and inexpensive.[78] The polymer molecular weight, solubility, and structure also greatly influence drug delivery. Unless the protein is protected, the oral bioavailability of proteins is usually less than 1–2%[79] due to the action of digestive proteases in the stomach and intestine, the acidic pH of the stomach and the physical barrier of the mucus, glycocalyx and protease-containing microvilli.

In brief, effective delivery systems for oral protein delivery should fulfill the following criteria:

- The pH-sensitive behavior to protect the drug at the pH of the stomach and release it at intestinal pH.
- The release should be 'site specific', i.e., close to the absorption surface to avoid intestinal proteases.
- The selective and reversible opening of the tight junctions is preferable.
- The release should be controlled to achieve the physiological insulin concentration in blood.
- The drug delivery vehicle should be biocompatible.

To summarize, a multi-functional drug carrier is required to surmount the multiple hurdles between orally delivered insulin and the systemic circulation. Specific performance criteria need to be met to make oral insulin formulations successful. Insulin absorbed in the intestinal environment is usually transported to the portal circulation via the liver, which is responsible for controlling hepatic glucose production. [80] The rapid pre-systemic destruction of orally delivered insulin coupled with poor absorption in the intestinal region results in low bioactivity.

Ensuring adequate bioavailability of oral insulin, preserving its bioactivity, and maximizing the desired effects in the body are the most essential criteria for successful insulin delivery. To fulfill these criteria, the preliminary objective is the effective protection of insulin in the harsh acidic stomach environment. Controlled release of the drug requires a prolonged residence time in the intestinal milieu for better absorption, which is usually achieved by enhancing the permeability of the drug carrier though the mucosal epithelial layer of the intestine. Various strategies for improving drug absorption have been investigated, including the use of pH-responsive polymeric vehicles,[81, 82, 83] enzyme inhibitors,[84, 85] permeation enhancers, absorption enhancers[86] and the introduction of chemical modifications [87] to insulin. The polymers used in these vehicles must be biocompatible, biodegradable, nontoxic and provide significant bioadhesion to achieve the oral administration of insulin to the systemic circulation.

The time-dependent approach to colon delivery relies on relatively consistent small intestinal transit time (SITT) [88]. Indeed, SITT was assessed at 3±1 h (mean±SE) irrespective of diverse size and density properties of the tested units and fasted or fed condition of the healthy volunteers, when they are emptied into the duodenum. Thus, the release phase would be programmed to occur after the target site is reached and, provided that its timescale is consistent with colonic transit, could be completed before the device is cleared from the body.[89], [90]

Insulin and Diabetes Mellitus

Insulin is a polypeptide hormone (MW 5808 Da) formed from A (21 amino acids) and B (30 amino acids) chains interconnected by two disulfide bridges, which help stabilize its globular three-dimensional conformation with prevailing polar exterior and hidden hydrophobic residues. [91] A further disulfide bridge is present within A chain. Insulin shows a marked tendency to self-association into soluble dimers and hexamers. [92] The latter, consisting of three dimers arranged around two divalent zinc cations, are the form under which the hormone is stored in the body. Though biologically inactive, they are readily converted to monomers when the concentration diminishes. Insulin stability is chiefly impaired by the formation of desamido derivatives, via hydrolysis of the side-chain AsnA21 or AsnB3 amide group, and of a split product resulting from backbone cleavage at the A8–A9 peptide bond. [93] In addition, higher molecular weight transformation can take place giving rise to covalent dimers, oligomers and polymers.[94] The relative contribution of each of these degradation pathways depends on various parameters, such as the hormone concentration, pH, temperature and composition of the medium. Finally, non-covalent aggregation of partially unfolded insulin molecules into insoluble fibrils may also occur, especially upon exposure to hydrophobic surfaces. Insulin is secreted by β-cells of the pancreatic islets of Langerhans into the portal circulation mainly in response to surges in glycaemia. It plays a vital role in maintaining glucose homeostasis. [95, 96, 97] By inhibiting gluconeogenesis and increasing glycogen synthesis, it suppresses the hepatic production and output of glucose while promoting the relevant uptake and utilization in extra-hepatic tissues, particularly the muscular and adipose ones. As an anabolic hormone, it also participates in the regulation of protein and fat metabolism. In type 1 diabetes mellitus, endogenous insulin production is deficient because of destruction, mostly on an autoimmune basis, of β-cells in the endocrine pancreas.[98] On the other hand, insulin resistance is observed in type 2 diabetes, followed by a compensatory hypersecretion of the hormone that may result in progressive β-cell exhaustion.[99] The number of diabetics has exceeded 200 million people worldwide, with a marked growing incidence per year. Type 1 diabetes mellitus accounts for approximately 5 to 10% of all diagnosed cases. Insulin replacement still represents the mainstay of type 1 diabetes therapy. In addition, type 2 diabetic patients may eventually require exogenous insulin when oral hypoglycaemic medications combined with diet and exercises are no longer effective in controlling blood glucose levels.

Insulin pH-Dependent Delivery Systems

Early attempts at colonic delivery of insulin were not only based on microbially-triggered systems, but also on various pH-controlled approaches. Touitou and Rubinstein[100] reported on small soft gelatine capsules filled with an oily porcine insulin and surfactant (sodium laurate and cetyl alcohol) dispersion, coated with mixtures of acrylic polymers with enteric (Eudragit® L and Eudragit® S) and pH-independent (Eudragit® RS) solubility at differing ratios. Because of negligible *in vitro* release at pH < 7, two formulations were selected for an *in vivo* rat study, showing their potential for lowering the blood glucose concentration after an initial lag time of approximately 2 h. Surprisingly, post-treatment administration of a placebo capsule that only contained the emulsifying agents turned out to improve the extent and duration of the observed hypoglycaemia, whereas no improvement was attained by pre-treatment administration. Such a finding was attributed to differing absorption rates of the hormone and the employed enhancers, which would result in nonoverlapping timescales of intestinal permeation upon concomitant intake. In another attempt Agrawal et al. have demonstrated human insulin-loaded Eudragit S-100 microspheres containing protease inhibitor which possessed good encapsulation efficiency, pH dependant controlled release carrying encapsulated insulin to its optimum site of absorption. This ultimately resulted in enhanced insulin absorption and biological response [101]. A combination of acrylic polymers with pH-dependent and independent solubility (Eudragit® S and Eudragit® NE at 3:7 ratio) was also used to coat formaldehyde-treated hard-gelatin capsules filled with a gelled insulin microemulsion [102,103]. The composition of the coating mixture was set up to provide a slow pH-controlled release of the hormone throughout the intestinal transit. The capsules, further coated with cellulose acetate phthalate for gastroresistance, were orally administered to dogs exhibiting a significantly greater pharmacological availability in comparison with a non-encapsulated liquid insulin microemulsion and an insulin-free colon-targeted capsule. The pharmacological availability was calculated as the ratio between the area above the percent reduction in blood glucose concentration vs. time curves obtained on oral and parenteral dosing, respectively, then corrected for strength and body weight. The inclusion of aprotinin, yet not of sodium lauryl sulfate, in the insulin capsule resulted in an additional improvement of the hypoglycaemic response. However, injected into isolated GI segments of rats, the microemulsion was able to lower blood glucose levels when delivered to the small bowel only. It was suggested that lipase and bile salts would play a pivotal role on the intestinal absorption of drugs conveyed in microemulsions. In a different instance, hard-gelatin capsules entirely coated with a methacrylic acid copolymer soluble at pH>7, formerly demonstrated to disintegrate in the caecum by an imaging investigation, were proposed as colon delivery carriers for bovine insulin associated with sodium taurocholate. [104] Elevated insulinaemia was revealed 4–5 h after intake by three healthy volunteers along with a concurrent decline in the C-peptide concentration, which pointed out the exogenous origin of the assayed hormone. Analogous recombinant human insulin capsules, administered intracaecally to diabetic pigs in the presence of aprotinin and 5-methoxysalicylic acid or sodium lauryl sulfate, yielded a rise in plasma insulin levels and a corresponding decrease in the glucose ones.[105, 106] The effects appeared later and were less pronounced in animals with streptozotocin-induced chronic diabetes than in others with acute illness caused by pancreatectomy. These differences were interpreted by a suppressed secretion of glucagon in pancreatectomized pigs. In the pharmacological chronic diabetes model, an eight-day treatment based on the enteric-coated capsules was notably effective in controlling glycaemia with no need for injective insulin supplementation. In addition, no significant differences were found in the serum glucose concentration following oral and subcutaneous once-daily administration regimens. However, chemical stability issues were anticipated through preliminary studies carried out during the first 6 months of storage.

Hard-gelatin capsules coated with Eudragit® S soluble at pH>7 were also used to convey to the large bowel porcine insulin-containing lipid microspheres prepared by the spinning disk method. [107] On the basis of a previous in situ investigation, which indicated that sodium glycocholate and disodium EDTA could enhance the colonic absorption of insulin, such adjuvants were incorporated into the contents of the capsule. Carbopol® 934, a mucoadhesive cross-linked polyacrylate, was added to extend the contact of drug molecules with the intestinal epithelium. Administered perorally to two non-diabetic dogs, the resulting device elicited a modest though prolonged reduction in the blood glucose concentration. Nonetheless, the significance of this finding might be impaired by the limited number of animals involved in the study, the lack of statistical analysis of data and failure to highlight the relevant variability. Alternatively, Eudragit® S was directly applied to bovine or human insulin-loaded tricalcium phosphate porous microspheres. [108] The use of a ceramic material as an insulin matrix carrier was suggested on the basis of inherent biocompatibility and resorbability characteristics that are well-established in the biomedical area. In vitro studies pointed out a slow release at pH 6.8, which was accelerated when the pH was switched to 7.4. Preserved conformational stability and lack of aggregation

of human insulin as retrieved from pH 6.8 medium samples were assessed by circular dichroism and dynamic light scattering. After oral administration to streptozotocin-induced diabetic rats, enteric-coated microspheres containing either human or porcine insulin gave rise to a fall in the blood glucose concentration of up to 50% of the initial value. A dose-dependency was observed in the case of porcine insulin. When human insulin was dealt with, the hypoglycaemic effect was delayed.

There has been a long history of research directed toward the development of novel routes of insulin delivery since recombinant DNA technology made insulin available at a reasonable cost in an injectable dosage form. Needle phobia and stress have encouraged scientists to investigate and exploit all promising routes of insulin delivery, ranging from oral to rectal, with a wide variety of devices and delivery systems. Many approaches have been used to study various strategies to overcome the inherent barriers to insulin uptake across the GIT and by transmucosal and transdermal routes. Each of the various routes of insulin administration has its own set of favorable and unfavorable properties. Most of the approaches represent long-term possibilities for insulin delivery, but difficulties in achieving adequate blood insulin concentrations are yet to be overcome. Over the last several decades, these formidable tasks have focused on oral insulin delivery. Our final achievement will be clinically therapeutic bioavailable oral insulin that bypasses the obstacles of the GIT and overcomes the challenges inherent in the physicochemical properties of the insulin molecule. In recent years, the development of innovative oral insulin delivery carriers that improve oral insulin absorption has thrown some promising light on the new horizon of oral insulin therapy. Although extensive human clinical studies are still required, especially of long-term clinical applications, researchers in academic institutions and several drug delivery pharmaceutical companies are actively involved in the development of an oral insulin delivery system. The new millennium promises a revolutionary change in the delivery of insulin for billions of sufferers who are currently reliant on subcutaneous administration.

Conflict of Interests

The authors of the paper have no direct financial relationship with any commercial identity mentioned in this paper that could lead to a conflict of interest for any of the authors.

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