

Insulin Delivery Systems for Controlling Diabetes

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Abstract: The goal of all drug delivery systems is to deploy medications intact to specifically targeted parts of the body through a medium that can control the therapy's administration by means of either a physiological or chemical trigger. The polypeptide insulin is the primary hormone responsible for controlling the transport, utilization and storage of glucose in the body. Due to the inconvenience of insulin injections, various approaches have been attempted to formulate insulin for administration by non-injectable routes. Different approaches to deliver insulin including transdermal, transmucosal, pulmonary route using dry aerosols and inhalers, smart hydrogels, nasal delivery, oral delivery, and treatment of diabetes with synthetic beta cells, has resulted in recent developments in treatment of diabetes. Among the latest patent approaches are delivering into the subject a genetic construct comprising a coding sequence for a human proinsulin operably connected a promoter functional in the host cells. Polypeptides having activity of human neurogenin3 (hNgn3), and nucleic acid encoding such polypeptide are among the other inventions that use of islet transcription factors such as hNgn3 to facilitate production of pancreatic islet cells from progenitor cells, and to facilitate insulin delivery by production of islet cells so produced.

Keywords: Insulin, Hydrogels, Nasal delivery, PEG-insulin, Blood glucose level, Aerosols, Transdermal.

INTRODUCTION

Diabetes mellitus, the most commonly encountered endocrinopathy, continues to increase dramatically in prevalence. Diabetes is the sixth most common cause of death in the United States and significantly affects other more common causes of death such as cardiac disease and stroke.

Diabetes mellitus (commonly referred to simply as diabetes) is a disease characterized by dysregulation of metabolism, particularly glucose metabolism. In normal individuals, a rise in blood glucose levels (such as that which occurs immediately following eating) triggers the islet beta cells of the pancreas to secrete insulin, a peptide hormone, into the bloodstream. The insulin binds to insulin receptors located on a number of cell types, notably muscle cells, and thereby signals the cells to increase the rate of glucose uptake into the cells. As the blood glucose returns to normal pre-prandial levels, the amount of insulin in the blood also drops. In the absence of insulin, blood glucose levels would rise to dangerously high levels (a condition termed hyperglycemia), possibly resulting in death. Too much insulin causes abnormally low blood glucose levels (hypoglycemia), which is also dangerous and possibly fatal. In a normal individual, built-in feedback loops regulating the secretion of insulin and its clearance from the systemic circulation prevent both hyperglycemic and hypoglycemic conditions from occurring.

Type I diabetes, or insulin-dependent diabetes mellitus (IDDM), usually begins in childhood. It is a disease affecting approximately one in 250 individuals in the United States. Type I diabetes is characterized by atrophy of the pancreatic

beta cells, resulting in a decrease or cessation of insulin production, and leaving the patient dependent on exogenous insulin for survival.

Far more common is Type II diabetes, or non-insulin-dependent diabetes mellitus (NIDDM), which generally occurs in patients older than 40 years. These patients may, at least initially, have normal or even high levels of insulin in their blood, but exhibit an abnormally low rate of cellular uptake of glucose in response to insulin. Although Type II diabetes often can be treated by controlling the patient's diet, administration of exogenous insulin to supplement that secreted by the patient's beta cells may also prove necessary.

Ideally, exogenous insulin would be administered at times and in doses that would yield a plasma profile which mimics the natural plasma profile of endogenously secreted insulin in a normal individual, thereby avoiding both hyperglycemic and hypoglycemic states. Insulin is typically administered at set times (e.g., before meals and/or bedtime), or, if blood glucose is monitored, whenever the patient's blood glucose level appears high. The standard method of administration is by subcutaneous injection of a saline solution of insulin, usually by the patient him/herself. This method deposits a reservoir of the insulin-containing solution under the patient's skin, and permits gradual absorption of the solution into the bloodstream via the dermal capillaries. Insulin formulated for s.c. injection reaches its maximum activity at 2 to 3 hours following injection; duration of effect is said to be 6 to 8 hours. Certain slow-acting formulations of insulin (e.g., Lente insulin) show an even more prolonged effect.

The polypeptide insulin is the primary hormone responsible for controlling the transport, utilization and storage of glucose in the body. The beta-cells of the pancreatic islets secrete a single chain precursor of insulin, known as proinsulin. Proteolysis of proinsulin results in removal of

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certain basic amino acids in the proinsulin chain and the connecting or C-peptide and provides the biologically active polypeptide insulin.

The insulin molecule has been highly conserved in evolution and generally consists of two chains of amino acids linked by disulfide bonds. In the natural human, two-chain insulin molecule (mw 5,800 Daltons), the A-chain is composed of 21 amino acid residues and has glycine at the amino terminus; and the B-chain has 30 amino acid residues and phenylalanine at the amino terminus.

Insulin may exist as a monomer or may aggregate into a dimer or a hexamer formed from three of the dimers. Biological activity, i.e., the ability to bind to receptors and stimulate the biological actions of insulin, resides in the monomer.

Treatment of diabetes typically requires regular injections of insulin. Due to the inconvenience of insulin injections, various approaches have been attempted to formulate insulin for administration by non-injectable routes.

For example Kitao *et al.* [1] proposes pharmaceutical compositions for rectal administration of insulin. The pharmaceutical compositions include insulin and fatty acids having 8 to 14 carbon atoms and nontoxic salts thereof.

Kidron *et al.* [2] proposes pharmaceutical compositions for the oral administration of insulin. The pharmaceutical compositions include insulin, a bile acid or alkali metal salt thereof, the bile acid being selected from the group consisting of cholic acid, chenodeoxycholic acid, taurocholic acid, taurochenodeoxycholic acid, glycocholic acid, glycochenocholic acid, 3.beta.-hydroxy-12-ketocholic acid, 12.alpha.-3.beta.-dihydrocholic acid, and ursodesoxycholic acid, and a protease inhibitor. The composition is provided with an enterocoating to assure passage through the stomach and release in the intestine.

Chiou [3] proposes compositions for systemic delivery of insulin through the eyes where the drug passes into the nasolacrimal duct and becomes absorbed into circulation. The composition includes insulin and an enhancing agent. The enhancing agents proposed include, either alone or in combination, surfactants such as polyoxyethylene ethers of fatty acids and bile salts and acids such as cholic acid, deoxycholic acid, glycocholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium cholate, sodium glycocholate, glycocholate, sodium deoxycholate, sodium taurodeoxycholate, chenodeoxycholic acid, and ursodeoxycholic acid. The enhancer is present in a concentration ranging from 0.1% to 5% (w/v).

Backstrom *et al.* [4] proposes a therapeutic preparation for inhalation that includes insulin and a substance, which enhances the absorption of insulin in the lower respiratory tract. The enhancer is preferably a sodium salt of a saturated fatty acid of carbon chain length 10 (i.e., sodium caprate), 12 (sodium laurate), or 14 (sodium myristate). Potassium and lysine salts of capric acid are also proposed. Backstrom *et al.* note that if the carbon chain length is shorter than about 10, the surface activity of the surfactant may be too low, and if the chain length is longer than about 14, decreased solubility of the fatty acid in water limits its usefulness. As an alternative to the proposed fatty acid enhancers, Backstrom

et al. propose the use of the following bile salts-sodium ursodeoxycholate, sodium taurocholate, sodium glycocholate, and sodium taurodihydrofusidate.

Enteric-coated compositions for oral administration of insulin are newly proposed [5]. The composition includes insulin, a bile salt or bile acid, and carbonate or bicarbonate ions, which are used to adjust the pH of the gut to a pH of from 7.5 to 9. Watts *et al.* [6] proposes drug delivery compositions for colonic delivery of insulin. The drug delivery compositions include insulin, an absorption promoter which (a) includes a mixture of fatty acids having 6 to 16 carbon atoms or a salt thereof and a dispersing agent, or (b) comprises a mixture of mono/diglycerides of medium chain fatty acids and a dispersing agent, and a coating to prevent the release of the insulin and absorption promoter until the tablet, capsule or pellet reaches the proximal colon. It is desirable to provide pharmaceutical compositions for administration of insulin that may provide improved bioavailability when compared to the conventional compositions described above.

NOVEL DRUG DELIVERY SYSTEMS FOR INSULIN

Biologically active agents such as nutritional supplements, hormones, and a variety of pharmaceutical preparations, which will generally be referred to as "drugs" are typically provided in oral (liquids or solids) or injectable dosage formulations, however there are many disadvantages associated with this type of administration. Many of the ingredients are degraded within the gastrointestinal (GI) tract or undergo first-pass metabolism in the liver. In addition, there exists a segment of the population who experience difficulty in swallowing pills or are unable to tolerate any solids. During the past three decades, however, formulations that control the rate and period of drug delivery (e.g., time-release medications) and target specific areas of the body for treatment have become increasingly common and complex. Some have provided solutions to the problem of administering different types of drugs but there are still a large number of medications that do not achieve maximum pharmaceutical effect because they do not reach the intended tissue targets either fast enough or in high enough concentrations.

The potency and therapeutic effects of many drugs are limited or reduced because of the partial degradation that occurs before they reach a desired target in the body. Further, injectable medications could be made less expensively and administered more easily if they could simply be dosed by other routes such as the oral mucosa, the pulmonary mucosa or through the vaginal and intestinal tract. However, this improvement cannot happen until methods are developed to safely shepherd drugs through these specific areas of the body, where different physiological environments (e.g. low pH values in the stomach) can destroy a medication or where absorption is not rapid or complete, or through an area where healthy tissue might be adversely affected. The goal of all drug delivery systems is to deploy medications intact to specifically targeted parts of the body through a medium that can control the therapy's administration by means of either a physiological or chemical trigger.

To achieve this goal, a number of researchers have turned to advances in micro and nanotechnology. One prominent area of endeavor is the production of so-called "nanoparticles" which act as chemical or physical "carriers" of drugs.

Although these approaches are the focus of intense research, other processes are also under consideration, including aerosol inhalation devices, transdermal methodologies, forced-pressure injectables, and biodegradable polymer networks designed specifically to transport new gene therapies.

1. BUCCAL DELIVERY

Transmucosal routes of drug delivery offer distinct advantages. Of the various routes, the mucosal linings of the nasal passages and the oral cavity are the most attractive. Although the nasal route has reached commercial success with several drugs, such as with allergy medications, potentially serious side-effects, such as irritation and possibly irreversible damage to the ciliary action of the nasal cavity from chronic application, have deterred health professionals from recommending their long-term use. Within the oral cavity, there are three generally recognized routes of administration of a biologically active agent. Local delivery is mainly limited to applications regarding disruptions occurring within the oral cavity itself, such as a canker sore. Sublingual delivery is achieved through the mucosal membranes lining the floor of the mouth. This route provides rapid absorption and has reached commercial status with biologically active agents such as nitroglycerin, which is placed under the tongue. Because of the high permeability and the rich blood supply, transport via the sublingual route results in a rapid onset of action, providing a delivery route appropriate for highly permeable drugs with short delivery period requirements and an infrequent dosing regimen. The negative however, is that it produces a saliva wash (swallowing) and in the case of nitroinqual it has been found to cause headaches as a result of administering excess of the drug needed to accomplish its task. The third generally recognized route is the buccal mucosa. This area encompasses the mucosal membranes of the inner lining of the cheeks. This area also has a rich blood supply, is robust, and provides a short cellular recovery time following stress or damage. Although the buccal mucosa is less permeable than the sublingual area, the expanse of smooth and relatively immobile mucosa provide a highly desirably absorption pathway for sustained-release and controlled-release delivery of biologically active agents. As with other transmucosal routes of administration, two major advantages include avoiding hepatic first-pass metabolism and pre-systemic elimination within the GI tract.

One of the major disadvantages associated with buccal mucosa delivery of a biologically active agent has been the relatively low passage of active agents across the mucosal epithelium, thereby resulting in low agent bioavailability, which translates into a substantial loss usable active agent within each dosage. Various permeation and absorption enhancers such as polysorbate-80, sorbitol, and phosphatidylcholine have been explored to improve buccal penetration. Studies have indicated that the superficial layers and protein domain of the epithelium may be responsible for maintaining the barrier function of the buccal mucosa [7].

Additionally, it is known that use of a permeation enhancer can increase the passage of a biomolecule. Furthermore, studies have suggested the feasibility of buccal delivery of even a rather large molecular weight pharmaceutical [8]. A further area of investigation includes the use of bioadhesive polymers in buccal delivery systems. Bioadhesive polymers have been developed to adhere to a biological substrate in order to maintain continual contact of an agent with the site of delivery. This process has been termed mucoadhesion when the substrate is mucosal tissue [9].

Prior artisans have explored a variety of avenues in an effort to produce a viable and efficient means for buccal mucosal delivery. Such avenues include the use of liposomal carriers to enhance uptake or facilitate the delivery of a product; decreasing the particle size of microspherical carriers, or employing a physical matrix, such as a sponge, to hold a medicinal product at the buccal area.

What is lacking in the art is a method for increasing the bioavailability of a biologically active agent, which may be administered via various routes, but particularly with regard to administration via the buccal mucosa; and a stable product useful for carrying out the method.

2. HYDROGELS

During the past decade, novel polymeric microspheres, polymer micelles, and hydrogel-type materials have been shown to be effective in enhancing drug targeting specificity, lowering systemic drug toxicity, improving treatment absorption rates, and providing protection for pharmaceuticals against biochemical degradation. These are all goals of drug delivery. In addition, several other experimental drug delivery systems show signs of promise, including those composed of biodegradable polymers, dendrimers (so-called star polymers), electroactive polymers, and modified C-60 fullerenes (also known as "buckyballs").

Polymer drug delivery systems are based on "carriers" which are composed of mixing polymeric chemical compounds with drugs to form complex, large molecules, which "carry" the drug across physiological barriers.

Illustrative examples of these polymeric compounds are poly(ethylene-glycol)-poly(alpha, beta-aspartic acid), carboxylates, and heterobifunctional polyethylene glycol, in addition to others.

Another type of nanotechnology revolves around the use of "hydrogels" as carriers of drugs. The principle behind this technology is to use a chemical compound which traps a drug and then releases the active compound by "swelling" or expanding inside of specific tissues, thus allowing a higher concentration of the drug in a biodegradable format. Hydrogels are very specialized systems and are generally formulated to meet specific needs for the delivery of individual drugs.

During the past two decades, research into hydrogel delivery systems has focused primarily on systems containing polyacrylic acid (PAA) backbones. PAA hydrogels are known for their super-absorbency and ability to form extended polymer networks through hydrogen bonding. In addition, they are excellent bioadhesives, which means that they can adhere to mucosal linings within the gastrointestinal

tract for extended periods, releasing their encapsulated medications slowly over time.

One example of the complexity of these systems is a glucose-sensitive hydrogel that could be used to deliver insulin to diabetic patients using an internal pH trigger. This system features an insulin-containing "reservoir" formed by a poly [methacrylic acid-g-poly(ethylene glycol)]hydrogel membrane into which glucose oxidase has been immobilized. The membrane itself is housed between nonswelling, porous "molecular fences".

The current growth of hydrogel applications in drug delivery and biosensors is ascribed in part to the biocompatibility of hydrogels, and in part to fast and reversible volume changes in response to external stimuli such as temperature, pH, electric and magnetic fields, or analyte concentration. Thus these hydrogels are sometimes called "stimulus responsive polymers" [10].

One approach is to use pH sensitivity to mediate changes in swelling. A pH-sensitive hydrogel undergoes very large and reversible volume changes in response to pH changes within the hydrogel. Two main types of pH-sensitive hydrogels are acidic hydrogels and basic hydrogels. Acidic hydrogels by definition will be ionized and hence swollen at high pH, and uncharged and unswollen at low pH [11,12]. Swelling behavior of a basic hydrogel has the opposite dependence on pH. The pH sensitivity is caused by pendant acidic and basic groups such as carboxylic acids, sulfonic acids, primary amines, and quaternary ammonium salts [11-13]. Carboxylic acid groups for example are charged at high pH and uncharged at low pH, whereas the reverse is true for primary amine groups and quaternary ammonium salts.

In a biosensor, the swelling and shrinking of the hydrogel is usually made to be responsive to changes in the level of a biological indicator or molecule of interest. This is generally achieved by incorporating into the hydrogel an enzyme, receptor, antibody, or other agent which binds the molecule of interest. Oxidoreductase enzymes are one category of such agents, which find particular use in biosensors. The characteristics of oxidoreductase enzymes of particular value in sensor applications is the production of oxygen by the enzyme reaction.

Among the oxidoreductase currently being investigated for use in biosensors are glucose oxidase (for sensing blood sugar levels), cholesterase (for sensing cholesterol levels), alcohol dehydrogenase (for sensing alcohol levels), and penicillinase (for sensing penicillin levels). Besides those named here, there are over 100 known oxidoreductase enzymes, and at least some of these are likely to find future use in biosensors.

A pH-sensitive hydrogel containing glucose oxidase (GOx) enzyme is called a glucose-sensitive hydrogel (GSH) due to its responsiveness to environmental glucose concentrations. Thermally stable GOx is a flavin-containing glycoprotein which catalyzes a reaction which is very specific for glucose, and which produces gluconic acid and hydrogen peroxide in the presence of glucose and oxygen as shown below. Therefore, increases in the environmental glucose concentration lower the pH value within the GSH.

Several attempts have been made to utilize this catalytic reaction in glucose biosensors [14-17]. Glucose biosensors based on amperometric methods are the most highly developed. In the amperometric method, an electrode is used which produces a current proportional to the diffusional flux of hydrogen peroxide to the electrode surface, or, alternatively, proportional to the diffusional flux of oxygen to the electrode surface [14-17]. At steady state, the diffusional flux of hydrogen peroxide to the electrode surface equals the rate at which hydrogen peroxide is produced by the GOx reaction in the hydrogel adjacent to the electrode. However, unlike the hydrogels considered here, the hydrogels in amperometric glucose biosensors do not swell in response to pH changes.

An important physical property of pH-sensitive GSHs is the ability to change volume in response to changes in environmental glucose concentrations, due to changes in pH within the hydrogel caused by the reaction of the GOx enzyme. This physical phenomenon has been applied in insulin delivery devices to control insulin permeability through GSHs [18-21].

pH-Sensitive glucose hydrogels are useful in devices using either amperometric means or pressure transducers to detect glucose concentrations. For such applications, two major problems with the GOx enzymatic process have been identified: insufficient oxygen supply for the reaction, and the decay of the GOx activity with time due to peroxide-induced degradation [15-17, 22, 23].

In summary, for both insulin delivery devices and glucose biosensors, GOx stability is essential for long term use *in vivo*. For insulin delivery devices and the pressure-based glucose biosensors, a rapid swelling kinetic is also important, to provide the best performance. The use of hydrogels containing oxidoreductase enzymes in biosensors and controlled drug delivery systems, and more particularly to the inclusion of catalase in such biosensors and drug delivery systems has been reported [24]. The invention comprises a hydrogels containing an analyte-sensitive enzyme which generates hydrogen peroxide, co-immobilized with catalase, with the catalase being present in amounts ranging from about 100 units/ml to about 1000 units/ml. The term "hydrogel" is intended to encompass any polymer matrix suitable for use in hydrated conditions. In one embodiment, the analyte is glucose and the analyte-sensitive enzyme is glucose oxidase. In addition to glucose oxidase, the invention is applicable any analyte-sensitive enzyme which generates hydrogen peroxide as part of the reaction. These include monoamine oxidase as well as many oxidoreductases. The invention further encompasses biosensors incorporating these hydrogels. The hydrogels may preferably be formulated such that swelling of the gel permits flow of a drug such as insulin out of the gel. Thus, in a further embodiment the invention encompasses analyte-responsive drug delivery devices containing hydrogels which meet the above description. The hydrogels may be used with biosensors or drug-delivery devices which use pressure transducers or amperometric means to register analyte concentration. Hydrogels according the invention may also be used with devices employing gas reservoirs or semi-permeable membranes. The invention further includes

methods for using catalase in hydrogels, biosensors and analyte-responsive drug delivery devices [24].

Drug delivery has been a subject of intense studies over recent years. The goal is to achieve sustained (or slow) and/or controlled drug release and thereby improve efficacy, safety, and/or patient comfort. A sustained and/or controlled release of the drug agents is achieved by the retardation of drug diffusion by and/or gradual disintegration of the polymer matrix following application.

In-situ gelation is a process of gel formation at the site of application after the composition or formulation has been applied to the site. In the field of human and animal medicine, the sites of application refers to various injection sites, topical application sites, surgical sites, and others where the agents are brought into contact with tissues or body fluids. As a drug delivery agent, the *in-situ* gel has an advantage related to the gel or polymer network being formed *in-situ* providing sustained release of the drug agent. At the same time, it permits the drug to be delivered in a liquid form. The *in-situ* gelation compositions using ionic polysaccharides have been reported [25], which consist of a drug, a polymer and a gel forming ionic polysaccharide which consist of two components, an ionic polysaccharide and a cross-linking ion capable of cross-linking the former. The *in-situ* gel formation is induced by the application of the cross-linking ions.

Thus, a great need exists for a simpler and more efficient *in-situ* gelling composition that employs only a low polymer concentration for the purposes of drug delivery. Pectin is a biodegradable acidic carbohydrate polymer. Pectin is commonly found in plant cell walls. The cell wall of a plant is divided into three layers consisting of the middle lamella, the primary wall and the secondary cell wall. The middle lamella is richest in pectin. The chemistry and biology of pectin have been extensively reviewed [26-28].

Current commercial pectins are mainly from citrus and apples. However, besides citrus and apples, pectins can also be isolated from many other plants. All vegetables and fruits that have been examined contain pectins. Pectins from sugar beets, sunflowers, potatoes, and grapefruits are just a few other well known examples. A pectic substance to provide a biodegradable *in-situ* gelling composition for animal and human use was prepared. The composition transformed from a liquid into a gel following administration to the target site. Preferably the pectic substance was *Aloe* pectin. This composition could control, or sustain, the release of a physiologically active agent in the body of an animal. It provided a transparent polymer solution wherein no dramatic increase in gel cloudiness was created beyond certain concentration ranges. Preferably the composition was capable of creating an *in-situ* gel at low concentrations. The polymer solution was transparent wherein a thickener is added. Preferably the composition is capable of creating an *in-situ* gel at low concentrations to be delivered in the liquid form and provide a composition for drug delivery a therapeutic or diagnostic agent incorporated into the formulation or composition. These agents can be small molecules as well as large ones such as proteins like insulin. Preferably the composition is capable of forming an *in-situ* gel at low concentrations [29].

Two classes of polymers that are currently receiving widespread attention in biosensor development are hydrogels and conducting electroactive polymers. The integration of two materials to produce electroactive hydrogel composites are reported that physically entrap enzymes within their matrices for biosensor construction and chemically stimulated controlled release [30]. Enhanced biosensing capabilities of these membranes have been demonstrated in the fabrication of glucose, cholesterol and galactose amperometric biosensors. All biosensors displayed extended linear response ranges (10^{-5} - 10^{-2} M), rapid response times (<60 s), retained storage stabilities of up to 1 year, and excellent screening of the physiological interferents ascorbic acid, uric acid, and acetaminophen. When the cross-linked hydrogel components of these composite membranes were prepared with the amine containing dimethylaminoethyl methacrylate monomer the result was polymeric devices that swelled in response to pH changes (neutral to acidic). Entrapment of glucose oxidase within these materials made them glucose-responsive through the formation of gluconic acid. When insulin was co-loaded with glucose oxidase into these "bio-smart" devices, there was a twofold increase in insulin release rate when the devices were immersed in glucose solutions. This demonstrates the potential of such systems to function as a chemically-synthesized artificial pancreas [30].

Glucose-sensitive hydrogel membranes have been synthesized and characterized for their rate-of-delivery of macromolecules. The mechanism for changing this rate is based on variable displacement of the affinity interaction between dextran and concanavalin A (con A). Membranes were constructed from crosslinked dextrans to which con A was coupled via a spacer arm. Changes in the porosity of the resulting hydrogel in the presence of glucose led to changes in the diffusion rate observed for a range of proteins. Gels of specified thickness were cast around to nylon gauze support (pore size, 0.1 mm) to improve mechanical strength. Diffusion of proteins through the gel membrane was determined using a twin-chamber diffusion cell with the concentrations being continuously monitored using a UV-spectrophotometer. Changes in the transport properties of the membranes in response to glucose were explored and it was found that, while 0.1M D-glucose caused a substantial, but saturable, increase in the rates of diffusion of both insulin and lysozyme, controls using glycerol or L-glucose (0.1M) had no significant effect. Sequential addition and removal of external glucose in a stepwise manner showed that permeability changes were reversible. As expected, diffusion rates were inversely proportional to membrane thickness. A maximum increase in permeability was observed at pH 7.4 and at 37 degrees C. The results demonstrate that this hydrogel membrane functions as a smart material allowing control of solute delivery in response to specific changes in its external environment [31].

A novel UV polymerised glucose-responsive mixture containing concanavalin A (con A) and dextran was synthesised and characterised as a "smart" biomaterial to form the basis of a closed-loop delivery device. Dextran and con A precursors were modified with acrylic side groups and then UV polymerised to produce covalently bonded mixtures which were examined by FTIR. The viscoelastic properties of these polymerised mixtures containing glucose concen-

trations between 0% and 5% w/w were also examined using oscillatory rheometry within the linear viscoelastic range across a frequency range of 0.01-50 Hz. As the formulation glucose concentration was raised, a graded decrease in storage modulus, loss modulus and complex viscosity when compared at 1 Hz was observed. Increasing the mixture irradiation time produced viscosity profiles at higher values throughout the glucose concentration range. The subsequent testing of such formulations in *in vitro* diffusion experiments revealed that the leaching of the mixture components is formulation dependent and is restricted significantly in the covalently bonded mixtures. Insulin delivery in response to glucose in the physiologically relevant glucose concentration range was demonstrated using the novel polymerised mixture at 37 degrees C. The performance of this covalently cross-linked glucose-responsive biomaterial has been improved in terms of increased mixture stability with reduced component leaching. This could, therefore be used as the basis of the design of a closed-loop drug delivery device for therapeutic agents used for the management of diabetes mellitus [32].

3. DRY POWDER INHALERS

A dry powder compositions comprising a pharmacologically active polypeptide and a surfactant, wherein at least 50% of the total mass of the polypeptide and the surfactant consists of primary particles having a diameter less than 10 microns. The compositions are suitable for inhalation from a dry powder inhaler device. It has been found that when a peptide or protein (hereinafter collectively referred to as polypeptides) is combined with an appropriate absorption enhancer and is introduced into the lung in the form of a powder of appropriate particle size, it readily enters the pulmonary circulation by absorption through the layer of epithelial cells in the lower respiratory tract. This is conveniently accomplished by inhalation of the powder from an inhaler device, which dispenses the correct dose of powdered polypeptide/enhancer in a particle size which maximizes deposition in the lower respiratory tract, as opposed to the mouth and throat. (For ease of reference, the polypeptide and enhancer are hereinafter collectively referred to as the "active compounds"). To accomplish this preferential delivery into the lung, as much as possible of the active compounds should consist of particles having a diameter less than approximately 10 μm (e.g., between 0.01-10 μm , and ideally between 1-6 μm). In preferred embodiments, at least 50% (preferably at least 60%, more preferably at least 70%, still more preferably at least 80%, and most preferably at least 90%) of the total mass of active compounds, which exits the inhaler device consists of particles within the desired diameter range. A pharmaceutical composition was prepared containing a mixture of active compounds (A) a pharmacologically active polypeptide and (B) an enhancer compound which enhances the systemic absorption of the polypeptide in the lower respiratory system (preferably the lungs) of a patient, the mixture being in the form of a dry powder suitable for inhalation, in which at least 50% of the total mass of active compounds (A) and (B) consists of primary particles having a diameter less than or equal to about 10 microns. The primary particles may be packaged as such, or may optionally be formed into agglomerates, which then are substantially deagglomerated prior to entry into the respiratory tract of the patient. The

composition may of course contain other ingredients as needed, including other pharmaceutically active agents, other enhancers, and pharmacologically acceptable excipients such as diluents or carriers. Therefore, the therapeutic preparation of the present invention may contain only the said active compounds or it may contain other substances, such as a pharmaceutically acceptable carrier. This carrier may largely consist of-particles having a diameter of less than about 10 microns so that at least 50% of the resultant powder as a whole consists of optionally agglomerated primary particles having a diameter of less than about 10 microns; alternatively the carrier may largely consist of much bigger particles ("coarse particles"), so that an "ordered mixture" may be formed between the active compounds and the said carrier. In an ordered mixture, alternatively known as an interactive or adhesive mixture, fine drug particles (in this invention, the active compounds) are fairly evenly distributed over the surface of coarse excipient particles (in this invention, the pharmaceutically acceptable carrier). Preferably in such case the active compounds are not in the form of agglomerates prior to formation of the ordered mixture. The coarse particles may have a diameter of over 20 microns, such as over 60 microns. Above these lower limits, the diameter of the coarse particles is not of critical importance so various coarse particle sizes may be used, if desired according to the practical requirements of the particular formulation. There is no requirement for the coarse particles in the ordered mixture to be of the same size, but the coarse particles may advantageously be of similar size within the ordered mixture. Preferably, the coarse particles have a diameter of 60-800 microns. The invention also includes processes for the manufacture of a pharmaceutical composition suitable for administration by inhalation. In one such process, a solution is first provided in which are dissolved (a) a pharmaceutically active polypeptide and (b) an enhancer compound which enhances the systemic absorption of the polypeptide in the lower respiratory tract of a patient. The solvent is then removed from the solution to yield a dry solid containing the polypeptide and the enhancer, and the dry solid is pulverized to produce a powder. A second such process involves dry mixing (a) a pharmaceutically active polypeptide and (b) an enhancer compound, and micronizing the obtained mixture. Yet a third suitable process includes the steps of providing a first micronized preparation containing a polypeptide and a second micronized preparation containing an enhancer compound, and mixing the two micronized preparations together. When a carrier is to be included other than when an ordered mixture is desired, this may be added to the solution, or to the dry-mixture of the pharmaceutically active polypeptide prior to micronization, or micronised carrier may be dry mixed with the other micronised components. In producing an ordered mixture, micronised polypeptide and enhancer are mixed with a suitable carrier [33].

It has been found that when insulin is combined with an appropriate absorption enhancer and is introduced into the lower respiratory tract in the form of a powder of appropriate particle size, it readily enters the systemic circulation by absorption through the layer of epithelial cells in the lower respiratory tract. This is conveniently accomplished by inhalation of the powder containing insulin and the absorp-

tion enhancer (hereinafter collectively referred to as the active compounds) from an inhaler device, which dispenses the correct dose of powdered active compounds in a particle size which maximizes deposition in the lower respiratory tract, as opposed to the mouth and throat. To accomplish this preferential delivery into the lower respiratory tract, as much as possible of the inhaled active compounds should consist of particles with a diameter less than approximately 10 μm (e.g., between 0.01-10 μm , and ideally between 1-6 μm). In preferred embodiments, at least 50% (preferably at least 60%, more preferably at least 70%, still more preferably at least 80%, and most preferably at least 90%) of the total mass of the active compounds which exits the inhaler device consists of particles within the desired diameter range. The plasma pharmacokinetics (i.e., the rate of appearance and disappearance in the plasma) of insulin delivered by the method of the invention has been found to resemble more closely the plasma pharmacokinetics of endogenous insulin secreted by a healthy individual in response to glucose challenge or a meal, than does the plasma pharmacokinetics of human insulin delivered by subcutaneous injection, the standard route of insulin delivery. This is believed to occur because a dose of insulin delivered in accordance with the invention is absorbed much more rapidly into the systemic circulation than is a dose of subcutaneously injected insulin. The method of the invention therefore offers the ability rapidly to produce a transient rise in blood insulin in accordance with the needs of the patient, without a concomitant persistence of artificially high blood insulin concentrations long after the transient requirement for insulin is satisfied. In addition, the method of the invention also has the advantage of simple and painless delivery of the insulin dosage.

The invention thus features a method of treating a patient in need of insulin treatment, which method includes the step of introducing into the lower respiratory tract of the patient an effective amount of active compounds (a) insulin (e.g., human insulin) and (b) an enhancer which enhances the absorption of insulin in the lower respiratory tract of the patient, which active compounds may be comprised in a dry powder suitable for inhalation. By "enhances absorption" is meant that the amount of insulin absorbed into the systemic circulation in the presence of the enhancer is higher than the amount absorbed in the absence of enhancer.

At the point the powder enters the respiratory system of the patient, at least 50% of the total mass of the active compounds therein preferably consists of particles having a diameter of about 10 microns or less. Where the powder is supplied as agglomerates of such particles, contained in an inhaler device, the agglomerates should be substantially deagglomerated prior to entry into the respiratory system of the patient. This may be accomplished, for example, by use of an inhaler device in which the agglomerates are substantially deagglomerated by air turbulence created within the device upon inhalation from the device by the patient. Where the powder is supplied as an ordered mixture of active compounds and pharmaceutically acceptable carrier, the active compounds should be released from the large particles preferably upon inhalation, either by mechanical means in the inhaler device or simply by the action of inhalation, or by other means, the active compounds then being deposited in

the lower respiratory tract and the carrier particles in the mouth.

The inhaler device is suitably a dry powder inhaler device and is preferably a single-dose, dry powder inhaler device. The enhancer of the invention is preferably a surfactant, such as a salt of a fatty acid, a bile salt, or a phospholipid. The enhancer may be, for example, a sodium, potassium, or organic amine (e.g., lysine) salt of the fatty acid, and the fatty acid is preferably capric acid or another fatty acid of 8-16 carbon atoms. The preferred fatty acid salt is sodium caprate. The ratio of insulin to enhancer will preferably vary from about 9:1 to about 1:1 [34].

4. PULMUNARY DELIVERY

Over the years, certain drugs have been sold in compositions suitable for forming a drug dispersion for oral inhalation (pulmonary delivery) to treat various conditions in humans. Such pulmonary drug delivery compositions are designed to be delivered by inhalation by the patient of a drug dispersion so that the active drug within the dispersion can reach the lung. It has been found that certain drugs delivered to the lung are readily absorbed through the alveolar region directly into blood circulation. Pulmonary delivery is particularly promising for the delivery of macromolecules (proteins, polypeptides and nucleic acids), which are difficult to deliver by other routes of administration. Such pulmonary delivery can be effective both for systemic delivery and for localized delivery to treat diseases of the lungs.

Pulmonary drug delivery can itself be achieved by different approaches, including liquid nebulizers, aerosol-based metered dose inhalers (MDI's), and dry powder dispersion devices. Aerosol-based MDI's are losing favor because they rely on the use of chlorofluorocarbons (CFC's), which are being banned because of their adverse effect on the ozone layer. Dry powder dispersion devices, which do not rely on CFC aerosol technology, are promising for delivering drugs that may be readily formulated as dry powders. Many otherwise labile macromolecules may be stably stored as lyophilized or spray-dried powders by themselves or in combination with suitable powder carriers. The ability to deliver pharmaceutical compositions as dry powders, however, is problematic in certain respects. The dosage of many pharmaceutical compositions is often critical so it is necessary that any dry powder delivery system be able to accurately, precisely, and reliably deliver the intended amount of drug. Moreover, many pharmaceutical compositions are quite expensive. Thus, the ability to efficiently deliver the dry powders with a minimal loss of drug is critical. It is also essential that the powder be readily dispersible prior to inhalation by the patient in order to assure adequate distribution and systemic absorption.

The respiratory tract encompasses the upper airways, including the oropharynx and larynx, followed by the lower airways, which include the trachea followed by bifurcations into the bronchi and bronchioli. The upper and lower airways are called the conducting airways. The terminal bronchioli then divide into respiratory bronchioli, which then lead to the ultimate respiratory zone, the alveoli, or deep lung [35]. The

deep lung, or alveoli, are the primary target of inhaled therapeutic aerosols for systemic drug delivery.

Inhaled aerosols have been used for the treatment of local lung disorders including asthma and cystic fibrosis [36] and have potential for the systemic delivery of peptides and proteins as well [37]. However, pulmonary drug delivery strategies present many difficulties for the delivery of macromolecules; these include protein denaturation during aerosolization, excessive loss of inhaled drug in the oropharyngeal cavity (often exceeding 80%), poor control over the site of deposition, lack of reproducibility of therapeutic results owing to variations in breathing patterns, the frequent too-rapid absorption of drug potentially resulting in local toxic effects, and phagocytosis by lung macrophages.

Local and systemic inhalation therapies can often benefit from a relatively slow controlled release of the therapeutic agent [38]. Slow release from a therapeutic aerosol can prolong the residence of an administered drug in the airways or acini, and diminish the rate of drug appearance in the bloodstream. Also, patient compliance is increased by reducing the frequency of dosing [35, 39].

Controlled release drug delivery to the lung may simplify the way in which many drugs are taken [40, 41]. Pulmonary drug delivery is an attractive alternative to oral, transdermal, and parenteral administration because self-administration is simple, the lungs provide a large mucosal surface for drug absorption, there is no first-pass liver effect of absorbed drugs, and there is reduced enzymatic activity and pH mediated drug degradation compared with the oral route. Relatively high bioavailability of many molecules, including macromolecules, can be achieved via inhalation [42-44]. As a result, several aerosol formulations of therapeutic drugs are in use or are being tested for delivery to the lung [45-48].

Drugs currently administered by inhalation come primarily as liquid aerosol formulations. However, many drugs and excipients, especially proteins, peptides [49], and biodegradable carriers such as poly(lactide-co-glycolides) (PLGA), are unstable in aqueous environments for extended periods of time. This can make storage as a liquid formulation problematic. In addition, protein denaturation can occur during aerosolization with liquid formulations [50]. Considering these and other limitations, dry powder formulations (DPF's) are gaining increased interest as aerosol formulations for pulmonary delivery [51-53]. However, among the disadvantages of DPF's is that powders of ultrafine particulates usually have poor flowability and aerosolization properties, leading to relatively low respirable fractions of aerosol, which are the fractions of inhaled aerosol that escape deposition in the mouth and throat [38]. A primary concern with many aerosols is particulate aggregation caused by particle-particle interactions, such as hydrophobic, electrostatic, and capillary interactions. An effective dry-powder inhalation therapy for both short and long term release of therapeutics, either for local or systemic delivery, requires a powder that displays minimum aggregation, as well as a means of avoiding or suspending the lung's natural clearance mechanisms until drugs have been effectively delivered. Therefore, a need exists for dry-

powders suitable for inhalation, which minimize or eliminate the above-mentioned problems.

Pulmonary delivery advantageously can reduce or eliminate the need for injection. For example, the requirement for daily insulin injections can be avoided. In an invention [54] the particles were prepared that could be delivered as a dry powder to the deep lung, upper or central airways. They could be used to provide controlled systemic or local delivery of therapeutic or diagnostic agents to the respiratory tract via aerosolization. The particles could be easily prepared from simple, lung-compatible compounds without requiring the use of large macromolecules such as polymers, proteins, polysaccharides and others. The formation of colloidal suspensions resulted in particles of desired shape and porosity. Compared to methods that require solubilizing, higher concentrations could be employed. Administration of the particles to the lung by aerosolization permitted deep lung delivery of relatively large diameter therapeutic aerosols, for example, greater than about 5 μm in mean diameter. The particles could be fabricated with a rough surface texture to reduce particle agglomeration and improve flowability of the powder. The spray-dried particle could be fabricated with features which enhance aerosolization via dry powder inhaler devices, and lead to lower deposition in the mouth, throat and inhaler device. In this invention particles having a tap density less than about 0.4 g/cm^3 were formed by spray drying from a colloidal solution including a carboxylic acid or salt thereof, a phospholipid, a divalent salt and a solvent such as an aqueous-organic solvent. The colloidal solution could also include a therapeutic, prophylactic or diagnostic agent. Preferred carboxylic acids included at least two carboxyl groups. Preferred phospholipids included phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylserines, phosphatidylinositols and combinations thereof. The particles were suitable for pulmonary delivery [54].

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delivering drugs that may be readily formulated as dry powders. Many otherwise labile macromolecules may be stably stored as lyophilized or spray-dried powders by themselves or in combination with suitable powder carriers. The ability to deliver pharmaceutical compositions as dry powders, however, is problematic in certain respects. The dosage of many pharmaceutical compositions is often critical so it is necessary that any dry powder delivery system be able to accurately, precisely, and reliably deliver the intended amount of drug. Moreover, many pharmaceutical compositions are quite expensive. Thus, the ability to efficiently deliver the dry powders with a minimal loss of drug is critical. It is also essential that the powder be readily dispersible prior to inhalation by the patient in order to assure adequate distribution and systemic absorption.

Accordingly, dispersible dry powder pharmaceutical-based compositions are provided, including methods for their manufacture and dry powder dispersion devices. A dispersible dry powder pharmaceutical-based composition is one having a moisture content of less than about 10% by weight (% w) water, usually below about 5% w and preferably less than about 3% w; a particle size of about 1.0-5.0 μm mass median diameter (MMD), usually 1.0-4.0 μm MMD, and preferably 1.0-3.0 μm MMD; a delivered dose of about >30%, usually >40%, preferably >50%, and most preferred >60%; and an aerosol particle size distribution of about 1.0-5.0 μm mass median aerodynamic diameter (MMAD), usually 1.5-4.5 μm MMAD, and preferably 1.5-4.0 MMAD. Such compositions are of pharmaceutical grade purity [55].

Biodegradable particles of low density and large size have been also used for drug delivery to the pulmonary system. Biodegradable particles have been developed for the controlled-release and delivery of protein and peptide drugs [56]. Examples include the use of biodegradable particles for gene therapy [57] and for 'single-shot' immunization by vaccine delivery [58].

Improved aerodynamically light particles for drug delivery to the pulmonary system, and methods for their synthesis and administration are provided. In a preferred embodiment, the particles are made of a biodegradable material, have a tap density less than 0.4 g/cm³ and a mean diameter between 5 μm and 30 μm . In one embodiment, for example, at least 90% of the particles have a mean diameter between 5 μm and 30 μm . The particles may be formed of biodegradable materials such as biodegradable polymers, proteins, or other water-soluble materials. For example, the particles may be formed of a functionalized polyester graft copolymer consisting of a linear alpha-hydroxy-acid polyester backbone having at least one amino acid residue incorporated per molecule therein and at least one poly (amino acid) side chain extending from an amino acid group in the polyester backbone. Other examples include particles formed of water-soluble excipients, such as trehalose or lactose, or proteins, such as lysozyme or insulin. The aerodynamically light particles can be used for enhanced delivery of a therapeutic agent to the airways or the alveolar region of the lung. The particles incorporating a therapeutic agent may be effectively aerosolized for administration to the respiratory tract to

permit systemic or local delivery of a wide variety of therapeutic agents. They optionally may be co-delivered with larger carrier particles, not carrying a therapeutic agent, which have for example a mean diameter ranging between about 50 μm and 100 μm [59].

5. AEROSOLS

Medicaments for treating respiratory and nasal disorders are frequently administered in aerosol formulations through the mouth or nose. One widely used method for dispensing such an aerosol formulation involves making a suspension formulation of the medicament as a finely divided powder in a liquefied gas known as a propellant. Pressurized metered dose inhalers, or (pMDI's) are normally used to dispense such formulations to a patient. Surface active agents, or surfactants, are commonly included in order to aid dispersion of the medicament in the propellant and to prevent aggregation of the micronised medicament particles, and to improve lubrication of the valve. Until recently, chlorofluorocarbon-containing propellants (CFC's) were accepted for use in all pharmaceutical aerosol formulations. Typical surfactant dispersing agents used in the CFC formulations were for example sorbitantriolate, oleic acid, lecithines, and ethanol. Since CFC's have been implicated in the destruction of the ozone layer, a new generation of propellants has emerged to take their place. The respiratory delivery of aerosolized aqueous insulin solutions is described in a number of references [60-67].

Pulmonary delivery of dry powder medicaments, such as insulin, in a large particle carrier vehicle is described in [68]. A metered dose inhaler (MDI) for delivering crystalline insulin suspended in a propellant is described by Lee and Sciara [69]. A MDI for delivering insulin into a spacer for regulating inhalation flow rate is described in [70]. The intrabronchial administration of recombinant insulin is briefly described by Schluter *et al.* [71]. Intranasal and respiratory delivery of a variety of polypeptides, including insulin, in the presence of an enhancer, are described in [72, 73]. Intranasal delivery of insulin in the presence of enhancers and/or contained in controlled release formulations are described in [74-81]. The preparation and stability of amorphous insulin were described by Rigsbee and Pikal [82].

It has now been found that certain specific classes of surfactant are particularly suitable for use with the new generation of propellant. Accordingly, a pharmaceutical aerosol formulation was prepared comprising a hydrofluoroalkane propellant or a mixture of hydrofluoroalkane propellants, a physiologically effective amount of a medicament for inhalation and a surfactant selected from a C.sub.8 -C.sub.16 fatty acid or salt thereof, a bile salt, a phospholipid or an alkyl saccharide [83]. The surfactants employed in this invention gave fine dispersions in the new propellants, with good stability. The inventive formulations were therefore useful for administering inhalable medicaments. The most preferred surfactants are bile salts. "A medicament for inhalation" means a medicament which is suitable for inhalation and which consists largely of particles in a size range appropriate for maximal deposition in the lower respiratory tract (i.e., under 10 microns). Therefore as much as possible of the medicament preferably

consists of particles having a diameter of less than 10 microns, for example 0.01-10 microns or 0.1-6 microns, for example 0.1-5 microns. Preferably at least 50% of the medicament consists of particles within the desired size range. For example at least 60%, preferably at least 70%, more preferably at least 80% and most preferably at least 90% of the medicament consists of particles within the desired size range. Alternatively a portion of the micronised surfactant may be cold-mixed with a portion of the propellant and optional ethanol, whereafter the micronised medicament may be added. After mixing in of the medicament the remaining surfactant and propellant and optional ethanol may be added and the suspension filled into appropriate containers. The aerosol formulation of this present invention is useful for the local or systemic treatment of diseases and may be administered for example via the upper and lower respiratory tract, including by the nasal route. As such the present invention also provides said aerosol formulation for use in therapy; the use of the aerosol formulation in the manufacture of a medicament for the treatment of diseases via the respiratory tract; and a method for the treatment of a patient in need of therapy, comprising administering to said patient a therapeutically effective amount of the aerosol formulation of the present invention [83].

6. TRANSDERMAL DELIVERY

Prior art efforts to develop a non-injectable transdermal insulin delivery system for the treatment of diabetes have not been successful to date. While insulin can be systemically delivered to a patient by the topical application of an insulin-containing vehicle, the systemic blood levels of insulin that are achievable using this delivery method have proven to be generally inadequate for meeting the demands of the diabetic patient. Various methods have been developed for enhancing the transdermal delivery of insulin including improved passive diffusion carriers for increasing the permeability of the epidermis, sonophoresis, iontophoresis and ionosonic transport. Passive diffusion through the outer layer of skin has been used successfully for the delivery of low molecular weight lipophilic drugs such as scopolamine, estradiol and nitroglycerine, but has been largely unsuccessful for the transdermal delivery of hydrophilic peptides such as insulin due to the low skin permeability of such peptides. Thus, mechanical vibrational energy and/or iontophoresis are employed to increase skin permeability and facilitate transdermal insulin delivery. Sibalis *et al.* [84] teaches an apparatus and method for the iontophoretically mediated transdermal delivery of insulin. Henley [85, 86], discloses an ionosonic apparatus suitable for the ultrasonic-iontophoretically mediated transport of therapeutic agents across the skin. Insulin has a tendency to form dimers and hexamers in pharmacological compositions, which are considered to be too large for transdermal delivery. Brange [87] suggests chemically modifying insulin to produce insulin analogs that resist intermolecular association and enable improved iontophoretic delivery. Jang *et al.* [88] discloses a patch containing insulin formulated in a gel for the iontophoretically driven transdermal delivery of insulin. Notwithstanding the advances in methods for the transdermal delivery of insulin described above, the transdermal delivery of insulin in a quantity sufficient to attain a therapeutic level

in the blood of diabetic patients has heretofore not been possible. Clinical use of transdermal drug delivery has been limited because very few drugs are able, at least by passive diffusion alone, to penetrate the skin at a sufficient rate to produce a useful systemic drug concentration in the patient. The outer layer of the skin, the stratum corneum, is a major barrier to diffusion of low and especially high molecular weight drugs across the skin to the bloodstream. One drug for which an effective transdermal delivery system has long been sought is insulin, a therapeutic agent useful in the control of both Type I (juvenile onset) and Type II (adult onset) diabetes. Insulin, unfortunately, constitutes an example of molecules which do not readily diffuse through the stratum corneum at a therapeutically useful rate. While there have been attempts in the prior art to develop transdermal "patches" which contain a particular amount of insulin, which may be transferred at a particular rate, these patches have numerous limitations. One specific limitation is that insulin users must often gauge their requirements relative to physical activity and ingestion of carbohydrates. Additionally, there are different types of insulin, e.g. long-acting and short-acting, and the patient must develop skill in blending both the type and quantity of insulin in order to adequately control their blood sugar levels. The use of multiple patches having variable dosage strengths and insulin response characteristics thus becomes problematic.

Thus there remains a longfelt need for a dermal delivery system for insulin in a convenient format, e.g. a gel or cream, which can be formulated with insulin compounds having varied release characteristics, and whereby the dosage could be determined as a function of the volume applied.

A dermal delivery system is invented composition comprising an aqueous base vehicle including American Emu oil, Isopropyl Palmitate (PROTACHEM IPP), PEG-8 (a polyethylene glycol available under the trade name PROTACHEM 400), methylsulfonylmethane (MSM) and SEPIGEL 305 (a combination including about 40% polyacrylamide, about 15% C.sub.13 C.sub.14 Iso-paraffin, about 5% Laureth-7 and sterile water sufficient to make 100%). To this base vehicle, one or more active insulin ingredients are added, e.g. HUMALOG. As opposed to the use of injected insulin, topical creams of the instant invention have the advantage of not requiring the patient or a caregiver to give an injection; nor must the patient carry and/or transport the necessary paraphernalia required for giving an injection. The dermal delivery system, as illustrated herein, is alcohol free and therefore does not suffer from the problems of decreased shelf-life associated with alcohol containing prior art formulations. Since alcohol is not utilized, the presence of glycerin is likewise not required. Thus, a unique alcohol-free dermal delivery system is provided which provides enhanced penetration via the dermal layers thereby enabling a safer, quick-acting, and easier-to-comply alternative to capsules and tablets [89].

Another invention relates to methods for administration of insulin into the intradermal compartment of subject's skin, preferably to the dermal vasculature of the intradermal compartment. The methods of the invention enhance the pharmacokinetic and pharmacodynamic parameters of insulin delivery and effectively result in a superior clinical

efficacy in the treatment and/or prevention of diabetes mellitus. The methods of the instant invention provide an improved glycemic control of both non-fasting (*i.e.*, post-prandial) and fasting blood glucose levels and thus have an enhanced therapeutic efficacy in treatment, prevention and/or management of diabetes relative to traditional methods of insulin delivery, including subcutaneous insulin delivery [90]

7. ORAL DELIVERY OF INSULIN

Attempts have been made to deliver insulin by oral administration. The problems associated with oral administration of insulin to achieve euglycemia in diabetic patients are well documented in pharmaceutical and medical literature. Digestive enzymes in the GI tract rapidly degrade insulin, resulting in biologically inactive breakdown products. In the stomach, for example, orally administered insulin undergoes enzymatic proteolysis and acidic degradation. Survival in the intestine is hindered by excessive proteolysis. In the lumen, insulin is barraged by a variety of enzymes including gastric and pancreatic enzymes, exo- and endopeptidases, and brush border peptidases. Even if insulin survives this enzymatic attack, the biological barriers that must be traversed before insulin can reach its receptors *in vivo* may limit oral administration of insulin. For example, insulin may possess low membrane permeability, limiting its ability to pass from the lumen into the bloodstream.

Gastrointestinal patch systems with integrated multifunctions could surmount the challenges associated with conventional drug delivery. Several gastrointestinal patch systems provide bioadhesion, drug protection and unidirectional release. This combination of function could improve the overall oral bioavailability of large molecules that can currently be delivered only by injection, for example, epoetin-alpha and granulocyte-colony-stimulating factor, which are commonly used to treat chemotherapy-associated anemia and leukopenia, respectively. Furthermore, self-regulated release and cell-specific targeting provide additional 'smart' characteristics to this innovative therapeutic platform [91].

Many peptides and proteins are potentially useful as therapeutic agents but lack an adequate method of administration. The usefulness of polypeptides as therapeutic agents is limited by the biological barriers that must be traversed before a polypeptide can reach its specific *in vivo* target. Parenterally administered polypeptides are readily metabolized by plasma proteases. Oral administration, which is perhaps the most attractive route of administration, is even more problematic. In the stomach, orally administered polypeptides risk enzymatic proteolysis and acidic degradation. Survival in the intestine is even more unlikely due to excessive proteolysis. In the lumen, polypeptides are continuously barraged by a variety of enzymes, including gastric and pancreatic enzymes, exo- and endopeptidases, and brush border peptidases. As a result, passage of polypeptides from the lumen into the bloodstream is severely limited. The problems associated with oral and parenteral administration of polypeptides are well known in the pharmaceutical industry. Various strategies have been used in attempts to improve oral and parenteral delivery of polypeptides. Attempts have been made to use emulsions as

matrices for drug delivery of labile drugs (e.g., drugs such as insulin, which are susceptible to enzymatic, chemical, or physical degradation). However, in spite of preliminary reports on the efficacy of emulsion formulations in promoting the intestinal absorption of insulin in rats and rabbits [92], subsequent research was abandoned because of the lability of the insulin and the need for excessive doses to maintain glucose homeostasis [93,94]. Therefore, there remains a needed the art for methods and compositions which enable the use of emulsions and microemulsions for delivering labile drugs, such as insulin. Pharmaceutically active polypeptides such as insulin have been conjugated with polydispersed mixtures of polyethylene glycol or polydispersed mixtures of polyethylene glycol containing polymers to provide polydispersed mixtures of drug-oligomer conjugates. For example, Davis *et al.* [95] proposes conjugating polypeptides such as insulin with various polyethylene glycols such as MPEG-1900 and MPEG-5000 supplied by Union Carbide.

Ekwuribe [96] proposes conjugating polypeptides such as insulin with polyethylene glycol modified glycolipid polymers and polyethylene glycol modified fatty acid polymers. The number average molecular weight of polymer resulting from each combination is preferred to be in the range of from about 500 to about 10,000 Daltons. It is desirable to provide non-polydispersed mixtures of insulin-oligomer conjugates where the oligomer comprises polyethylene glycol. It has unexpectedly been discovered that a non-polydispersed mixture of insulin-oligomer conjugates comprising polyethylene glycol exhibits higher *in vivo* activity than a polydispersed mixture of similar conjugates having the same number average molecular weight. This heightened activity may result in lower dosage requirements. Moreover, a non-polydispersed mixture of insulin-oligomer conjugates comprising polyethylene glycol is typically more effective at surviving an *in vitro* model of intestinal digestion than polydispersed mixtures of similar conjugates. Furthermore, non-polydispersed mixtures of insulin-oligomer conjugates comprising polyethylene glycol also typically result in less inter-subject variability than polydispersed mixtures of similar conjugates [97].

In a preferred aspect, the pharmaceutical formulations are emulsions or microemulsions. The drug-oligomer conjugates have the important advantage that they are more readily incorporated into emulsion and microemulsion formulations. Furthermore, the lipophilicity/hydrophilicity of the conjugates can be readily adjusted by varying the molecular weight and structure of the hydrophilic and lipophilic components of the oligomer, in order to facilitate solubility in a specific emulsion or microemulsion formulation. A hydrolyzable drug-oligomer conjugate of insulin, PEG, and oleic acid was invented that can be orally administered [98].

Pharmaceutical compositions that include an insulin drug-oligomer conjugate, a fatty acid component, and a bile salt component are described [99]. The insulin drug is covalently coupled to an oligomeric moiety. The fatty acid component and the bile salt component are present in a weight-to-weight ratio of between 1:5 and 5:1. Methods of treating an insulin deficiency in a subject in need of such treatment using such pharmaceutical compositions are also

provided, as are methods of providing such pharmaceutical compositions [99].

8. NANOSUSPENSIONS FOR INSULIN DELIVERY

Another method to formulate drugs for delivery has been the use of nanosuspensions of drugs for reducing size and creating uniform suspensions. The use of commercial devices such as mill processors, microfluidizers and homogenizers has allowed the formulation of nanosuspensions of various substances. Nanosuspended drugs can also be wrapped in liposomes or made into micellar mixtures by mixing the drug preparations with appropriate chemical compounds. The instant inventor has utilized a microfluidization technique for the production of microsuspensions of aqueous and oil-based solutions for use in drug delivery systems. The instant process does not require encapsulation in polymers or the use of hydrogels or other supporting or encapsulating substances. Chemicals prepared in this manner are called "nanosuspensions." This process allows molecules to be embedded into microdroplets of between about 87 nm to about 10 μm in size, which are used to create stable and uniform emulsions and dispersions.

The nanosuspensions of the instant invention are effective in providing higher concentrations of a molecule in the bloodstream over a longer period of time as compared to molecules prepared by other pharmacological methods and similarly delivered, e.g. by a buccal mucosal route, intestinal absorption, or the like. While not wishing to be bound to any particular theory of operation, it has been hypothesized that the nanosuspensions of the instant invention allow molecules to be delivered across tissue barriers at a more even rate than non-microfluidized preparations. A method of manufacturing a stable nanosuspension for delivery of a biologically active agent into the bloodstream was invented. A microfluidizable mixture was initially formed and processed via a microfluidization process to form the stable nanosuspension, which may be administered via the buccal mucosa or other suitable routes of administration. This product demonstrates increased bioavailability, enhanced period of onset, and enhanced stability for a controlled-release product [100].

9. NASAL INSULIN DELIVERY

Many of peptide/proteinaceous drugs are not readily absorbed into body mainly because it is readily decomposed by proteolytic enzymes in the gastrointestinal tract when orally administered. Therefore, it is often forced to administer such a drug by injection in order to use it for therapy. Unfortunately, injection imposes burden such as pain, attendance to the hospital, etc., to a patient. Accordingly, it is desired to develop a pharmaceutical preparation for noninvasive administration such as nasal administration which can substitute injection.

Nasal administration, by which a drug is transferred into circulating blood through the nasal mucosa, is being energetically studied as a method for non-injection type administration together with transdermal administration, transocular administration, transrectal administration, transpulmonary administration, etc. Among these non-injection type administration methods, the nasal administration is easy to administer a drug. Moreover, nasal administration is considered to be superior in the absorption of a drug among

the non-injection type administration methods since the blood vessel system in the nasal mucous membrane is more developed compared with the skin, the ocular mucous membrane, the rectal mucous membrane, etc. Therefore, a pharmaceutical preparation for nasal administration has been put into practice in some drugs. Further, the transfer of a drug into blood in nasal administration is faster than that in oral administration, and it can be expected that nasal administration has immediate effect similar to injection. On the other hand, the absorption of a drug through the nasal mucosa depends on physical properties such as lipophilicity of the drug and also on the molecular weight, etc. It is pointed out that a drug having a high solubility in water, a drug having a high lipophilicity, a peptide/proteinaceous drug having a large molecular weight, etc., is generally low in absorption through the nasal mucosa. Under these circumstances, some contrivances to improve the absorption of such a drug through the nasal mucosa has been proposed.

In a long acting pharmaceutical preparation for nasal cavity of said patent publication, it is assumed that a high pernasal absorption ratio is hardly achieved for a drug having a high solubility in water, a drug having a high lipophilicity or a peptide/proteinaceous drug having a large molecular weight. Under these circumstances, the development of a pharmaceutical preparation for administering such a drug on the nasal mucosa, which can utilize it effectively in terms of curing effect and curing efficiency, is strongly desired.

Nolte, *et al.* [101], and Bruice *et al.* [102], reported insulin preparations for nasal administration containing sodium glycolate or sodium taurofusidate as an absorption promoter. However, these absorption promoters have problems in irritation on the nasal mucosa, and the preparations have not been put in practice yet.

On the other hand, a powdery composition for nasal administration excellent in absorption through the nasal mucosa comprising a polypeptide and a water-absorbing and water-insoluble base material was prepared [103]. It is claimed that the nasal absorption of the polypeptide without using an absorption promoter had been achieved in the composition.

However, even in the composition of the above patent publication, none of the nasal absorption ratios of polypeptides [the area under blood concentration-time curve (AUC) after nasal administration] exceeds 10-20% of AUC in injection. For instance, in Example 4 of the patent publication, the maximum blood concentration of insulin was less than 200 $\mu\text{U}/\text{ml}$ when 10 units of insulin had been administered to a rabbit, and it was about 20% of the maximum blood concentration obtained in injection with same amount of insulin. The absorption ratio of the nasal preparation determined from AUC is estimated to be less than 10% of the absorption ratio of injection. A patent publication describes the combined use of a water-absorbing and water-insoluble base material with a water-absorbing and water-soluble base material in an amount of 0.1-60 wt % based on the water-absorbing and water-insoluble base material, especially preferably 1-50 wt % [104]. This invention is a powdery composition for nasal administration, which is characterized in that (1) the composition contains (i) a drug, (ii) a water-absorbing and gel-forming base material

of one kind or more selected from the group comprising hydroxypropyl cellulose, hydroxypropylmethyl cellulose, methyl cellulose, hydroxyethyl cellulose and sodium carboxymethyl cellulose and (iii) a water-absorbing and water-insoluble base material of one kind or more selected from the group comprising crystalline cellulose, .alpha.-cellulose, cross-linked sodium carboxy-methyl cellulose, cross-linked starch, gelatin, casein, tragacanth gum, polyvinyl pyrrolidone, chitin and chitosan, (2) the content of the water-soluble and gel-forming base material is about 5-40 wt % based on the total of the water-absorbing and water-insoluble base material and the water-absorbing and gel-forming base material, and (3) the drug is unevenly dispersed more on/in the water-absorbing and water-insoluble base material than on/in the water-absorbing and gel-forming base material. Thus, this invention provides a powdery composition for nasal administration excellent in absorption from the nasal cavity and capable of exhibiting extreme increase in maximum blood concentration compared with a conventional composition for nasal administration even for a drug having a high solubility in water, a drug having a high lipophilicity or a peptide/proteinaceous drug having a large molecular weight [104].

Chitosan gels [105] and microspheres [106] have also been reported for nasal delivery of insulin. As chitosan itself acts as an absorption enhancer, could promote the insulin AUC highly near 45% of the intravenous administration in rats.

10. TREATMENT OF DIABETES WITH SYNTHETIC BETA CELLS

This method relates to treatment of diabetes with synthetic beta cells, and specifically to a method of utilizing non-islet cells comprising a genetic construct that has a coding sequence for a proinsulin expressible in the cells in response to glucose levels. The proinsulin synthesized in the cells is further processed into a secretable, active insulin. Insulin is normally produced in and secreted by the beta cells of the islets of Langerhans in the pancreas. Mature insulin is a protein having two polypeptide chains, A and B, held together by disulfide bonds. The glucose responsive release of insulin from the beta cells is a complex event including gene expression, posttranslational modification and secretion. The initial protein product and insulin precursor is preproinsulin, a single polypeptide chain having an N-terminal signal sequence and an intervening sequence, the C-peptide, between the A and B chains. The signal sequence is cleaved during transport from the rough endoplasmic reticulum to form proinsulin. The proinsulin is packaged into secretory granules along with specific enzymes required for its processing. Proinsulin folds into a specific three-dimensional structure, forming disulfide bonds. Mature insulin results from removal of the C-peptide. In beta cells, this function is catalyzed by endopeptidases that recognize the specific amino acid sequences at the junction of the A chain and the C peptide (C-A junction) and at the junction of the B chain and the C peptide (B-C junction). Mature insulin, stored in secretory granules, is released in response to elevated blood glucose levels. The detailed mechanism of insulin release is not completely understood, but the process

involves migration and fusion of the secretory granules with the plasma membrane prior to release.

In normally functioning beta cells, insulin production and release is affected by the glycolytic flux. Glucokinase and glucose transporter 2 (GLUT-2) are two proteins that are believed to be involved in sensing changes in glucose concentration in beta cells. A reduction in GLUT-2, which is involved in glucose transport, is correlated with decreased expression of insulin; loss of glucokinase activity causes a rapid inhibition of insulin expression.

Autoimmune destruction of pancreatic beta cells causes insulin-dependent diabetes mellitus or Type I diabetes. As a consequence of partial or complete loss of beta cells, little or no insulin is secreted by the pancreas. Most cells, with the exception of brain cells, require insulin for the uptake of glucose. Inadequate insulin production causes reduced glucose uptake and elevated blood glucose levels. Both reduced glucose uptake and high blood glucose levels are associated with a number of very serious health problems. In fact, without proper treatment, diabetes can be fatal.

One conventional treatment for diabetes involves periodic administration of injectable exogenous insulin. This method has extended the life expectancy of millions of people with the disease. However, blood glucose levels must be carefully monitored to ensure that the individual receives an appropriate amount of insulin. Too much insulin, can cause blood glucose levels to drop to dangerously low levels. Too little insulin will result in elevated blood glucose levels. Even with careful monitoring of blood glucose levels, control of diet, and insulin injections, the health of the vast majority of individuals with diabetes is adversely impacted in some way.

Replacement of beta cell function is a treatment modality that may have certain advantages over insulin administration, because insulin would be secreted by cells in response to glucose levels in the microenvironment. One way of replacing beta cell function is by pancreas transplantation, which has met with some success. However, the supply of donors is quite limited, and pancreas transplantation is very costly and too problematic to be made widely available to those in need of beta cell function. There have been many other proposed alternatives for beta cell replacement, including replacing beta cell function with actual beta cells or other insulin-secreting, pancreas-derived cell lines [107]. Because the immune system recognizes heterologous cells as foreign, the cells have to be protected from immunoreactive cells (e.g., T-cells and macrophages mediating cytolytic processes). One approach to protect heterologous cells is physical immunoisolation; however, immunoisolation itself poses significant problems. Newgard [108] discloses another approach to beta cell replacement. This patent describes an artificial beta cell produced by engineering endocrine cells of the At-T-20 ACTH secreting cells. A stably transfected cell, At-T-20, is obtained by introducing cDNA encoding human insulin and the glucose transporter gene, i.e. the GLUT-2 gene, driven by the constitutive CMV promoter. The cell line already expresses the correct isoform of glucokinase required for glucose responsive expression of the proinsulin gene. Although the cell line is responsive to glucose, it is secretagogue-regulated at concentrations below the normal

physiological range. Therefore, use of these cells in an animal would likely cause chronic hypoglycemia; furthermore, these cells are derived from a heterologous source and bear antigens foreign to the recipient host.

Laurance *et al.* [109] discloses another approach to obtaining a cell line in which insulin production is secretagogue-regulated. Subpopulations of beta-TC-6 cells having an increased internal calcium concentration, a property associated with insulin secretion, were selected using a cell sorter. After successive passages, a subpopulation of cells that produce insulin in response to glucose in the physiological range (4-10 mM) was selected, and the cells were encapsulated for therapeutic use in alginate bounded by a PAN/PVC permeable hollow fiber membrane according to the method of Dionne [110].

Valera *et al.* [111], describes transgenic mouse hepatocytes expressing insulin under the control of the PEPCK promoter driven by P-enolpyruvate. The PEPCK promoter is sensitive to the glucagon/insulin ratio and is activated at elevated glucose levels. The PEPCK/insulin chimeric gene was introduced into fertilized mouse eggs. Under conditions of severe islet destruction by streptozotocin (SZ), the production and secretion of intact insulin by the liver compensated for loss of islet function. A method for obtaining glucose-regulated expression of active insulin in the cells of a mammalian subject is described in [112]. The method involves delivering into the subject a genetic construct comprising a coding sequence for a human proinsulin operably connected a promoter functional in the host cells. The construct includes a glucose responsive regulatory module having at least one glucose inducible regulatory element comprising a pair of CACGTG motifs linked by a five base nucleotide sequence, which confers glucose inducible expression of the proinsulin coding sequence. To ensure proper processing of the proinsulin to active insulin, the coding sequence was modified to direct the synthesis of a mutant proinsulin polypeptide having amino acid sequences that can be cleaved to mature insulin in suitable host cells, such as hepatocytes.

Polypeptides having activity of human neurogenin3 (hNgn3), and nucleic acid encoding such polypeptide are invented [113]. The invention also features use of islet transcription factors such as hNgn3 to facilitate production of pancreatic islet cells from progenitor cells, and to facilitate insulin delivery by production of islet cells so produced.

CURRENT & FUTURE DEVELOPMENTS

Various types of insulin are available in the market, including newer analogs (lispro, aspart, glargine). Although insulin analogs seem to be more physiological, controlled studies suggested either similar efficacy to regular insulin or only a minor benefit in favor of insulin analogs. Noninvasive insulin deliveries are now in development. It does appear that the most clinically viable non-invasive system to date may be pulmonary delivery. In the future, patients with type 1 diabetes will receive insulin in optimal quantities at optimal times by way of optimal routes into the body because of needle-free routes of administration in order to achieve optimal blood glucose control. These new

technologies will facilitate proper treatment of type 1 diabetes and improve the lives of affected patients. Among the noninvasive ways for controlling diabetes in the future, the nanoparticulate systems seem to be promising potential drug carriers for oral insulin delivery. The ultimate goal for the treatment of diabetes remains the development of a fully automated glucose-controlled device.

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