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Title: ORAL INSULIN DELIVERY SYSTEMS FOR CONTROLLING DIABETES

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ORAL INSULIN DELIVERY SYSTEMS FOR CONTROLLING DIABETES

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

This application claims priority to Indian Provisional Patent Application No. 856/MUM/2009 filed 31 March, 2009, the entire content of which is incorporated by reference.

TECHNICAL FIELD

The present invention relates to the development of controlled release (CR) formulations containing insulin and description of its methods. More specifically, it relates to the development of an oral CR preparation of insulin encapsulated into microspheres using a pH-sensitive, biocompatible polymer/blends of polymer and produced by double emulsion solvent evaporation method.

BACKGROUND ART

Oral administration of therapeutic agents like proteins and peptides is the preferred means of delivering drugs compared to other routes because of ease of administration, low-cost production and high patient compliance: However, formulating biologically active protein or peptide drugs for oral delivery is a complicated process due to the poor intrinsic protein permeability - as a result of proteins' high molecular weight, ease of degradation by proteolytic enzymes in the stomach and in the small intestine as well as the chemical instability. Major hurdles in developing effective oral formulations for delivering peptides and proteins have been addressed. (Ramesh et al, Expert Opinion, Drug Delivery, 5, 2008, pp. 403-415; Mundargi et al., J. Controlled Release, 125, 2008, pp. 193-209, Emisphere Technologies, Inc., Tarrytown, NY, USA. Diabetes Technology & Therapeutics, 6, 2004, pp. 510-517). Protein/peptide denaturation or degradation can be overcome by designing suitable carriers, which would protect insulin from harsh environments of the stomach before releasing the protein or peptide cargo into more favorable regions of gastrointestinal (GI) tract, specifically lower region of the intestine. In order to control the release of the biactive ingredient in vivo over the specified period of time and in order for the drug to safely and effectively exhibit its pharmacological
efficacy, a sustained or controlled release of drug can be achieved by the retardation of drug diffusion by gradual disintegration of the polymer matrix following its application.

In case of small peptides, even if decomposition scarcely occurs, the stability becomes an important issue. Therefore, microencapsulation process of protein-based pharmaceutical products must be free from excessive heat and shear stress, sharp changes in pH, aggressive organic solvents, excessive freezing and drying. It is possible that microencapsulated proteins may be hydrated even during the storage and proteins are prone to denaturation and aggregation under these circumstances. The polymer may degrade after administering, thus creating highly concentrated acidic microenvironment inside and around the carrier device due to the decomposed acidic monomer. Under these circumstances, proteins are prone to aggregation, hydrolysis and chemical change, leading to denaturation, or inactivation of proteins, thereby affecting the delivery rate. Particularly, insulin becomes the target of a protease and is prone to chemical, physical denaturation in a solution or a suspension (Brange et al., J. Pharm. Sci., 86, 1997, pp 517-525).

Utilizing micro or nanotechnology, novel hydrogels have been developed and extensively evaluated as carriers for the peptide insulin by Peppas et al., (WO 99/43615, Oct.8, 1998. "method for oral delivery of proteins"). The principle behind this technology and those of others (Lin et al., Nanotechnology, 18, 2007, pp. 1-11 and references therein) is to utilize a suitable polymer, which traps the drug such as insulin and releases it by swelling or deswelling mechanisms in a controlled manner within the specific tissues. This allows higher concentration of the peptide in a chosen biodegradable format. These hydrogels are very specialized systems and drug release from such systems is triggered by changes in pH of the medium or temperature or magnetic field as the case may be. One example of the complexity of such systems is the glucose-sensing hydrogel used to deliver insulin to diabetic patients (Lee et al., Peptide and Protein Drug Delivery. 2000, pp. 691-740).

Another approach is to use pH-sensitive nature of the delivery device, to mediate the changes in swelling of the hydrogel, since a pH-sensitive hydrogel undergoes large and reversible changes in volume in response to pH changes within a biological environment (Peppas et al., J. Controlled Release 62, 1999, pp.81-87; Bures et al., Eur. J. Pharm. Biopharm. 50, 2000, pp. 27-66).

Therefore, most efforts have been concentrated on developing therapeutic strategies for optimizing oral insulin absorption and its delivery using pH-sensitive hydrogels, micro or nanoparticles prepared from biodegradable/biocompatible polymers or a combination
thereof with other types of synthetic polymers. Insulin has been encapsulated in these carriers to be released at specified time intervals over the required period of time. Specifically engineered delivery carriers that would transport across the biological cell membranes, including GI tract are useful in these applications and many such systems have been proposed in the prior art, (Yu-Hsin Lin et al., Nanotechnology, 18, 2007, pp. 1-11) yet none of these devices are available in the market. Insulin delivery is therefore one of the major targets for the controlled-release (CR) delivery particle preparation that is being investigated vigorously.

Various research groups in the prior art have proposed and developed many CR formulations containing insulin that are capable of reducing the concentration of serum glucose levels continuously for a long time after in vivo administration (Ramesh et al, Expert Opinion, Drug Delivery, 5, 2008, pp. 403-415; Mundargi et al., J. Controlled Release, 125, 2008, pp. 193-209). However, most insulin delivery carriers currently used are based on the reaction between glucose-oxidase tied up into a polymer in a drug delivery system and glucose present in the blood. If the reaction between glucose and glucose-oxidase results in a decrease of pH of the microenvironment, the polymer system is swollen such that the amount of released insulin increases.

Bioavailability of insulin after oral administration is normally low, due to its instability in the GIT, low partition coefficient and physical barrier of the intestinal epithelium. Innumerable attempts in the prior art to develop the oral insulin formulations have met with two hurdles that are to be overcome: (i) insulin transport across the mucosal barrier is restricted and (ii) insulin degradation by proteolytic enzymes of the stomach as well as intestinal lumen. Such formulations are described in detail in US patent numbers 7018980, 5824638, 6258377, 6368619, PCT patent application 97/34581 and 99/43615.

In a recent report by Singh et al., (Indian Pat. Appl. 2008, 35 pp. IN 2006DE01437A 20080104, Application: IN 2006-DE1437 20060616) the hydrogel copolymer microparticles of P(MAA-co-PEGDMA), P(MAA-co-PEGDA), P(AA-co-PEGDA) and P(AA-co-PEGDMA) by copolymerization of poly(ethylene glycol) dimethacrylate (PEGDMA) and poly(ethylene glycol) acrylate (PEGDA) of various molecular weights with methacrylic acid (MAA)/acrylic acid (AA), respectively are reported.

Hassan et al., in an Egyptian patent (EG 2002-827 20020720) reported the inventions on the controlled release of insulin from oral capsule formulations containing chitosan, hydroxypropyl cellulose, methyl cellulose, and methacrylic acid copolymers providing optimum pH for insulin release.

Novel type of nanoparticles (NPs) coated with chitosan which allow insulin to be administered orally were reported in a recent finding (Lin et al., Nanotechnology, 18, 2007, pp. 1-11). The NPs could transiently and reversibly open the tight junctions in Caco-2 cell monolayers, thus increasing their paracellular permeability. After oral administration of FITC-labeled NPs, fluorescence signals, co-localized with ZO-I proteins, were observed at cell-to-cell contact sites in the small intestine of rats. The intensity of fluorescence signals observed at the duodenum was stronger and appeared at a deeper level than at the jejunum and the ileum. The insulin-loaded NPs suspended in water were stable in typical storage conditions to investigate the release pattern of insulin-loaded formulation as a function of pH. Oral administration of insulin in the form of NPs in diabetic rats demonstrated sustained effect of decreasing the blood glucose level for at least 10h, indicating the effect of NPs to enhance the absorption of fully functional insulin.

Several examples utilizing chitosan and/or its various types of derivatives have been reported and these were used as insulin delivery devices through oral route (Prego et al., Pharm. Res. 23, 2006, pp. 549-56; Pan, Int. J. Pharm. 249, 2002, pp. 139-147; Ramadas et al., J. Microencapsulation 17, 2000, pp. 405-411; Ma et al., Int. J. Pharm. 93, 2005, pp. 271-280; Ma et al., Pharm. Res. 20, 2003, pp. 1812-1819; Mao et al., Pharm. Res. 22, 2005, 2058-2068).

Development and characterization of new insulin containing polysaccharide nanoparticles is the subject of another publication (Sarmento et al., Colloids and Surfaces B: Biointerfaces, 53, 2006, pp 193-202). Yet other studies on P(MAA-g-EG) hydrogels have dealt with their potential capacity to bind calcium (Nakamura et. al., J. Control. Rel., 95,
2004, pp 589-599; Aragoa et al., Eur. J. Pharm. Sci., 11, 2000, pp 333-341; J. Control. Rel., 97, 2004, pp 115-124) and affect the proteolytic activity of calcium-dependent enzymes, such as trypsin. Other reports on oral insulin delivery devices include (i) biodegradable poly(glycolic acid), (PGA), poly(lactic acid), (PLA), poly(lactic acid-co-glycolic acid), (PLGA), poly(lactic acid-co-poly(ethylene glycol), (PLA-PEG), dextran-PEG; (ii) pH-sensitive polymers like poly(acrylic acid) i.e., PAA and poly(methacrylic acid), PMAA; and (iii) complexing hydrogel graft polymers like P(MAA-g-EG) i.e., P(PAA-g-EG) in addition to biopolymers like chitosan, cyclodextrin, etc., in various combinations with methacrylic or acrylic-polymers. According to the most recent prior art (Teply et al., Biomaterials, 29, 2008, pp 1216-1223), a new formulation strategy was developed for prolonging the intestinal retention of protein drug giving substantial absorption. This method involved using the negatively charged PLGA microparticles, which were subsequently mixed with the positively charged micromagnets to form stable complexes through the electrostatic interactions.

Paul and Sharma (J. Pharma. Sci., 97, 2008, pp 875-882) attempted to load insulin in tricalcium phosphate (TCP) microspheres coated with a pH-sensitive polymer of methacrylate derivative to study the stability and conformational variations of insulin as well as their biological activity in diabetic rats. The microspheres were coated with Eudragit S100, a pH-dependent anionic copolymer of methacrylic acid and methyl methacrylate, solubilizing above the pH of 7.4 for target delivery to large intestine.

According to another report, Dave et al., (J. Chromatography A, 1177, 2008, pp 282-286) have developed a conjugated insulin product (IN-105) exhibiting high bioavailability, wherein the recombinant human insulin was conjugated covalently with a monodisperse short chain methoxy polyethylene glycol derivative.

In another report (Agarwal, et. al., Int. J. Pharmaceutics, 225, 2001, pp 31-39), a coprecipitation technique was developed for preparing microparticles of insulin using Eudragit L100 (polymethacrylate). The effect of variables like the addition of salts in the precipitating medium and ratio of polymeric solution to volume of precipitating medium on dissolution and encapsulation efficiency of insulin microparticles was tested. The pH-sensitive graft copolymers of poly(methacrylic acid) and poly(ethylene glycol) i.e., P(MAA-g-EG) were reported for insulin delivery (Lowman and Peppas, Macromolecules 30, 1997, pp 4959-4965). Tuesca et al., (J. Pharma. Sci., 97, 2008, pp 2607-2618) developed hydrogels of poly(methacrylic acid) grafted onto poly(ethylene glycol)
P(MAA-g-EG) for oral insulin release. Insulin absorption of this study was dependent on the amount of polymer as well as the concentration of insulin, giving a maximum bioavailability of 8.0%.

Polypelectrolyte complexes of cationic chitosan (CS) with anionic polymers like sodium carboxy methylcellulose, sodium alginate, PAA/PMAA, etc., have been developed for drug delivery (Hari et al., J. Appl. Polym. Sci, 59, 1996, pp 1795-1801; Yoshioka et al., Biotechnol. Bioeng., 35, 1990, pp 66-72; Du et al., J. Biomed. Mater. Res., B: Appl. Biomater. 72B, 2005, pp 299-304; Hu et al., Biomaterials, 23, 2002, pp 3193-3201), under mild conditions without the surfactants, organic solvents or steric stabilizers. MAA can be polymerized in the presence of CS and PEG by optimizing polymer compositions. Damge et al., (J. Control. Rel., 117, 2007, pp 163-170) developed NPs of poly(ε-caprolactone) (biodegradable) and anion-biodegradable Eudragit® RS blends that gave encapsulation efficiency of 96% applicable for oral insulin administration; the authors prepared NPs using poly(ε-caprolactone) and Eudragit RS100 polymers with poly(vinyl alcohol) as a surfactant. Poly(ε-caprolactone) is a biodegradable polymer and it took longer time to degrade, so it may not be useful for developing short-acting oral insulin delivery formulations.

Thus, formulation and delivery are the most important factors in developing protein/peptide-based pharmaceuticals. In general, proteins or peptides have high molecular weights and a tertiary structure on which their activity is greatly dependent. These biomacromolecules are prone to denaturation making their formulation difficult. Insulin is easily denatured during microencapsulation and generating deaminated products with result in approximately 50% of loss of activity.

**OBJECTIVES OF THE INVENTION:**

It is aim of the present invention to provide a method of preparing microspheres comprising insulin without any denaturation of the protein.

**SUMMARY OF INVENTION**

Since careful consideration must be taken into the stability of the formulated products, the present invention provides microsphere-based encapsulation method to attain the stability of insulin contained in the formulations.
The present invention provides a pH sensitive polymer. The present invention provides a pH sensitive blend polymer of Eudragits.

The present invention provides oral formulations of pH sensitive active ingredient encapsulated by the polymers of the present invention. The present invention provides hydrogel compositions of the insulin loaded formulation. There is negligible release of the insulin in the acidic pH, particles according to the present invention provides to avoid burst release of the insulin in neutral pH.

The present invention provides increased encapsulation efficiency and optimization of insulin loading/release.

The present invention provides a novel pH sensitive biodegradable copolymer blends for microencapsulation of insulin. Examples of these biocompatible polymers include Eudragit L100, Eudragit RS100 and their blends.

The present invention also provides Eudragit L100 and blend of Eudragit L100 with Eudragit RS100 for oral delivery of active ingredient i.e. encapsulated pH sensitive formulation, for the CR of the active ingredients.

In one embodiment, the present invention provides a method of preparation of microspheres containing insulin in liquid form.

The present invention provides a novel method of encapsulating insulin into polymeric microspheres by a double emulsion followed by solvent evaporation technique in the presence of paraffin oil.

In one embodiment, the present invention provides the composition by using different methods such as ‘double’ emulsion solvent evaporation.’ In one preferred embodiment, the present invention provides a method of preparation of formulation composition for encapsulating insulin.

In one embodiment, the present invention provides compositions for the oral delivery of active ingredient such as insulin using pH sensitive polymer of the present invention. In one embodiment, the present invention provides compositions for oral delivery of insulin.

In one embodiment, the present invention provides oral formulations of pH sensitive active ingredient using polymer blend of the present invention. In one preferred embodiment, the oral formulation is a pH-sensitive.
In one embodiment, the present invention provides a pH sensitive polymer and/or blend polymer that does not release the active ingredient in acidic pH, but swell in neutral pH to release the active ingredient. In one preferred embodiment, the formulation of insulin retains insulin at pH 1.2 and releases insulin in pH 7.4.

In one embodiment, the present invention provides a formulation of insulin composition, which does not alter the stability of the active ingredient.

BRIEF DESCRIPTION OF DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure, the inventions of which can be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1(A) shows Scanning electron microscopic images of group of microspheres of Eudragit L100 prepared by method LTIG. FIG. 1(B) shows Scanning electron microscopic images of group of microspheres of Eudragit L100 prepared taken at different magnifications by method II.

FIG. 2(A) shows Circular dichroism spectra of pure bovine insulin. FIG. 2(B) shows Circular dichroism spectra of encapsulated bovine insulin. FIG. 2(C) shows Circular dichroism spectra of released bovine insulin.

FIG. 3(A) shows Circular dichroism spectra of pure human insulin. FIG. 3(B) shows Circular dichroism spectra of released human insulin.

FIG. 4 shows in vitro release of insulin-loaded Eudragit L100 microspheres in pH 1.2 and 7.4 media.

FIG. 5 shows in vitro release of Eudragit L100-coated insulin-loaded Eudragit L100 tablet in pH 1.2 and 7.4 media.

FIG. 6 shows in vitro release of insulin-loaded Eudragit L100/Eudragit RSIOO (50:50) blend in pH 1.2 and 7.4 media.

FIG. 7 shows in vitro release of bovine insulin-loaded Eudragit L100 microspheres prepared by method II.

FIG. 8 shows in vitro release of human insulin-loaded Eudragit L100 microspheres prepared by method II.
FIG. 9 shows *in vivo* experiments performed on (♦) control, (■) insulin-loaded Eudragit L100 microspheres (20 IU/200 g of body weight of the rats) and (A) insulin-loaded Eudragit L100/RS100 (50:50) blend microspheres (20 IU/200 g of body weight of the rats).

FIG. 10 shows the % inhibition of orally-fed formulations of insulin-loaded Eudragit L100 and Eudragit L100/RS100 (50:50) blend microspheres.

**DESCRIPTION OF EMBODIMENTS**

The present invention focuses on developing controlled-release (CR) formulations of insulin that minimize denaturation of insulin and increase its stability during microencapsulation. In general, emulsifying a polymer matrix with a drug or protein produces microspheres. The size of microspheres, which is an important parameter to determine internal behavior of drug or protein, can be adjusted by selecting appropriate formulation conditions. In the present invention, different biocompatible polymers were used to develop insulin-loaded formulations by microencapsulation. Examples of these include Eudragit L100, Eudragit RS100, and their blends.

**DEFINITIONS:**

The term "MAA" as used herein refers to methacrylic acid.

The term "pH sensitive copolymer" as used herein refers to Eudragit L100

The term "acidic pH" as used herein refers to 1.2

The term "neutral pH" as used herein refers to 7.4

The term "insulin" as used herein refers to human insulin, porcine insulin, bovine insulin as well as their analogues, such as recombinants

The term "derivatives", used herein comprises polymers having substitution of chemical groups like alkyl, alkenylene, hydroxylation, oxidation, addition and other similar modifications made by the one skilled in the art in a conventional manner.

The present invention provides biocompatible polymers used for the controlled release of bioactive ingredients like drugs, proteins, peptides as the case may be. Examples of these biodegradable polymers include Eudragit L100, Eudragit RS100.

Biodegradable polymers in general, are degraded *in vivo* both by enzymatic and non-enzymatic hydrolysis, surface or bulk erosion. Microspheres of the present invention
prepared by microencapsulating insulin are used for the CR preparations of insulin by which pharmaceutical efficacy of insulin can be continuously retained in vivo for a long period of time. The CR preparation of insulin may, therefore, include the pharmaceutically acceptable diluents, carriers or additives. Preferably, the CR preparation according to the present invention can be made in various forms suitable in oral delivery form, which is the most preferred route.

To this end, the present invention provides the CR formulations containing insulin by which microspheres are prepared. The insulin-loaded CR formulations according to the present invention are, therefore, a release device, which can help to reduce the number of administrations of insulin due to the continuous exhibition of pharmaceutical efficacy. Also, the extent of initial burst of insulin can be controlled, minimized or completely stopped to prevent the sharp decrease in serum glucose concentration.

Biodegradable polymers used for microencapsulation differ in decompositions depending on their physical properties or compositions thereof, so a considerable time is required for their complete decomposition. Thus, if a microencapsulated formulation using a biodegradable polymer as a carrier is continuously administered, the accumulation of polymer in the living body will occur. Hence, the understanding of their toxicity aspects is of great importance. This problem becomes important particularly in case of polymers that have not been identified or classified as completely biodegradable.

Generally, microspheres of this study can be freeze-dried for storage purpose and dispersed prior to their characterization or actual usage. However, in case of protein-based pharmaceutical products, denaturation may occur due to the surface partition of the microspheres caused possibly during the process of drying. Among peptides for therapeutic purposes, insulin becomes a target of a protease and is prone to chemical, physical denaturation in a solution or suspension (Brange, et al., J. Pharm. Sci., 86, 1997, pp 517-525). Therefore, consideration must be taken into the stability of pharmaceutical products in view of the formulation as well. Thus, in order to facilitate insulin administration and to prevent the initial burst, the present invention provides a method of preserving the formulation by freeze-drying of the encapsulated product. The other objects and advantages of the invention will be further described and these will be apparent by the detailed embodiments of the present invention. This invention relates to a microencapsulation method while attaining stability of insulin contained in the formulation.
Microspheres of the present invention can be used for targeted delivery to the colon not only for local colonic pathologies to avoid systemic effects of drugs or inconvenient and painful trans-colonic administration of drugs, but also for systemic delivery of drugs like proteins and peptides, which are otherwise degraded and/or poorly absorbed in the stomach and small intestine, but may be better absorbed from the more benign environment of the colon.

As has been discussed before, hydrogels have been generally used to deliver hydrophilic, small-molecular weight drugs, which have high solubilities in both hydrophilic hydrogel matrix and surrounding aqueous media: In the matrices of the type invented herein, it is possible to achieve good encapsulation efficiency into the swollen hydrogel matrix and subsequently release the hydrophilic drug payload into an aqueous environment.

The matrices of the present invention may be potential use in encapsulation of drugs for the treatment of diseases sensitive to circadian rhythms such as asthma, angina and arthritis. Furthermore, colon delivery of drugs that are absorbable in the colon, such as steroids, which would increase the efficiency and enable reduction of the required effective dose, can be administered using these matrices. The treatment of disorders of the large intestine, such as irritable bowel syndrome, colitis, Crohn's disease and colon disease, where it is necessary to attain a high concentration of the drug, may be efficiently achieved by colon-specific delivery using the type of matrices developed in this art.

Overall, the present invention relates to a system or systems for releasing a drug or drugs specifically in the colon of the GI tract. More particularly, it relates to a colon-specific drug release system, which comprises a drug, encapsulated in an organic acid-soluble polymer material and/or polysaccharide (Mundargi et al., Drug Development and Industrial Pharmacy, 33, 2007, pp 1-10).

The present invention can be used not only for insulin delivery, but also can effectively be used for various polypeptides, proteins and derivatives thereof that are easily degraded in the upper part of the GI tract and are absorbed in the lower part of the GI tract to exhibit their pharmacological activities. Examples of such drugs may include insulin, calcitonin, angiotensin, vasopressin, desmopressin, luteinizing hormone-releasing hormone (LH-RH), somatostatin, glucagon, oxytocin, gastrin, ciclosporin, somatomedin, secretin, human antil natriuretic peptide (h-ANP), melanocyte-stimulating hormone, (MSH), adrenocorticotropic hormone (ACTH), β-endorphin, muramyl dipeptide, enkephalin, neuromtensin, bombesin, vasoactive intestinal polypeptide (VIP), parathyroid hormone
(PTH), calcitonin gene-related peptide (CGRP), cholecystokinin-8 (CK-8), thyrotropin-releasing hormone (TRH), endocerine, human growth hormone (hGH), cytokines (e.g., interleukin, interferon, colon-stimulating factor, and tumor necrosis factor), as well as derivatives thereof.

The above-mentioned peptides and proteins include not only naturally occurring substances, but pharmacologically active drugs, derivatives thereof and the analogues thereof (Mundargi et al., J. Control. ReL, 125, 2008, pp 193-209). For example, insulin used in the present art includes human insulin, porcine insulin, bovine insulin as well as their analogues, such as recombinants.

Drugs effective on diseases of lower part of GI tract, such as Crohn's disease, ulcerative colitis, irritable colitis, amoebiasis and colon cancer are also useful in the present invention. Examples of such drugs include: salazosulfapyridine, 5-aminosalicylic acid, cortisone acetate, triamcinolone, dexamethasone, budesonide, tegafur, budesonide, metronidazole, mesalazine, sulfasalazine, fluorouracil (Rokhade et al, J Microencapsulation, 24, 2007, pp 274-288) and derivatives thereof.

In addition to the above drugs of interest, the inventions of this patent will also cover physiologically active substances that can be used as the main active ingredient that is absorbed efficiently from lower part of GI tract. These include for instance, antitussive expectorants, such as theophylline (Rokhade et al, Carbohydrate Polymers 69, 2007, pp. 678-687), vasodilators, such as nicardipine hydrochloride (Soppimath et al, Drug Dev Ind Pharm, 27, 2001, pp. 507-515) and nifedipine (Shelke and Aminabhavi, Int. J. Pharma. 345, 2007, pp. 51-58), atenolol and carvedilol (Mundargi et al., Carbohydrate Polymers, 69, 2007, pp. 130-141, diltiazem hydrochloride (Aminabhavi et al., Designed Monomers and Polymers, 1, 1998, pp. 347-372), coronary vasodilators, such as iso-sorbide nitrite; antipyretic analgesics, such as acetaminophen, indomethacin (Sairam et al., J App. Polym. Sci., 104, 2007, pp. 1860-1865), hydrocortisone, ibuprofen (Mundargi et al., J. Microencapsulation, 25, 2008, pp. 228-240), salazopyrin, etc.

As confirmed from the above-described invention and examples therein, the developed oral CR formulations of insulin in which insulin was microencapsulated, can reduce the denaturation of insulin that may possibly occur during microencapsulation step as well as reduce the initial burst of insulin in a living body and thereby, preventing the risk of hypoglycemia. According to the present invention, insulin-loaded micron level
formulations are suitably prepared for successful oral delivery of insulin. Further, increased encapsulation efficiency and % inhibition of insulin on fasted as well as diabetic induced rat experiments suggest the success in developing the development of the devices for oral insulin delivery in a living body. The CR formulation according to the present invention exhibits stable pharmaceutical efficacy in a living body continuously for a long period of time. It is thus possible to adjust the serum glucose concentration of a diabetic patient in a more stable and controllable manner, while reducing the number of administrations and avoiding the sub-cutaneous (s.c) route injections. Further, the method developed is so simple that it can easily be scaled-up for large-scale applications.

EXAMPLES

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skilled in the art that the techniques disclosed in the examples, which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1: Insulin-loaded Eudragit L100 particles by solvent evaporation method

Solvent evaporation is a popular, simple and commercially accepted method of producing microparticles/microspheres/nanoparticles/nanospheres of uniform size. The method is well known in the prior art (Mundargi et. al. J. Control. ReL., 125, 2008, pp 193-209) and involves selecting the suitable solvent to dissolve the polymer. In the present invention, methanol was used for dissolving Eudragit L100. In the next step, insulin solution prepared in 0.1 M HCl was added. The insulin containing polymer solution was then homogenized using a high speed homogenizer. This solution was poured into the light liquid paraffin oil. The total solution was stirred to evaporate the solvent to obtain microspheres of uniform size. The method was repeated several times to obtain the desired size of the final particles.

In the present invention, we have adopted two different approaches and accordingly, we have developed two different methods, referred herein this work as method I and method II. While these methods do not differ significantly in their approaches, method II is more
appropriate to produce the microspheres of the formulations containing insulin giving higher encapsulation efficiency, uniform spherical particle size distribution, smooth surface morphology and offer minimal release of insulin in acidic media.

Method I

In this method, 500 mg of Eudragit L100 was dissolved in a mixture of 5 mL of methanol and 15 mL dichloromethane (DCM) under constant stirring in a 50 mL beaker. To this, 20 mg of human insulin dissolved in 0.5 mL of 0.1 M HCl was added and homogenized at 11,000 rpm for 2 min. The entire solution was transferred to a 250 mL beaker containing 100 mL of light liquid paraffin oil and 0.5% of span-80 surfactant. The solution was stirred at 600 rpm for 3 h to evaporate the methanol. After 3 h, particles were filtered and washed with Milli Q water to remove excess paraffin oil and span-80. The completely washed particles were dried at ambient temperature (25°C) for 24 h and stored at -20°C before characterization and further analysis.

Following the same method as above, blend microspheres of Eudragit L100 and Eudragit S100 were prepared. Here, 250 mg of Eudragit L100 was dissolved in 10 mL of methanol, while 250 mg of Eudragit RS100 was dissolved in 10 mL of DCM under constant stirring in a 50 mL beaker. Both solutions were mixed with constant stirring. To this, 20 mg of human insulin dissolved in 0.5 mL of 0.1 M HCl was added and homogenized at 11,000 rpm speed for 2 min. The solution was transferred to a beaker containing 100 mL of light liquid paraffin oil and 0.5% of Span-80 surfactant. The solution was stirred at 600 rpm speed for 3 h to evaporate methanol and DCM. After 3 h, the microspheres were filtered and washed with Milli Q water to remove the excess paraffin oil and Span-80. The washed particles were dried at ambient temperature for 24 h and stored at -20°C before characterization.

Method II

In this method, 720 mg of Eudragit L100 was dissolved in 8 mL of methanol under constant stirring in 15 mL falcon tube. To this, 36 mg of bovine/human insulin dissolved in 0.5 mL of 0.1 M HCl was added and homogenized at 11,000 rpm for 70 sec. The entire solution was transferred to a 1 liter plastic beaker containing 100 mL of light liquid paraffin oil and 0.5% of Span-80. The emulsion was stirred at 500 rpm for 4 h to evaporate methanol. After 4 h, particles were filtered and washed with 50 mL petroleum ether (60-80°C) and 8 mL Milli Q water to remove the excess paraffin oil and Span-80.
The microspheres were then dispersed in 2 mL water, freeze dried at -41°C for 20 h and stored at -20°C before characterization and further analysis.

Following the same protocol as above, microspheres of Eudragit S100 were prepared by taking 720 mg of Eudragit S100 dissolved in 8 mL of DCM at constant stirring in 15 mL falcon tube. To this, 36 mg of bovine insulin dissolved in 0.5 mL of 0.1 M HCl was added and homogenized at 11,000 rpm for 70 sec. The solution was transferred to 1 liter plastic beaker containing 100 mL of light liquid paraffin oil and 0.5 % of Span-80. The emulsion was stirred at 500 rpm for 4 h to evaporate methanol. After 4 h, the particles were filtered and washed with 50 mL of petroleum ether (60-80 °C) and 8 mL Milli Q water to remove the excess paraffin oil and Span-80. The microspheres were then dispersed in 2 mL water, freeze dried at -41°C for 20 h and stored at -20°C before characterization and further analysis.

EXAMPLE 2: Characterization of the copolymer:

(A) Size, shape and morphology analysis

Size measurement of the microspheres was done by ocular micrometer. Three sets of dry microspheres were randomly chosen and their size was measured by calibrated ocular micrometer. Mean size of the microspheres was considered in the present invention. The size of microspheres prepared by Method I is 1-5 µm, while those prepared by Method II vary from 10 to 40 µm.

For scanning electron microscopy (SEM), insulin-loaded microspheres were mounted on metal stubs using double-sided adhesive tape, drying in a vacuum chamber, sputter-coating-with a gold layer and viewing under the SEM (JSM-840J Jeol·Instruments, Tokyo, Japan) to characterize shape and morphology as well as to confirm the particle size.

SEM images of human insulin-loaded microspheres prepared by method I at 5000 X and by method II at 4000 X and 2000 X magnifications are shown in Fig. 1 (A) and 1 (B), respectively. Microspheres prepared by Method I (2.5 % polymer concentration) are spherical in shape with agglomerations having slight rough surfaces. The microspheres prepared by Method II (9 % polymer concentration) are also spherical but without any agglomerations and are having smooth surfaces. Further, process parameters viz., polymer concentration, insulin loading and solvent plays significant roles in the preparation of microspheres (Mundargi et al., J Control Release 119, 2007, pp 59-68).
EXAMPLE 3: Determination of encapsulation efficiency of insulin

Microspheres (50 mg) were dissolved in 5 mL methanol and insulin was extracted using 25 mL 0.1 M HCl in a separating funnel, shaken for few minutes and solution was filtered through a filter of 0.22 µm diameter. The insulin content was estimated by HPLC and the % encapsulation efficiency was determined. Both the methods are discussed hereunder.

HPLC Method I

Insulin was separated on C18 Vydac 218MS54 column (4.6 x 250 mm) having the pore size of 300 Å and particle size of 5 µm. The buffer solution for mobile phase was prepared by dissolving 28.4 g of anhydrous sodium sulfate in 1000 mL double distilled water and pH of the buffer was adjusted to 2.3 by adding 2.7 mL of orthophosphoric acid. The mobile phase consisted of (A) 82:18 of acetonitrile buffer and (B) 50:50 acetonitrile:buffer solutions. The HPLC run was carried out in gradient mode (see Table 1) at flow rate 0.5 mL/min, injection volume of 100 µL and detection wavelength of 210 nm.

Table 1. Gradient mode HPLC conditions

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Conc. B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>16</td>
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<tr>
<td>22</td>
<td>60</td>
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<tr>
<td>24</td>
<td>100</td>
</tr>
<tr>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td>32</td>
<td>16</td>
</tr>
</tbody>
</table>

HPLC Method II

In this method, insulin was analyzed using MN C18 column (250 x 4.6 mm). HPLC run was carried out in a gradient mode (see Table 2) at a flow rate of 1 mL/min, injection volume of 100 µL and detection wavelength of 210 nm. Mobile phase consisted of (A) water (0.05 % trifluoroacetic acid, TFA,v/v) and (B) acetonitrile: water (80:20) (0.05 % TFA v/v).

Table 2 Gradient mode HPLC conditions

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Conc. B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- 16 -
Weight of drug in microspheres\[\% \text{ loading} = \left(\frac{\text{Weight of drug in microspheres}}{\text{Weight of microspheres}}\right) \times 100\]

\[\% \text{ Entrapment efficiency} = \left(\frac{\text{Drug loading}}{\text{Theoretical loading}}\right) \times 100\]

Microspheres produced by the Method I have rough surfaces giving encapsulation efficiency (EE) of 52 % and 26 % for Eudragit L100 and Eudragit L100/RS100 (50:50) blend respectively. Lower EE is due to loss of insulin in the external phase of paraffin oil. When insulin in 0.1 M HCl is homogenized with a mixture of methanol and DCM, low EE of 33 % was observed. Further, change in solvent from methanol + DCM mixture to only methanol and increasing the polymer concentration from 5 % to 9 % in the primary emulsion as per method II, increased the yield of microspheres to 95 % and the EE of 63 %, whereas for human insulin, the yield and EE values are 92 % and 64 %, respectively. With bovine insulin-loaded Eudragit RS100 microspheres, the yield is 94 %, while EE was 33 %.

**Table 3. Formulation parameters of insulin-loaded systems**

<table>
<thead>
<tr>
<th>Method type</th>
<th>Insulin</th>
<th>Polymer type</th>
<th>% Yield</th>
<th>% EE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method I</td>
<td>Human</td>
<td>Eudragit L100</td>
<td>86</td>
<td>52</td>
</tr>
<tr>
<td>Method I</td>
<td>Human</td>
<td>Eudragit L100 and Eudragit RS100</td>
<td>80</td>
<td>26</td>
</tr>
<tr>
<td>Method II</td>
<td>Bovine</td>
<td>Eudragit L100</td>
<td>95</td>
<td>63</td>
</tr>
<tr>
<td>Method II</td>
<td>Human insulin</td>
<td>Eudragit L100</td>
<td>92</td>
<td>64</td>
</tr>
<tr>
<td>Method II</td>
<td>Bovine</td>
<td>Eudragit RS100</td>
<td>94</td>
<td>33</td>
</tr>
</tbody>
</table>
EXAMPLE 4: Circular Dichroism (CD)

The insulin released samples were filtered (0.22 µm; Millipore; Ireland) prior to CD measurement (for insulin integrity) to remove particulate matter and possible protein aggregates. CD spectra at 25°C were obtained using a Jasco J-180 spectropolarimeter using a 1 cm path length quartz cell at the protein concentration of 2 mg/mL. Analysis conditions were as follows: 0.5 nm bandwidth, 10-mdeg sensitivity, 0.2-nm resolution, 2s response, 10 nm/min scanning speed and 200-240 nm measuring range. Each spectrum is the average of at least three runs, being the buffer baseline subtracted from the average spectra. Final spectra are presented in mean residual ellipticity. Deconvolution of CD spectra was obtained by the SELCON method (Hua et al., J Biol Chem 279 (2004) pp 21449-2146).

CD spectra provide qualitative as well as quantitative information about protein conformations (Prieto et al., J Mol Biol 268, 1997, pp 760-778). In this work, CD is used to probe the unfolding and folding of protein secondary structure either at equilibrium or kinetically. Figure 2 displays different CD spectra viz., (A) pure bovine insulin, (B) for encapsulated bovine insulin and (C) released bovine insulin from the developed formulation at pH 7.4. Figure 3 shows the CD spectra of (A) pure human insulin, while (B) shows that of released human insulin from Eudragit formulations in pH 7.4. The CD spectra in neutral pH revealed no significant difference in the secondary structure of released insulin compared to the native insulin.

EXAMPLE 5: In vitro release of insulin-loaded microspheres

The in vitro release experiments were done by taking 100 mg of insulin-loaded Eudragit L100 or Eudragit L100/RS 100 blend particles in a flask containing 25 mL of buffer solution. Particles were placed in buffer solution to observe insulin release. The dissolution was carried out in an incubator maintained at 37°C under constant stirring at 200 rpm. At regular intervals of time, aliquot samples (2 mL each time) were withdrawn and analyzed for insulin using the HPLC at the λmax value of 210 nm employing the gradient method. In order to simulate the stomach and intestinal environments, all release experiments were performed in solutions of pH of 1.2 and 7.4, respectively. The formulations were kept in 1.2 pH media for the first 2 h and later, in pH of 7.4 media to follow the intestinal environment.
In vitro release experiments were done on the formulated microspheres prepared by Method II. In this case, 50 mg of the microspheres in pH 1.2 and pH 7.4 media were separately taken in 15 mL falcon tubes. Dissolution was performed in an incubator at 37°C under stirring at 100 rpm. Then, 1 mL sample was withdrawn each time at fixed interval of time and analyzed using HPLC method II.

In vitro release profiles of insulin-loaded Eudragit microspheres at pH 1.2 and 7.4 shown in Fig. 4 indicate that at pH 1.2, polymer shrinks, while at pH 7.4, it swells to release the insulin. At pH 1.2, Eudragit L100 has released 9% insulin, but at pH 7.4, almost 100% release of insulin occurred within 5 h. To minimize the amount of insulin released in pH 1.2, insulin-loaded microspheres were formulated as tablet using poly(vinyl pyrrolidone), magnesium stearate and microcrystalline cellulose as excipients. Further, the formulated tablet was coated with 5% Eudragit L100 solution in isopropyl alcohol by dip-coating. By this process, insulin release in pH 1.2 is negligible, but maximum release occurred in pH 7.4 as shown in Fig. 5.

The release profile of Eudragit formulations changes with pH. For instance, insulin release starts from third hour in pH 7.4, but not during the first two hours in pH 1.2. A maximum amount of insulin was released in about 5 h. In case of Eudragit L100, only 9% insulin was released at pH 1.2, while a burst release was observed in pH 7.4. On the other hand, blending of Eudragit RS100 with Eudragit L100 prevented insulin release at pH 1.2 completely followed by a maximum release in pH 7.4 as shown in Fig. 6.

Figure 7 displays the in vitro release of bovine insulin-loaded Eudragit L100 microspheres prepared by Method II. In this formulation, insulin release in pH 1.2 is 0.3%, while in pH 7.4, it is 93% in 3 h. In the case of human insulin-loaded Eudragit L100 formulation, insulin release in pH 1.2 is 0.8%, whereas in pH 7.4, it is 76% in 3 h as displayed in Fig. 8.

**EXAMPLE 6: Methods for testing in vivo efficacy of insulin-loaded microspheres on diabetes-induced rats**

Male Wistar rats (250 g) were housed in a 12-12 h light-dark cycle, constant temperature environment of 22°C, relative humidity of 55 and allowed free access to water and food during acclimatization. To minimize the diurnal variance of blood glucose, all experiments were performed in the morning. Diabetes was induced with intravenous injection of 150 mg/kg alloxan in saline (0.9% NaCl). Ten days after the treatment, rats...
with frequent urination, loss of weight and blood glucose levels higher than 300 mg/dL were included in experiments. Blood glucose levels were determined by glucose oxidase/peroxidase method using a glucometer. A 5 % dextrose solution was given in feeding bottle for a day to overcome the early hypoglycemic phase. After 72 hours blood glucose was measured by glucometer. The diabetic rats (glucose level > 300 mg/dl) were separated.

In order to investigate the effects of oral insulin-loaded particles, 12 h fasted diabetic rats were fed with insulin-loaded particles (20 IU) or placebo particles as control. Glucose was measured on a drop of blood collected from the tail vein before and at different intervals up to 4 h after oral administration. Results were expressed as means ± standard deviation (SD) or means ± standard errors of means.

Group 1 received the saline by the intraperitoneal (IP) route. Group 2 rats received the 20 IU of insulin-loaded particles Eudragit L100. Particles dispersed in a mixture of 9 ml of 5 % carboxy methyl cellulose (CMC) and 1 ml of 1M HCl solution through the oral route using oral feeding needle. Group 3 rats received the 20 IU of insulin-loaded particles Eudragit L100/RS 100 blend particles dispersed in a mixture of 9 ml of 5 % CMC and 1 ml of 1 M HCl through oral route using oral feeding needle.

After 30 min of the above administrations, rats of all groups were orally treated with 2 g/kg of glucose. Blood samples were collected from the rat tail vein/retro orbital just prior to glucose administration i.e., 0 min and at 30, 60 and 90 min after glucose loading. Blood glucose levels were measured immediately using a glucometer.

Estimation of blood glucose level:

The pulsatom gluco-strips (stored in refrigerator) were used for estimation. The glucometer is calibrated to 660 units or as according to the specifications mentioned in the strips. The blood removed iron the rat is immediately spread on the marked end of the strip. The strip is inserted in the glucometer where two electrodes are situated. After few seconds, the glucometer displays the blood glucose level.

Figure 9 displays in vivo efficacy (oral glucose tolerance test) of insulin-loaded Eudragit L100 and Eudragit RS100 blend microspheres. In this, insulin-loaded Eudragit L100 microspheres administered by oral route dropped the glucose level from 455 to 62 with 86 % inhibition, whereas insulin-loaded Eudragit L100 and RS 100 blend microspheres administered by oral route dropped glucose level from 319 to 258 with 42 % inhibition.
in 3 h (Fig. 10). On the other hand, the insulin injected through intra-peritoneal (IP) route has shown 65-85 % inhibition, a value that is still lower than that of Eudragit L100 microspheres within one hour of injection. However, it showed increased inhibition at 3 h, but the inhibition is still lower than the formulation.

EXAMPLE 7: Storage data:

The insulin-loaded Eudragit L100 and Eudragit L100/RS 100 microspheres prepared were stable up to three months. There was no change in color or any other physical characteristics.

DISCUSSION OF EXPERIMENTAL PROTOCOLS


Yet another report (Agarwal et al., Intl. J. Pharm. 225, 2001, pp 31-39) deals with the use of cp-precipitation technique to prepare insulin-loaded microspheres of Eudragit L100 (polymethacrylate) to investigate the effect of parameters like addition of salts in the precipitating medium and ratio of polymeric solution to volume of precipitating medium on the dissolution and encapsulation efficiency. Damge et al., (Damge et al., J. Control. ReL, 117, 2007, pp 163-170) developed the nanoparticles of biodegradable poly(ε-caprolactone) and a non-biodegradable Eudragit® RS 100 blends using poly(vinyl alcohol) as a surfactant to obtain high encapsulation efficiency up to 96 %. Since, poly(ε-caprolactone) even though it is biodegradable, it required longer time to degrade, and hence, was not recommended for short-acting oral insulin delivery.

Suitability of Eudragit L100 microspheres prepared by double emulsion-solvent evaporation method was evaluated in a recent report by Jain and Majumdar (Jain et al., J Biomat App, 21, 2006, pp 195-211). Various parameters were optimized to attain the maximum EE and optimum in vitro release profile. The microspheres retarded insulin release at gastric pH and provided its slow release in neutral pH of the upper intestine. In
a study by Gowthamarajan et al., (Gowthamarajan et al., Indian J Pharm Science 65, 2003, pp 177), microspheres of Eudragit L100 and S100 loaded with insulin, protease inhibitor and bile salts were prepared by solvent diffusion technique and the microspheres have shown the delayed release of insulin. The ideal batches of microspheres (prepared from Eudragit L100 alone and S 100 alone as carriers and 1% aprotinin and 1% sodium glycocholate) were used for in vivo evaluation of hypoglycemic effect. The in vivo evaluation of microspheres of Eudragit L-100, 1% aprotinin and 1% sodium glycocholate showed the prolonged hypoglycemic effect for 3 h compared to the intravenous injection of bovine insulin.

In order to achieve the bioavailability of insulin after oral administration, two hurdles needed to be overcome: (i) insulin transport across mucosal barrier is restricted and (ii) insulin degradation by proteolytic enzymes of the stomach as well as intestinal lumen. Present formulations meet these requirements. With the above-mentioned general criteria for a successful development of insulin-loaded formulation, the present invention dealt with Eudragit L100 and RS100, both individually as well as their blends.

In conclusion, all of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the methods described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents that are chemically or physiologically related may be substituted for the agents described herein, while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.
We claim:

1. A particle comprising a pH-sensitive copolymer comprising a Eudragit L100, or Eudragit RS100, or blends thereof, wherein a moiety encapsulated in the particle is not released at acidic pH, and is released at neutral pH.

2. The particle of claim 1, wherein a dimension of the particles is between 10-40 µm.

3. The particle of claim 1, wherein a dimension of the particles is between 1-5 µm.

4. The particle of claim 1, wherein the particle has an encapsulation efficiency of at least about 26%, at least about 33%, at least about 52%, at least about 63% and at least about 64%.

5. The particle of claim 1, wherein at least 90% of the encapsulated moiety is released within 3-5 hours at neutral pH.

6. The particle of claim 1, wherein the neutral pH corresponds to the pH in the lower gastrointestinal (GI) tract of a human.

7. The particle of claims 5 or 6, wherein the neutral pH is about 7.4.

8. The particle of claim 1, wherein the acidic pH corresponds to the pH in the upper gastrointestinal (GI) tract or stomach of a human.

9. The particle of claim 8, wherein the acidic pH is about 1.2.

10. The particle of claim 1, wherein the copolymer is formed by a method selected from the group consisting of emulsion polymerization, dispersion polymerization, solvent evaporation, in situ gel formation, precipitation, and free radical polymerization.

11. A controlled-release (CR) composition for oral delivery of a biologically active moiety, the composition comprising:
   a particle according to any of claims 1-10; and
   a biologically active moiety.

12. The CR composition of claim 11, wherein the copolymer does not alter the stability of the biologically active moiety.
13. The CR composition of claim 11, wherein the biologically active moiety is intended to be delivered to the colon and not delivered to the stomach or small intestine of a mammalian subject.

14. The CR composition of claim 13, wherein the biologically active moiety is a protein or polypeptide or derivative thereof.

15. The CR composition of claim 13, wherein the biologically active moiety is insulin, calcitonin, angiotensin, vasopressin, desmopressin, luteinizing hormone-releasing hormone (LH-RH), somatostatin, glucagon, oxytocin, gastrin, cyclosporin, somatomedin, secretin, human artial natriuretic peptide (h-ANP), melanocyte-stimulating hormone, (MSH), adrenocorticotropic hormone (ACTH), β-endorphin, muramyl dipeptide, enkephalin, neurotensin, bombesin, vasoactive intestinal polypeptide (VIP), parathyroid hormone (PTH), calcitonin gene-related peptide (CGRP), cholecystokinin-8 (CK-8), thyrotropin-releasing hormone (TRH), endocerine, human growth hormone (hGH), cytokines, interleukin, interferon, colon-stimulating factor, tumor necrosis factor, or derivatives thereof.

16. The CR composition of claim 15, wherein the biologically active moiety is insulin in liquid solution form.

17. The CR composition of claim 15, wherein the biologically active moiety is natural or recombinant human insulin, porcine insulin, bovine insulin, or their analogues.

18. The CR composition of claim 13, wherein the biologically active moiety is a drug suitable for treatment of a disorder of the large intestine selected from irritable bowel syndrome, colitis, ulcerative colitis, irritable colitis, amoebiasis, Crohn's disease and colon cancer.

19. The CR composition of claim 18, wherein the biologically active moiety is selected from the group consisting of salazosulfapyridine, 5-aminosalicylic acid, cortisone acetate, triamcinolone, dexamethasone, budesonide, tegafur, budesonide, metronidazole, mesalazine, sulfasalazine, fluorouracil and derivatives thereof.

20. The CR composition of claim 18, wherein the biologically active moiety is selected from the group consisting of antitussive expectorants, theophylline, vasodilators, nicardipine hydrochloride, nifedipine, atenolol, carvedilol, diltiazem hydrochloride, coronary vasodilators, iso-sorbide nitrite, antipyretic analgesics, acetaminophen, indomethacin, hydrocortisone, ibuprofen, salazopyrin, and derivatives thereof.
21. A pharmaceutical formulation comprising:
   a composition according to any of claims 11-20; and
   a pharmaceutically acceptable excipient.

22. The pharmaceutical composition of claim 21, wherein the excipient is selected from
   the group consisting of: poly(vinyl pyrrolidone), magnesium stearate and
   microcrystalline cellulose.

23. The pharmaceutical composition of claim 21 or 22, wherein the formulation is
   suitable for oral consumption.

24. A method for encapsulating insulin in a particle according to any of claims 1-10, the
   method comprising:
   encapsulating insulin into polymeric microspheres by a double emulsion; and then
   subjecting to solvent evaporation technique in the presence of paraffin oil.

25. A method for reducing insulin levels in an individual, the method comprising:
   measuring a level of glucose in an individual; and
   administering a controlled-release composition according to any of claims 11-20,
   wherein administration of the composition reduces glucose levels in the individual.

26. The method of claim 25, wherein the glucose level is reduced by at least about 42%.

27. The method of claim 25, wherein the glucose level is reduced by at least about 86%.

28. The method of claim 25, wherein the reduction of glucose level is comparable to that
   achieved by subcutaneously injected insulin.

29. A method for treating Type I diabetes in an individual, the method comprising:
   administering to an individual an amount of a controlled-release composition
   according to any of claims 11-20, wherein administration of the composition reduces
   a symptom of Type I diabetes.

30. A method for treating Type II diabetes in an individual, the method comprising:
   administering to an individual an amount of a controlled-release composition
   according to any of claims 11-20, wherein administration of the composition reduces
   a symptom of Type II diabetes.

31. The method according to claims 24 or 25, wherein the symptom of Type I or Type II
   diabetes is an elevated glucose level.
32. The method of claim 26, wherein the glucose level is reduced by at least 30% within 3-5 hours following administration of the controlled-release composition.

33. A particle comprising a pH-sensitive copolymer comprising a Eudragit L100, or Eudragit RS100, or blends thereof, and its application as claimed above exemplified herein substantially in the examples and figures.
Fig. 1(A). Scanning electron microscopic images of group of microspheres of Eudragit L100 prepared by method I.

Fig. 1(B). Scanning electron microscopic images of group of microspheres of Eudragit L100 prepared by method II.
Fig. 2 (A). CD spectra of pure bovine insulin, (B) CD Spectra of encapsulated bovine insulin and (C) CD spectra of released bovine insulin
Fig. 3 (A). CD spectra of pure human insulin and (B) CD Spectra of released human insulin.

Fig. 4. *In vitro* release of insulin-loaded Eudragit L100 in pH 1.2 and 7.4.
Fig. 5. *In vitro* release of Eudragit L100-coated insulin-loaded Eudragit L100 tablet in pH 1.2 and 7.4.

![Graph showing % Cumulative Release vs. Time (h) with pH 1.2 and pH 7.4 markers.]

Fig. 6. *In vitro* release of bovine insulin-loaded Eudragit L100/Eudragit RS100 microspheres prepared by method I.

![Graph showing % Cumulative Release vs. Time (h) with pH 1.2 and pH 7.4 markers.]
Fig. 7. *In vitro* release of bovine insulin-loaded Eudragit L100 microspheres prepared by method II.

![Graph showing % cumulative release over time for pH 1.2 and pH 7.4](image)

Fig. 8. *In vitro* release of human insulin-loaded Eudragit L100 microspheres prepared by method II.

![Graph showing % cumulative release over time for pH 1.2 and pH 7.4](image)
Fig. 9. *In vivo* animal experiments on (●) control, (■) insulin loaded Eudragit L100 particles (20 IU/200 g) and (▲) insulin-loaded Eudragit L100/RS100 blend particles (20 IU/200 g).

Fig. 10. The % inhibition of oral insulin delivery formulations of insulin-loaded Eudragit L100 and Eudragit L100/RS100 blend microspheres.