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(71) Applicant *(for all designated States except US)*: **RELIANCE LIFE SCIENCES PVT. LTD.** [IN/IN]; Dhirubhai Ambani Life Sciences Centre, R-282, TTC Area of MIDC, Thane Belapur Road, Rabale, Navi Mumbai 400 701, Maharashtra (IN).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **RANGASWAMY, Vidhya** [IN/IN]; Dhirubhai Ambani Life Sciences Centre, R-282, TTC Area of MIDC, Thane Belapur Road, Rabale, Navi Mumbai 400 701, Maharashtra (IN). **AMINABHAVI, Tejraj, Malleshappa** [IN/IN]; Dhirubhai Ambani Life Sciences Centre, R-282, TTC Area of MIDC, Thane Belapur Road, Rabale, Navi Mumbai 400 701, Maharashtra (IN). **BABU, Vadde, Ramesh** [IN/IN]; Dhirubhai Ambani Life Sciences Centre, R-282, TTC Area of MIDC, Thane Belapur Road, Rabale, Navi Mumbai 400 701, Maharashtra (IN). **MUNDARGI, Raghavendra, Chaluvayya** [IN/IN]; Dhirubhai Ambani Life Sciences Centre, R-282, TTC Area of MIDC, Thane Belapur Road, Rabale, Navi Mumbai 400 701, Maharashtra (IN).

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(54) **Title**: NOVEL COPOLYMERS FOR CONTROLLED RELEASE DELIVERY SYSTEM

(57) **Abstract**: The present disclosure relates to pH-sensitive copolymers poly(methacrylic acid-*co*-*N*-vinylcaprolactam), designated as P(MAA-co-VCL). The disclosure in particular provides the synthesis of a copolymer of poly(methacrylic acid-*co*-*N*-vinylcaprolactam) and its application for pH sensitive oral delivery of active ingredients *in vitro* and *in vivo*. The invention in particular provides microparticles comprising the copolymers for controlled release of drug delivery. Methods for delivering drug to the lower GI tract, while avoiding exposure to the stomach acids is provided, methods for treating diabetes using controlled release insulin delivery particles are provided.

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NOVEL COPOLYMERS FOR CONTROLLED RELEASE DELIVERY SYSTEM

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

This application claims priority to Indian Provisional Patent Application No. 857/MUM/2009 filed 31 March, 2009, the entire content of which is incorporated by reference.

TECHNICAL FIELD OF THE INVENTION

The present invention relates to polymeric microparticles for drug delivery. In particular, the invention relates to a pH-sensitive copolymer for oral delivery of active ingredients in the intestine. The invention in particular relates to the synthesis of copolymer of poly(methacrylic acid-*co*-*N*-vinylcaprolactam), designated as P(MAA-*co*-VCL) for oral delivery of active ingredients.

BACKGROUND OF THE INVENTION

Oral drug formulations or compositions require efficient delivery of the active ingredient to the site of absorption. Controlled delivery formulations loaded with bioactive molecules have been widely developed and used for a variety of applications for delivering the active ingredient. Such devices may include a simple capsule or microcapsules for oral administration, injections, microspheres, microparticles, nanospheres, nanoparticles, liposomes, implants, etc.

The current trend in drug delivery technology is towards the development of various polymers, which are biocompatible, biodegradable, and promises to deliver the active ingredient at a particular pH. The development of pH-sensitive polymers therefore, has gained impetus in the recent years, which comprise mainly copolymers developed for release of the active ingredient in the desired pH.

There has been considerable research activity in developing hydrogels that are sensitive to the surrounding physiological pH and these are found to be the ideal systems for site specific delivery of the active ingredient.

The various biopolymers available in developing these formulations are viz., biodegradable poly(glycolic acid), poly(lactic acid), poly(lactic acid-*co*-glycolic acid), poly(lactic acid-co-poly(ethylene glycol)), dextran–PEG, and pH-sensitive polymers like poly(acrylic acid) and poly(methacrylic acid) as well as complexing hydrogel graft polymers like poly

methylmethacrylate with ethylene glycol, chitosan, cyclodextrin, etc. These polymers have been evaluated for the most challenging task of developing oral delivery system for insulin (see review paper by Ramesh et al., Expert Opinion, Drug Delivery, 5, 2008, 403-415).

Recent advances in the various polymers for encapsulation have enabled efficient delivery of active ingredients without any loss in the activity or untoward side effects.

The pH-sensitive hydrogels are made up of polymers, which can exhibit reversible formation of inter-polymer complexes, which are sensitive to pH. These complexes are usually developed to be insoluble at lower or gastric pH and as the polymer passes through the stomach into the intestine, the increase in pH causes swelling and dissociates these complexes thereby, leading to the release of the active ingredient in the intestine.

One such example for pH-sensitive and colonic drug delivery extensively studied is controlled delivery of insulin. The prior art cites many examples, but the research efforts of Nakamura and Yakuzaigaku (Nippon Yakuzai Gakkai, 64, 2004, pp 350-353) dealt with the development of insulin-loaded oral delivery system using the copolymer of methacrylic acid and ethylene glycol. These systems when tested for insulin absorption by small intestine showed the dependence of polymer size and ionic strength of the medium on controlled release of insulin.

Wood et al., (AIChE Annual Meeting, Conference Proceedings, San Francisco, CA, United States, Nov. 12-17, 2006, American Institute of Chemical Engineers, New York) developed another method using stimuli-responsive complexation hydrogels of methacrylic acid grafted onto ethylene glycol chains functionalized with wheat germ agglutinin (WGA). In this study, insulin entrapment was unaffected by WGA functionalization .

Singh et al., (Indian Pat. Appl. 2008, 35 pp. IN 2006DE01437 A 20080104, Application: IN 2006-DE1437 20060616) invented hydrogel copolymeric microparticles of poly(methacrylic acid-*co*-poly(ethylene glycol) dimethacrylate), poly(methacrylic acid-*co*-poly(ethylene glycol) acrylate), poly(acrylic acid-*co*- poly(ethylene glycol) acrylate) and poly(acrylic acid-*co*-poly(ethylene glycol) dimethacrylate) by the 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.

Hassan (Egypt. 2004, pp 19, EG 23187 A, 20040731, Application: EG 2002-827 20020720) developed the CR insulin oral capsule formulations containing chitosan,

hydroxypropyl cellulose, methyl cellulose, and methacrylic acid copolymers providing optimum pH for insulin release.

Conventional methods of insulin encapsulation have several limitations such as high shear stress, exposure to organic solvents and extreme temperatures that degrade the insulin (Cairno and Mathiowit, Adv. Drug Deliv. Rev. 35, 1999, pp 249-257). In order to prevent insulin for oral administration from being denatured in the digestive system, micro or nanoparticles of biodegradable polymers have been developed. Recent review by Mundargi et al., (J. Controlled Release, 125, 193-209, 2008) covers many examples of carrier devices and related issues on the controlled release of proteins and peptides through biopolymers.

Other studies in the prior art on grafted polymer of methacrylic acid with polethylene glycol i.e., P(MAA-g-EG) hydrogels have dealt with their potential 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) thereby affecting the proteolytic activity of calcium-dependent enzymes, such as trypsin.

Insulin-loaded polymer (ILP) microparticles composed of poly(methacrylic acid) and poly(ethylene glycol) having pH dependent complexation and mucoadhesive properties were suggested to be the potential carriers for insulin via oral route. (Morishita, et al., J. Control. Rel., 97, 2004, pp 115-124). Nevertheless, further optimization of the polymer delivery system is required to improve clinical application. Therefore, the effect of particle size of the ILP on insulin absorption was studied in the *in situ* loop system, hypothesizing that smaller particle sizes of ILP could induce bigger hypoglycemic effects due to increase in mucoadhesive capacity. To verify the hypothesis, the adhesive capacities of differently sized ILPs to the mucosal tissues were evaluated. Additionally, the intestinal site-specificity of ILP for insulin absorption was investigated. Intra- and inter-cellular integrity and/or damage were also examined by lactate dehydrogenase leakage and membrane electrical resistance change to ensure the safety of ILP as a carrier for oral route.

Other oral insulin delivery devices based on (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) and P(PAA-g-EG) in addition to biopolymers like chitosan, cyclodextrin, etc., in various combinations with methacrylic or acrylic-polymers have been

reported (Ramesh et al., Expert Opinion, Drug Delivery, 5, 2008, 403-415). According to the most recent prior art (Teply, 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 electrostatic interactions

Insulin was loaded 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. (Paul and Sharma, J. Pharma. Sci., 97, 2008, pp 875-882)

Mahkam (J. Biomed. Mater. Res. Part B Appl. Biomater. 75B, 2005, 108-112) developed the copolymeric hydrogels of 2-hydroxyethyl methacrylate (HEMA) and methacrylic acid (MAA) that contained 2, 4, and 6 % of cubane-1,4-dicarboxylic acid as the cross-linking agent by the free radical copolymerization at 70°C.

The pH-sensitive graft copolymers of poly(methacrylic acid) and poly(ethylene glycol) i.e., P(MAA-*g*-EG) were invented for insulin delivery (Lowman and Peppas Macromolecules 30, 1997, pp 4959-4965). In acidic environment of the stomach, these hydrogels stayed in a complexed state to protect the insulin, but at intestinal pH (alkaline condition), the complex was immediately dissociated releasing the insulin.

Tuesca et al., (J. Pharma. Sci., 97, 2008, pp 2607-2618) developed hydrogels of poly(methacrylic acid) grafted onto poly(ethylene glycol) i.e., 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 %.

Hydrogels of PMMA-CS (Sajeesh and Sharma, J. Appl. Polym. Sci., 99, 2006, pp 506-512) prepared by free-radical polymerization using water-soluble initiator were also evaluated for oral insulin delivery. The *in vitro* experiments of these systems exhibited pH-dependent insulin release in alkaline pH up to 3-4 h.

Foss et al., (Eur. J. Pharm. Biopharm., 57, 2004, pp 163-169) reported the cross-linked networks of MAA grafted onto PEG (size, 200 nm) by free-radical precipitation/dispersion methods. The size of particles increased by increasing the pH of the medium i.e., the size ranged from 200 nm at the pH of 2.0 to 2 mm at the pH of 6.0. Insulin was encapsulated into

these copolymers at 9.33 and 9.54 mg/140 mg of solid hydrogel matrix by partitioning from the concentrated insulin solution.

In the 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, organic solvent, excessive freezing and drying. It is possible that the microencapsulated proteins may be hydrated even during storage and proteins are prone to denaturation and aggregation under these circumstances. The polymer degrades after being administered, thereby creating highly concentrated acidic microenvironment inside and around the polymer due to the possible decomposed acidic monomer. Under these circumstances, proteins are prone to aggregation, hydrolysis and chemical change, thereby affecting the delivery rate, leading to denaturation, aggregation or inactivation of the proteins.

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). Therefore, careful consideration must be taken into the stability of the formulated products. The present invention relates to the development of microparticle-based encapsulation to attain the stability of insulin contained in the formulations.

Among the examples of many pH-sensitive polymers, the choice by the majority of inventors is poly(methacrylic acid) and poly(ethylene glycol), which form inter-polymer complexes in acidic media (stomach) and dissociate in neutral media (intestine). However, the most challenging aspect of these polymers is to avoid the burst release of the peptide in the neutral pH. This is accomplished by the judicious selection of the particle size of the polymers in addition to various other parameters.

Thus, looking into the need for an ideal copolymer system, the present invention has focused on the development of a copolymer of poly(vinyl caprolactam) with methacrylic acid. The invention in particular provides a copolymer of *N*-vinylcaprolactam and methacrylic acid. The most important application of these copolymers was studied in the formulation of insulin-loaded hydrogel microparticles. The novel hydrogel prepared from *N*-vinylcaprolactam and methacrylic acid was sensitive to pH changes of the surrounding media *in vitro*.

OBJECTIVES OF THE INVENTION:

It is the aim of the present invention to provide oral formulations of pH-sensitive active ingredient encapsulated by the copolymer of the present invention.

It is the aim of the present invention to provide oral formulations of insulin encapsulated by copolymer of the present invention.

SUMMARY OF THE INVENTION

The present invention provides the development of a novel copolymer of *N*-vinyl caprolactam and methacrylic acid. This copolymer is sensitive to pH changes and thus, enables efficient formulations of pH-sensitive active ingredients. The present invention also provides pH-sensitive hydrogel formulation of insulin.

The present invention provides a novel route of the synthesis of a pH-sensitive copolymer. The present invention relates to a pH-sensitive copolymer of *N*-vinylcaprolactam and methacrylic acid.

The present invention relates to hydrogel compositions of the insulin that allow negligible release of the insulin in certain pH ranges and is able to provide sufficient release of insulin in the intestine.

The present invention provides a method to prepare novel pH-sensitive copolymer. In particular, the present invention provides a copolymer of *N*-vinylcaprolactam and methacrylic acid. The copolymer prepared can be used for oral delivery of pH-sensitive active ingredients.

In one embodiment, the present invention provides copolymer of *N*-vinylcaprolactam and methacrylic acid developed with two different proportions. In one preferred embodiment, the copolymer has vinyl caprolactam (VCL) and methacrylic acid (MAA) components in proportions of 75:25 to 50:50.

In one embodiment, the present invention provides methods of preparation of composition by using different methods such as emulsion polymerization, dispersion polymerization, solvent evaporation, *in situ* gel forming, and precipitation method. In one preferred embodiment, the present invention provides a method of preparation of composition using free radical polymerization.

In one embodiment, the present invention provides a copolymer of *N*-vinyl caprolactam and methacrylic acid, whose formulations are available in the size range of 50-100 microns.

In one embodiment, the present invention provides methods for characterization of the copolymer. In one preferred embodiment, the present invention provides FTIR, differential scanning calorimetry, thermogravimetry, size analyzer and scanning electron microscopy for establishing the chemical structure, thermal properties, size, shape and morphology of the copolymer.

In one embodiment, the present invention provides compositions for the oral delivery of active ingredient using the pH-sensitive copolymeric formulations of the present invention.

In one preferred 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 copolymer of the present invention. In one preferred embodiment, the oral formulation is a hydrogel.

In one embodiment, the present invention provides hydrogel, which does not release the active ingredient in acidic pH, but swells in neutral pH to release the active ingredient. In one preferred embodiment, the hydrogel of insulin shrinks at pH 1.2 and swells in pH 7.4 to release the insulin.

In one embodiment, the present invention provides a novel copolymer 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 shows FTIR spectra of (1A) monomer vinyl caprolactum and (1B) copolymers of poly(VCL-co-MAA).

FIG. 2. shows DSC thermogram of poly(VCL-co-MAA).

FIG.3 shows particle size distribution curve of microparticles after 20 passes through a Microfluidizer

FIG. 4 shows a TGA thermogram of poly(VCL-co-MAA).

FIG. 5 shows photographs of scanning electron microscope, illustrating the group of microparticles of poly(VCL-co-MAA).

FIG. 6 shows HPLC chromatograms of standard human insulin released at pH 1.2 and at pH 7.4.

FIG. 7 shows in vitro release profile of insulin-loaded microparticles of poly(VCL-co-MAA) in simulated gastric medium (pH 1.2) and intestinal medium (pH 7.4). Results presented are the representative means of ± SD of three experiments.

FIG. 8 (A) shows in vivo OGTT tests performed with (♦) carboxymethyl cellulose in 0.1 N HCl, (■) standard human insulin and (▲) insulin-loaded poly(VCL-co-MAA) particles through oral route and % inhibition given in the bar diagrams.FIG. 8 (B) shows the % reduction in glucose levels (bar diagram) in fasted rats fed with insulin-loaded poly(VCL-co-MAA) microparticles.

FIG. 9 (A) shows the in vivo efficacy of orally fed insulin-loaded microparticles on alloxan-induced diabetic rats using (♦) carboxymethyl cellulose in 0.1 N HCl, (■) standard human insulin, and (■) insulin-loaded poly(VCL-co-MAA) microparticles through oral route.

FIG. 9 (B) shows the % reduction in glucose on alloxan-induced diabetic rats fed with insulin-loaded poly(VCL-co-MAA) microparticles.

DETAILED DESCRIPTION OF EMBODIMENTS

Definitions:

The term "copolymer" as used herein refers to *N*-vinylcaprolactam and methacrylic acid abbreviated as "poly(VCL-*co*-MAA)".

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

The term "pH-sensitive copolymer" as used herein refers to poly(VCL-*co*-MAA).

The term "acidic pH" as used herein refers to pH value lower than 7.4, and in some embodiments pH 1.2.

The term "neutral pH" as used herein refers to pH about 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 present invention provides a novel pH sensitive copolymers of poly(VCL-*co*-MAA) for the controlled delivery of insulin. The copolymer of the present invention in particular is prepared from *N*-vinylcaprolactam and methacrylic acid monomers.

The hydrogel was prepared by varying the proportions of monomer and % of the insulin loading. The methacrylic acid moiety was varied from 10 % to 50 % in relation to vinyl caprolactam monomer in the total composition of the copolymer.

The particle size of hydrogel microparticles was measured by particle size analyzer (3000HS, Malvern, UK). The particles were produced by sieving the freeze-dried hydrogel through 200 micron size mesh. The particle size of the hydrogel microparticles are in the range of 50-100 μm. It was possible to further decrease the size of these hydrogels to 0.2 μm using a micro fluidizer. As the size of the particle increased, the agglomeration also increased, thereby leading to absorption of moisture and affecting the oral dosing of the formulation when administered to the animals. For this reason, the particle size was reduced by passing through 200 μm mesh, which resulted in a uniform distribution of particles that eased the oral administration of formulation in animal experiments.

The pH sensitivity of the copolymer alone was performed gravimetrically (i.e., measuring the weight uptake vs time) by swelling experiments in pH 1.2 and 7.4 at 37°C. To perform swelling experiments, the copolymer was soaked in 1.2 pH and 7.4 pH media separately; at certain interval of time, the copolymer was removed from the swelling media, taken in test bottles and blotted carefully (without pressing hard) using soft tissue paper to remove the surface-adhered water droplets. The copolymer was then weighed on an electronic microbalance accurate to ± 0.0001 g. The weight gain of the copolymer as observed in pH 7.4 varied from 0.100 g to 0.160 g in 4 h, whereas no weight gain was observed in pH 1.2 during the entire process of swelling, which confirms that the copolymer is sensitive to pH; this property is well correlated with insulin *in vitro* release experiments.

The insulin-loaded poly(VCL-*co*-MAA) hydrogel microparticles were stored at 4^0C for 4 months. After 4 months, the formulated samples were evaluated for their *in vitro* release profile in acidic pH 1.2 for 2 h and later in 7.4 pH phosphate buffer. The *in vitro* release samples were analyzed by HPLC at 210 nm. In this study, the chromatogram shows the sharp peak of insulin without any deamidation peak confirming that insulin was stable and retained its activity even after 4 months.

The present invention describes the various compositions of monomer ratios, % insulin loading and its effects on *in vitro* release as well as encapsulation efficiency. Monomer ratio was varied from 10:90 to 50:50 of MAA:VCL and insulin loading was varied from 1-2 %. The variation in monomer ratio has shown an effect on insulin encapsulation efficiency, but the release pattern remained the same for all the formulations. As the methacrylic acid content increased, the encapsulation efficiency decreased. The *in vitro* release samples were also analyzed by the Western blot method using acrylamide gel. The blot showed the presence of a band in all the released samples, which confirmed the integrity of insulin. As mentioned in the literature, the formulations are biocompatible, but not biodegradable. As regards the safety issues, the polymer does not cause harmful effects to the human body because it is being used in a commercial formulation for facial application.

The present invention also covers the utility of the developed copolymer for encapsulation of other classes of biomolecules. For example, nifedipine (NFD) is a prototype 1,4-dihydropyridine calcium channel blocker and doxycycline (DXY), a member of the tetracycline antibiotics group. NFD and DXY drugs were encapsulated into poly(MAA-*co*-VCL) by *in situ* method using free radical polymerization technique. Firstly, the monomers viz., solid powder of *N*-vinyl caprolactam (VCL) and liquid methacrylic acid (MAA) were mixed in appropriate weights to obtain a total solution of 1 g at the fixed ratio of 75:25 of VCL:MAA. The mixture was dissolved in 5 mL Milli Q water at room temperature. After complete dissolution of the monomers, nitrogen gas was bubbled through the well-mixed homogeneous solutions for 30 min to remove the dissolved oxygen, a free radical scavenger, which will otherwise act as an inhibitor. To this solution, 0.1 wt. % (with respect to the total solution of 1 g) of the catalyst viz., ammonium persulfate and 50 mg of NFD was added with stirring. The total solution was allowed to react to form the hydrogel for 4 h. After 4 h, the hydrogel was washed with water to remove excess of monomers and freeze-dried at - 47^{0}C for overnight. In the same way, DXY was encapsulated into poly(MAA-*co*-VCL) hydrogel.

Encapsulation efficiency of the formulation of the present invention fall in the range of 37-52%. Previously (Cheng K and Lim L.Y., Drug Dev. Ind. Pharm. 2004, 30, 359-67), the formulation using pectin under varying pH conditions was prepared. The desired formulation with pH ranging from 2.2 to 3.4, which gave the maximum EE was obtained by adding 0.25 M HCl (20 to 100 mL) or 0.25 M NaOH (50 mL) to the pectin solution (0.1% in water) prior to mixing with the insulin solution. In addition, the concentration of the insulin solution was increased to 80 U/mL to enhance insulin EE to 92 %, while that of $CaCl_2$ solution was

lowered to 0.1% to reduce the particle agglomeration; whereas in the present invention, the insulin was loaded by *in situ* method. Since *N*-vinylcaprolactam is slightly hydrophobic, it reduces the amount of insulin encapsulation into the matrix.

Biodegradability of Copolymer Components:

Poly(vinyl caprolactam), PVCL is a non-biodegradable polymer and is known to absorb numerous organic compounds from water. The characteristics of PVCL have been studied and some of its applications in the area of biotechnology and biomedicine have been described in the prior art (Makhaeva et. al., Makromol. Chem. Phys. 197, 1996, 1973-1982). Enzyme immobilization has been achieved with the help of PVCL, wherein the stability of enzymes has been increased with PVCL-based hydrogel that protects the enzymes from denaturation by entrapment (Gao et. al.,. Macromolecules 32, 1999, 3674-3677). PVCL has been utilized in multi-layered glass materials in the 1960's, as an ingredient in wound-healing film (Patent, 1998, RU2117492; wound-healing and osteoplastic agent). Commercially, PVCL is available as a hair-fixative excipient under the trade name, Luviskol® Plus by the BASF company. Poly(methacrylic acid) is partially biodegradable and its biodegradability can be increased by copolymerizing with a biodegradable polymer, such as poly(ε-caprolactone), poly(ethylene glycol), etc. A rinse aid formulation containing a low foam non-ionic surfactant, a low molecular weight poly(methacrylic acid) and high molecular weight stabilizing polymer has been desrcibed before.

Biocompatibility of Copolymer Components:

PVCL is a homologue of poly(*N*-vinylpyrrolidone), (PVP), a biocompatible polymer, widely used in medicine and pharmaceutics. PVCL has been toxicologically assessed for its suitability in cosmetic preparations. On the basis of the information available at our disposal and provided that the recommended concentrations and fields of application are adhered to, there is no evidence of any toxicological risks associated with its use. PVCL is also known to be very stable against hydrolysis and owing to its stability, is expected to be a biocompatible polymer. The amide group in the ring is directly connected to the carbon-backbone chain. Hence, PVCL does not break easily hydrolytically, but if the PVCL is hydrolyzed, a polymeric carboxylic acid builds up and small toxic amide compounds will not form.

Poly(methacrylic acid) is a derivative of polycarbophil and carbomer. Carboxylic acid groups in poly(methacrylic acid) binds divalent ions like calcium/zinc and this may lead to reduction in integrity of epithelial tight junctions. Moreover, divalent ions (calcium and zinc) are

essential enzyme cofactors for serine proteases and binding of these ions may lead to a significant reduction in their activity. Furthermore, excellent mucoadhesivity and pH-sensitivity make these systems promising for oral peptide delivery applications (Peppas N.A. Hydrogels. In: Ratner B.D., Hoffman A.S., Schoen F.S., Lemons, J.E., Eds., Biomaterials Science, 2 nd Edn., New York: Elsevier Academic Press; 2004, pp 100–107; WO 99/43615, October 8, 1998 by Peppas et. al., under the title, "method for oral delivery of proteins"). Poly(methacrylic acid) was used in the manufacture of methacrylate resins and plastics in the form of pellets and granules, as absorbent for biological materials and as filters; also, as biological membranes and as hydrogels.

In vitro drug release from both formulations was investigated in phosphate buffer solution (PBS) of pH 7.4. Microparticles (50 mg) were suspended in 1 mL of PBS and placed inside the dialysis bag. The sample inside the dialysis bag was kept in a conical flask containing 50 mL PBS as the dissolution medium on a shaker at 100 rpm at 37°C (New Brunswick Scientific Innova 4230, MN, USA). The amount of drug released was determined by withdrawing 2 mL aliquots at the selected time intervals. The volume withdrawn was replenished with an equal volume of fresh and prewarmed PBS at 37°C. Samples were analyzed by UV spectrophotometer (UV-1650 PC, Shimadzu, Duisburg) at the $\square_{max}$ value of 275 nm for DXY and 238 nm for NFD using PBS as the blank. The release profiles of NFD and DXY from the formulations were observed up to 8 h. The burst release was observed at the earlier time points and almost 50 % of drug was released in 2 h, but 95 % of drug was released in 8 h. These data show that the formulations are useful for short-acting drugs.

The copolymer of the present invention can also 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 the 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 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 or any bioactive ingredient thereof, specifically in the colon of the gastrointestinal (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, 1-10).

The present invention and the matrices developed are not only useful for insulin delivery, but also can be effectively used in case of 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, cyclosporin, somatomedin, secretin, human artial natriuretic peptide (h-ANP), melanocyte-stimulating hormone, (MSH), adrenocorticotropic hormone (ACTH), β-endorphin, muramyl dipeptide, enkephalin, neurotensin, bombesin, vasoacive 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 the naturally occurring substances, but pharmacologically active drug derivatives thereof and the analogues thereof (Mundargi et al., J Control Release, 125, 2008, 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, 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 the lower part of the GI tract. These include for instance, anti-tussive expectorants, such as theophylline (Rokhade et al, Carbohydrate Polymers, 69, 2007, 678-687), vasodilators, such as nicardipine hydrochloride (Soppimath et al, Drug Dev Ind Pharm, 27, 2001, 507-515) and nifedipine (Ramesh et.al., Carbohydrate Polymers, 69, 2007, 241-250), atenolol and carvedilol (Mundargi et al., Carbohydrate Polymers, 69, 2007, 130-141), diltiazem hydrochloride (Aminabhavi et al., Designed Monomers and Polymers, 1, 1998, 347-372), coronary vasodilators, such as isosorbide nitrite; antipyretic analgesics, such as acetaminophen, indomethacin (Sairam et al., J. App. Polym. Sci., 104, 2007, 1860-1865), hydrocortisone, ibuprofen (Mundargi et al., J. Microencapsulation, 25, 2008, 228-240).

As confirmed from the above-described invention and examples therein, the developed oral controlld-release (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, the insulin-loaded micron or submicron level formulations are suitably prepared for the 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 of this approach in developing 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 subcutaneous 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 the preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent the 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: Process of preparation of copolymers

The copolymers of this invention were prepared by free radical polymerization, whose general protocols are known in the prior art. Firstly, the monomers viz., solid powder of *N*-vinylcaprolactam (VCL) and liquid methacrylic acid (MAA) were mixed in appropriate weights to obtain the total solution of 1 g to maintain the ratios of 50:50 and 75:25 compositions of VCL:MAA. This mixture was dissolved in 5 mL of Milli Q water taken in 50 mL beaker at room temperature. After complete dissolution of the monomers, nitrogen gas was bubbled through the well-mixed homogeneous solutions for 30 min to remove any dissolved oxygen, a free radical scavenger, which will otherwise act as an inhibitor. To this solution, 0.1 wt. % (with respect to the total solution of 1 g) of ammonium persulfate (APS) catalyst was added with stirring.

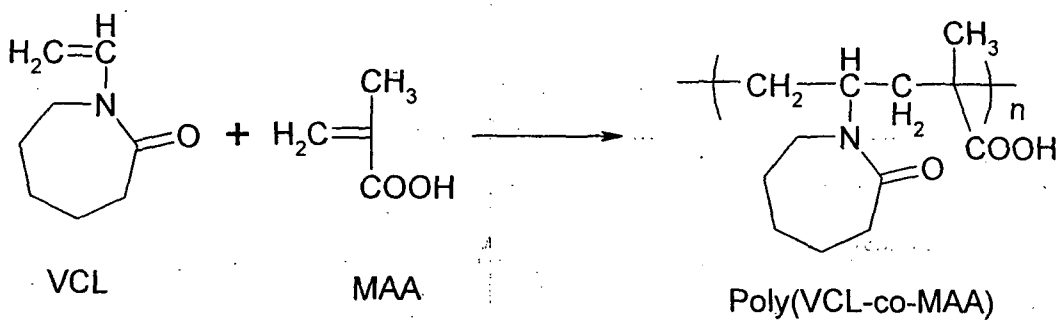
Separately, 10 mg of insulin dissolved in 600 μL of 0.1 M HCl was added to the above solution along with 0.05 wt. % of TEMED to activate the APS initiation at room temperature. The reaction mixture was allowed to react for 3 h to form the hydrogel that was washed thrice with milli Q water to remove the unreacted monomers and the hydrogel was freeze-dried at -47^{0}C overnight. The freeze-dried particles were obtained in microparticles ranging in size from 50 to 100 microns. The microparticles were characterized before further analysis. The copolymeric hydrogel microparticles thus formed were designated as: P(VCL-*co*-MAA) (50:50) and P(VCL-*co*-MAA) (75:25). The above compositions were fixed based on several trial experiments. However, of these two compositions, the one that gave the optimum results viz., the formulation containing 75:25 matrix was selected for the detailed final study.

The effect of insulin loading on encapsulation efficiency in this hydrogel preparation was studied by varying insulin from 1 to 2 %. Insulin-loaded P(VCL-*co*-MAA) hydrogel microparticles were prepared at room temperature (22°C) because of the thermal instability of insulin at higher temperatures. In case of thermo-stable drug molecules, such as NFD and DXY, the copolymeric hydrogel microparticles were prepared both at room temperature (i.e., 22°C) and at 50°C. The encapsulation efficiency and *in vitro* release of thermo-stable drug formulations prepared at room temperature and at 50°C are the same, indicating that the property of the copolymer remains unchanged at these two temperatures investigated.

EXAMPLE 2: Characterization of the copolymer:

(A) Fourier transform infrared spectroscopy (FTIR)

The polymerization reaction leading to the formation of copolymer is given in Scheme 1.



Scheme 1. Synthesis of the poly(VCL-co-MAA) copolymer.

FTIR spectrum of methacrylic acid has been reported in the previous literature (Mundargi, et al., J App Polym Sci. 101, 618– 623, 2006). The characteristic absorption bands at 2616 cm^{-1} and 1696 cm^{-1} were observed, which are assigned to carboxylic acid groups and carbonyl stretching vibrations respectively. Additional characteristic absorption bands of MAA appear at 1449 cm^{-1} and 1301 cm^{-1} because of COC multiple bond stretching and COH bending vibrations, respectively. VCL (Fig. 1A) showed characteristic bands due to different functional groups. For instance, a band appearing at 3571 cm^{-1} is due to O-H stretching vibrations, while those observed at 2928 and 2857 cm^{-1} are due to the C–H stretching vibrations. Carbonyl (C-O) stretching vibrations are seen at 1665 cm^{-1}, but the bands at 1390 cm^{-1} and 1347 cm^{-1} are due to C-H stretching and C–H bending vibrations, respectively. The bands at 1259 cm^{-1} and 1185 cm^{-1} belong to C–N stretching vibrations. In case of the copolymer (Fig. 1B) the region 1500 – 1700 cm^{-1} corresponds to absorption bands of VCL and the copolymer. The amide band splits into a doublet at 1621 cm^{-1} and

1615 cm^{-1}. The formation of carbonyl-stretching band in the copolymer indicates hydrogen-bonding interactions between VCL and MAA in the copolymer.

The C=O peak is observed at 1714 cm^{-1} and the amide low-frequency splitting is likely to be caused by the formation of new hydrogen bonds between carboxyl O-H groups and the amide groups. The high-frequency shift of C=O occurs due to the destruction of H-bonds between MAA carboxyl groups, which suggest involvement of carboxyl groups in H-bonding. In the spectrum of VCL/MAA, the OH band appearing at 3398 cm^{-1} is shifted to lower frequencies, implying change in the nature of H-bonding, which further confirms the formation of H-bonds between amide and carboxyl groups.

(B) Thermal Analysis

DSC was used to determine the crystallinity and melting temperature (T_m) of the copolymer and DSC thermogram of the copolymer is shown in Fig. 2. The copolymer exhibits an endothermic peak at 70°C, indicating its melting temperature. In addition, the copolymer exhibited two endothermic transitions, one at 125°C and other at 135°C, probably due to enhanced intramolecular interaction between the carbonyl groups in the copolymer.

Thermal stability of the copolymer was studied by TGA in the temperature range from 0°C to 800°C under inert nitrogen atmosphere. Thermal degradation patterns of copolymer (Fig. 3) has three weight loss stages occurring in the regions 60-140°C, 190-310°C and 350-460°C. The first stage of weight loss between 60-140°C corresponds to a loss of water molecules/moisture present in the polymer. The maximum weight of 54 % has been occurred between 350-460°C.

(C) Size, shape and morphology analysis

The drug-loaded sample was successfully processed using a Microfluidizer processor. The mean particle size for the insulin-loaded sample after twenty passes was reduced from its original size of 50 microns to about < 10 microns. The instrument used was an M-110S microfluidizer processor, using the G10Z interaction chamber at 23,000 psi at various numbers of passes. Prior to exit, the sample passed through a cooling coil. Graphic results are shown in Figure 4. Size distribution analysis was done by laser diffraction spectroscopy using Malvern zeta sizer (3000HS Malvern Instruments, UK). Mean diameters of the aqueous suspensions were determined in triplicate and the size distribution was estimated based on the number.

For scanning electron microscopy (SEM), the insulin-loaded micro and nanoparticles 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-840, Joel Instruments, Tokyo, Japan) to characterize the shape and morphology as well as to confirm the particle size. SEM photographs are given in Figure 5. The scanning electron micrographs suggest rough surfaces of the particles. The size of the particles is in the range of 50-100 μm.

EXAMPLE 3: Properties of the formulation:

(A) Extent of encapsulation of insulin

Insulin was encapsulated into the hydrogel during the preparation of hydrogel by free radical polymerization technique. Its encapsulation was found to be around 52 %

Encapsulation efficiency (EE) by HPLC analysis

Microparticles of this study loaded with insulin were evaluated for encapsulation efficiency (EE). The EE was estimated by sonicating a known amount of insulin-loaded microparticles and extracting the insulin from the particles. The amount of insulin released from the hydrogels was collected by taking 100 μL samples at predetermined time intervals of 1 h up to 8 h and analyzed using HPLC. Insulin was separated on a C18 Vydac 218MS54 column (4.6 x 250 mm) having a pore size of 300 A^0 and a particle size of 5 microns. The buffer for mobile phase was prepared by dissolving 28.4 g of anhydrous sodium sulfate in 1000 mL of double distilled water and adjusting the pH of the buffer to 2.3 with 2.7 mL of orthophosphoric acid. The mobile phase consisted of (A) 82:18 of acetonitrile:buffer and (B) 50:50 acetonitrile:buffer. The flow rate was 1 mL/min and the injection volume was 100 μL; the wavelength used for detection was 210 nm. The retention time of insulin was at 11.5 min. Typical HPLC chromatograms of insulin and insulin released in acidic and alkaline conditions is given in Figure 6. The % EE was calculated as:

% Insulin Loading = [Weight of insulin in microparticles/Weight of microparticles] x 100

% Encapsulation efficiency = [Actual loading/Theoretical loading] x 100

EE of the formulations developed varied from 37 to 56 %. Table 1 depicts the mean values of EE. Of the many trial experiments using both bovine insulin and human insulin taken in polymer systems, human insulin was used in the final studies that gave 52 %.

Table 1: Encapsulation efficiency of different formulations

Codes	VCL (g)	MAA	*N,N*-	Insulin (mg)	EE (%)

		(mL)	methylenebis acrylamide		
Human Insulin					
INS-1	0.5	0.5	10	10	37
INS-2	0.75	0.25	10	10	52

(B) Stability of the active ingredient in the formulation:

In vitro released samples on HPLC analysis showed no degradation/deamidation indicating that the insulin was stable. Similar results were observed with 4 months old formulation. The insulin-loaded formulation stored at 4^0C for 4 months was studied for *in vitro* release. The *in vitro* release data by HPLC confirmed that there was no degradation/deamidation of insulin. The *in vitro* release samples were also analyzed by the Western blot method using acrylamide gel. The blot showed the presence of a protein band corresponding to insulin in all the released samples, which confirmed that the insulin in the formulation is intact during the storage.

Example 4: *In vitro* release of insulin-loaded particles

The *in vitro* release experiments were done by taking 100 mg of insulin-loaded P(VCL-*co*-MAA), particles in a flask containing 50 mL of buffer solution. Particles were placed in buffer solution to allow 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 λ_{max} 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 simulate the intestinal environment. The *in vitro* data of formulation prepared are graphically displayed in Figure 7.

P(VCL-*co*-MAA) exhibit pH-dependent swelling due to reversible formation of inter-polymer complexes stabilized by the hydrogen-bonding between ethereal groups of the grafted VCL, and carboxylic acidic protons of PMAA network. This type of complex formation is very sensitive to the pH of media as well as copolymer composition and graft chain length. In acidic environment of the stomach, such hydrogels are in a complexed state such that insulin cannot readily diffuse through the membrane barrier. As the polymer passes through the stomach into the intestine, the pH increases above the transition pH of

the hydrogel and the complex immediately dissociates; the network pore size will then rapidly increase, leading to the release of insulin.

The *in vitro* release of insulin hydrogel particles displayed in Figure 7 represent unique pH-responsive property of P(VCL-*co*-MAAc) hydrogels in which inter-polymer complexes are formed in acidic media and dissociated in neutral environment. The insulin release can be significantly retarded in acidic media, while rapidly releasing it in neutral/basic conditions. In contrast, at higher amount of MAA in the polymer, the EE of insulin within the hydrogel would greatly reduce. In these hydrogel formulations, there was no release at pH 1.2, but the burst release occurred at pH 7.4. The content of methacrylic acid in the copolymer was varied from 25 to 50 % with respect to the total weight of the monomer content. In the present invention, the P(VCL-*co*-MAAc) copolymeric hydrogel was prepared by employing lower quantity of MAA., because of the possible toxicity of byproducts generated during degradation. The MAA proportion has a profound effect on the encapsulation of insulin into the copolymeric hydrogel and it was found that lower content of MAA favors higher encapsulation into the hydrogel.

EXAMPLE 5: Oral efficacy of insulin formulations for oral glucose tolerance test (OGTT) and antidiabetic activity in rats

Oral Glucose Tolerance Test (OGTT)

For insulin preclinical studies, the fasted rats were divided into three groups of six rats each. Group 1 is the control group, which received saline. Group 2 rats received the formulation equivalent to 20 IU of insulin-loaded poly(methacrylic acid-co-*N*-vinyl caprolactam) particles dispersed in a mixture of 9 mL of 5 % carboxy methyl cellulose (CMC) and 1 mL of conc. HCl solution through the oral route using the oral feeding needle. Group 3 received 400 µL of 5 IU of pure insulin solution through the IP route.

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 was calibrated to 660 units or as according to the specifications mentioned in the strips. The blood removed from the rat is immediately spread on the marked end of the strip. The strip

was then inserted in the glucometer where two electrodes are situated. After few seconds the glucometer displayed the blood glucose level.

Figure 8A represents the *in vivo* efficacy (oral glucose tolerance test; OGTT) of insulin-loaded poly(*N*-vinylacaprolactam-*co*-methacrylic acid). In this test, the standard insulin gave glucose drop from 211 to 137 with 64 % inhibition, and the insulin-loaded particles administered by the oral route gave glucose drop from 170 to 80 with 53 % inhibition as shown in Figure 8B. The inhibition rate was 53 % owing to the mucoadhesive property of *N*-vinylcaprolactam and other enzymes in the stomach.

In vivo efficacy of insulin-loaded particles on diabetic rats

Male Wistar rats (250 g) were housed in a 12-12 h light-dark cycle, constant temperature environment of 22^0C, relative humidity of 55 % and allowed free access to water and food during acclimatization. To minimize the diurnal variance of blood glucose, all the 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 the experiments. Blood glucose levels were determined by glucose oxidase/peroxidase method using a glucometer. The 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 the oral administration. Results were expressed as means ± standard deviation (SD) or means ± standard errors of means.

The Figure 9A represents the *in vivo* efficacy of orally fed insulin-loaded (VCL-*co*-MAA) on alloxan-induced diabetic rats. In this, the standard insulin gave glucose drop from 217 to 44 with 80 % inhibition, whereas the insulin-loaded particles (VCL-*co*-MAA) by the oral route gave glucose drop from 219 to 86 with 60 % inhibition as shown in Figure 9B.

Release profile with respect to pH

At pH 1.2, the hydrogel shrinks and there is no release of insulin, while at pH of 7.4, the hydrogel swells to release the insulin as shown in Figure 4.

Release profile with respect to time.

The release profile of hydrogel changes with time and pH. The *in vitro* release was carried out at both pH 1.2 and 7.4. At pH 1.2, from 1 to 2 hrs, there is a suppression of swelling of the hydrogel and there is no release. As the hydrogel is shifted to pH 7.4, it starts swelling and releases the insulin immediately. After the burst release in first hr, there is a gradual increase in the % of insulin release as observed at 2^{nd}, 3^{rd} and 4^{th} hr (Figure 4). Since all the insulin is released within 4 hrs at pH 7.4, the formulation is useful for short-acting.

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in the 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.

CLAIMS

We claim:

1. A particle comprising a pH-sensitive copolymer comprising a methacrylic acid monomer and a N-vinyl caprolactam monomer,
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 the ratio of methacrylic acid monomers to N-vinyl caprolactam monomers are between 10:90 to 50:50.

3. The particle of claim 1, wherein the particle has an encapsulation efficiency between 30% and 60%.

4. The particle of claim 1, wherein the size of the particles is in the range of 50-100 μm.

5. The particle of claim 1, wherein the encapsulated moiety is essentially released within 4 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 claim 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 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 composition of claim 11, wherein the copolymer does not alter the stability of the biologically active moiety.

13. The 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 composition of claim 13, wherein the biologically active moiety is a protein or peptide.

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

17. The 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.

18. The composition of claim 17, 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.

19. A pharmaceutical formulation comprising:
a composition according to any of claims 11-17; and
a pharmaceutically acceptable excipient.

20. 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 11-17, wherein administration of the composition reduces glucose levels in the individual.

21. The method of claim 20, wherein the glucose level is reduced by at least 50%.

22. The method of claim 20, wherein the reduction of glucose level is comparable to that achieved by subcutaneously injected insulin.

23. The methods of claims 20-22, wherein the glucose level is monitored by an Oral Glucose Tolerance Test (OGTT).

24. 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 11-17, wherein administration of the composition reduces a symptom of Type I diabetes.

25. 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 11-17, wherein administration of the composition reduces a symptom of Type II diabetes.

26. The method according to claims 24 or 25, wherein the symptom of Type I or Type II diabetes is an elevated glucose level.

27. The method of claim 26, wherein the glucose level is reduced by at least 60% within 4 hours following administration of the controlled-release composition.

28. A particle comprising a pH-sensitive copolymer comprising a methacrylic acid monomer and a N-vinyl caprolactam monomer and its application as claimed above exemplified herein substantially in the examples and figures.

FIG.1(A): FTIR spectra of monomer vinyl caprolactam

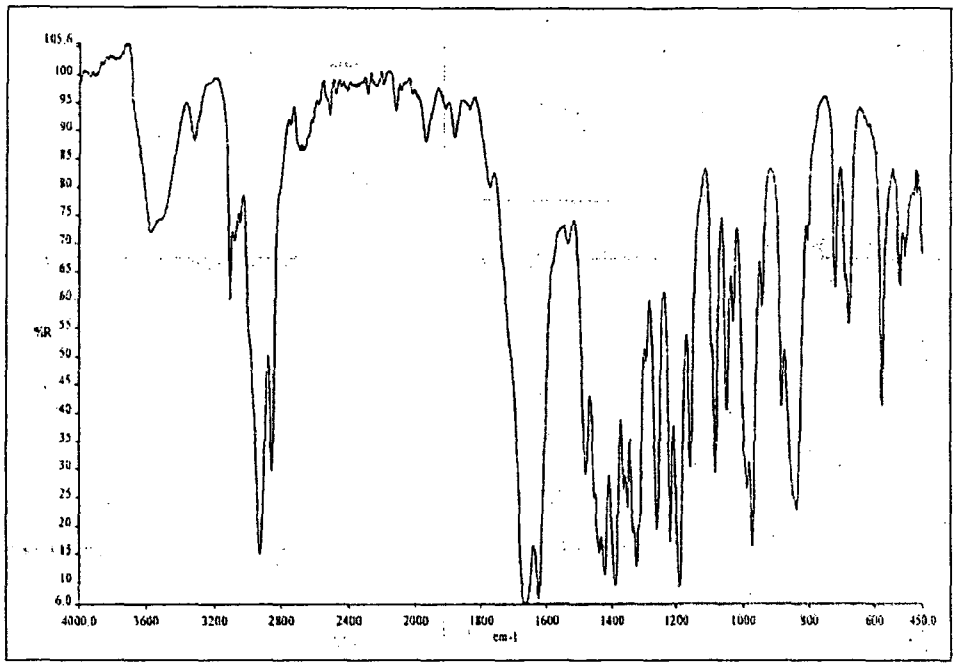


FIG. 1(B): FTIR spectra of copolymer of Poly(VCL-*co*-MAA)

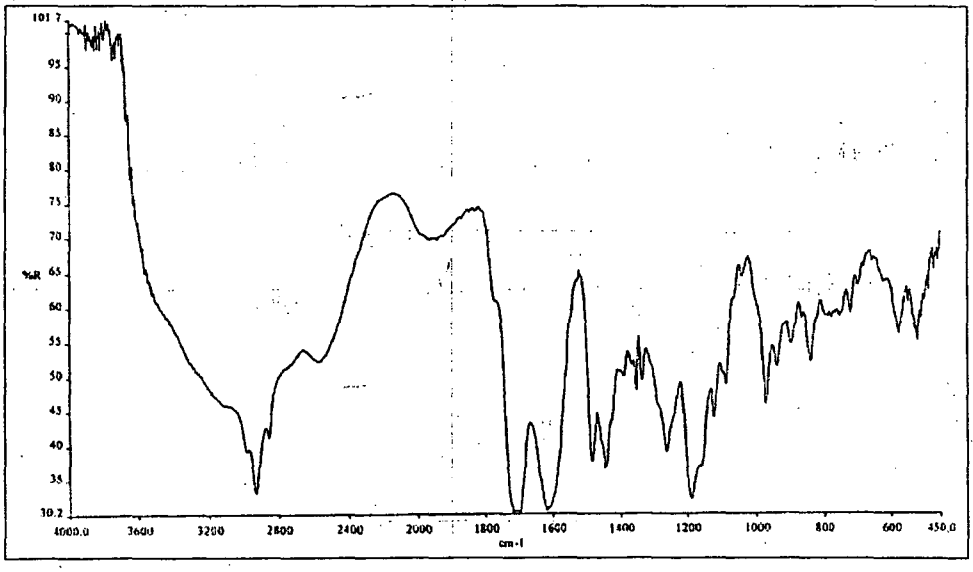


FIG. 2: DSC thermogram of Poly(VCL-*co*-MAA)

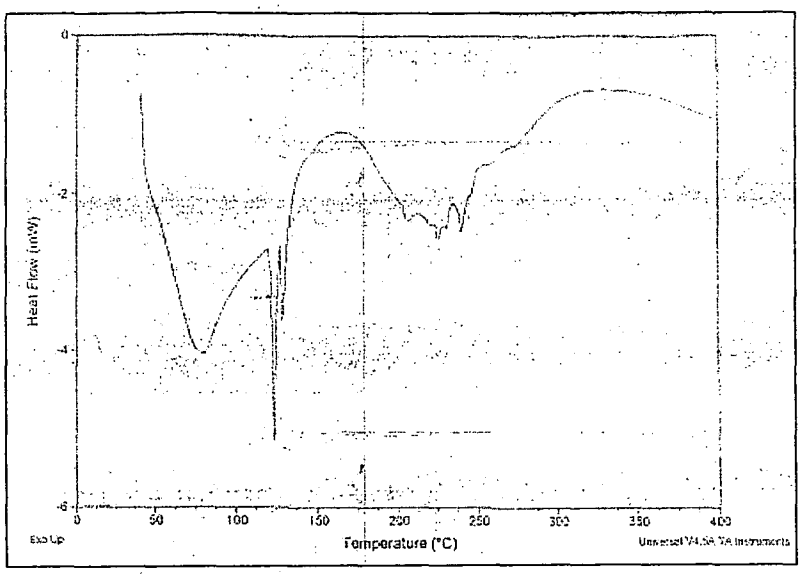


FIG. 3. TGA thermogram of Poly(VCL-*co*-MAA)

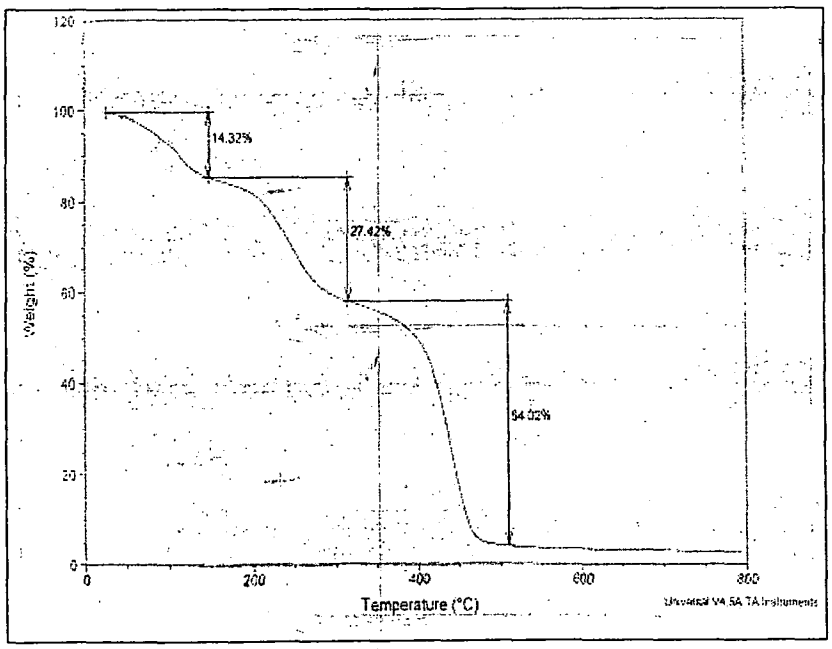


FIG.4: Particle size distribution curve of microparticles after 20 passes through Microfluidizer

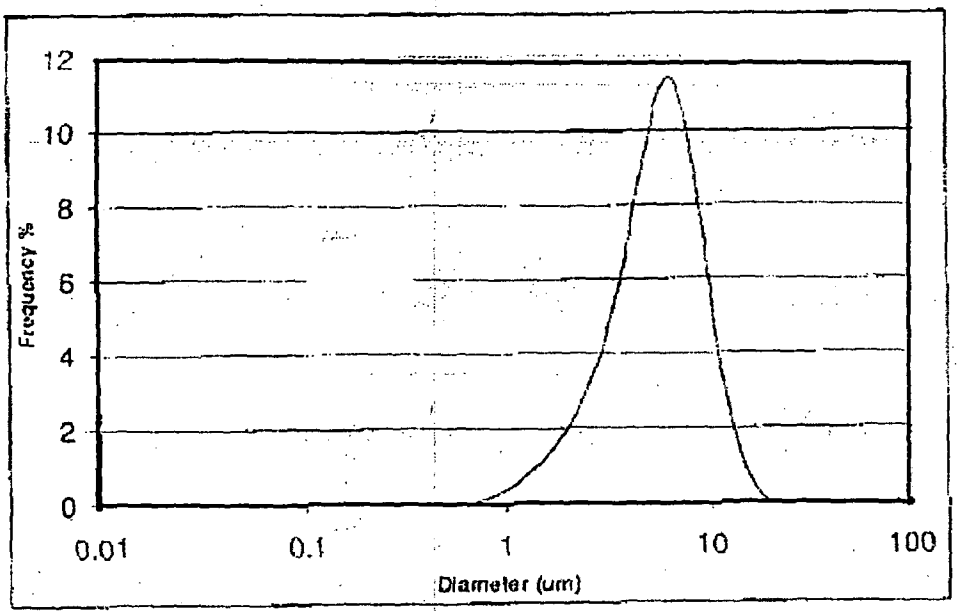


FIG. 5: SEM images of Poly(VCL-*co*-MAA) microparticles

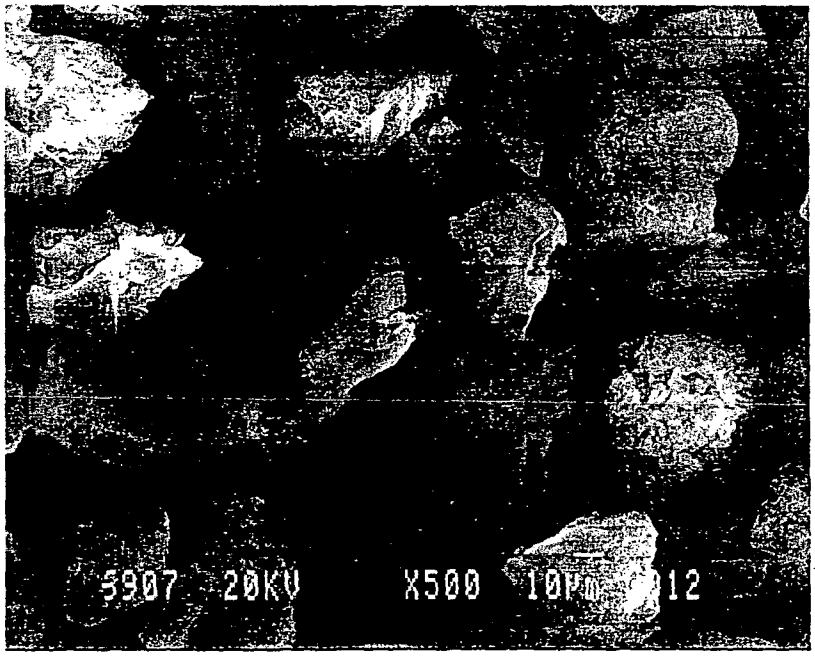


FIG. 6: HPLC chromatograms of standard human insulin released at pH 1.2 and 7.4.

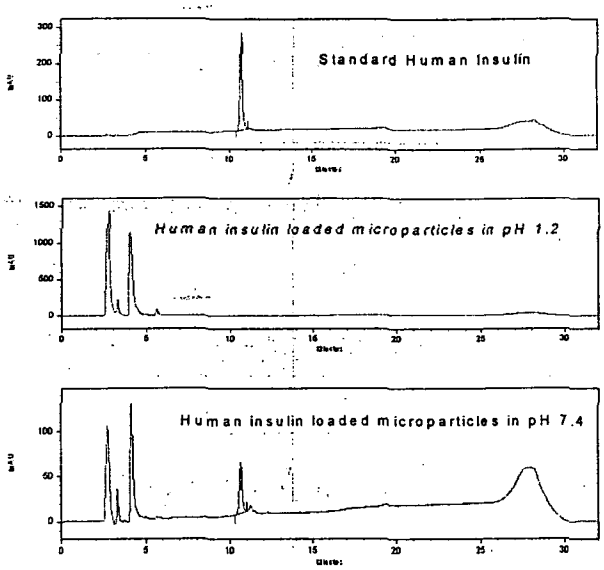


FIG. 7: *In vitro* release profile of insulin-loaded microparticles of Poly(VCL-*co*-MAA) in simulated gastric medium (pH 1.2) and intestinal medium (pH 7.4)

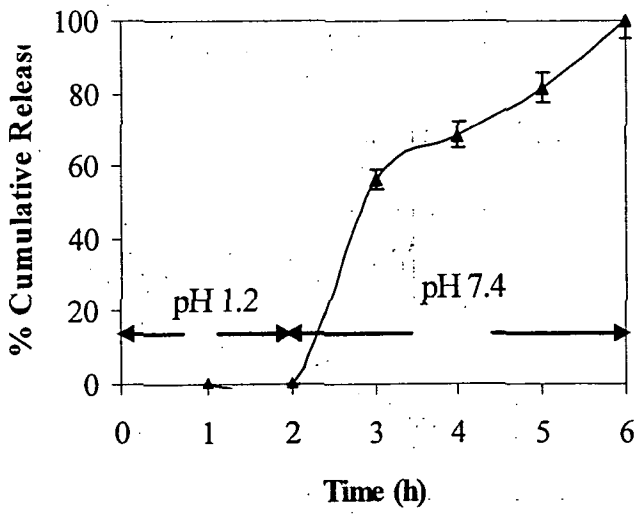


FIG. 8(A): *In vivo* OGTT tests

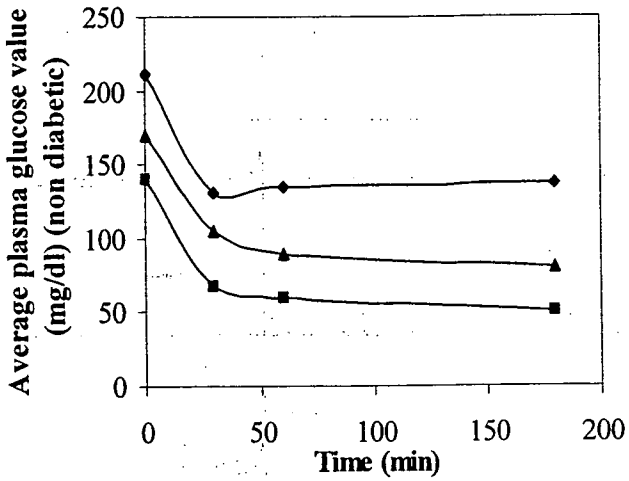


FIG. 8(B): The % reduction in glucose levels

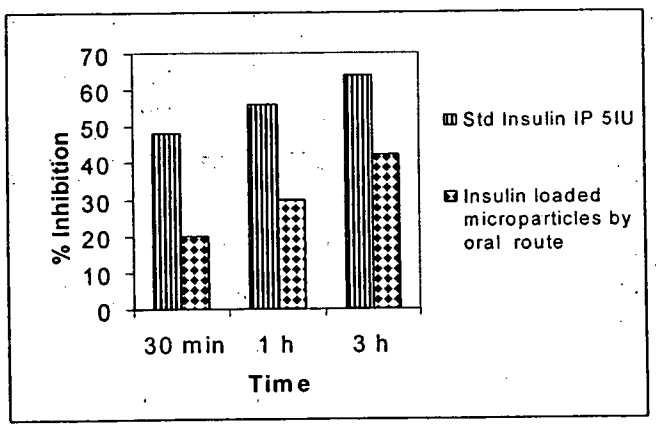


FIG. 9(A): *In vivo* efficacy of orally fed insulin-loaded microparticles

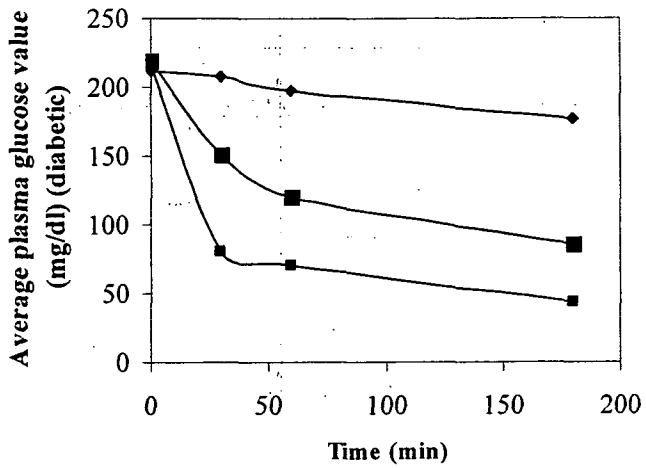


FIG. 9(B): The % reduction in glucose on alloxan-induced diabetic rats