Review Article [Yadav *et al.*, 4(6): June, 2013]
CODEN (USA): IJPLCP **ISSN: 0976-7126**

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Application of natural polysaccharide for delivery of biopharmaceuticals

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Abstract

Recently, the world communities are moving towards the utilization of herbal products. Instead of pharmacological effect herbal material has been identified in cosmetology as well as pharmaceutical excipients. Literatures confirm the applicability of herbal product as a binder, suspending agent, thickener, disintegrating agent etc. The world market for biopharmaceutical products increases since last 10 years in their application and production. Biopharmaceutical products are recombinant of various biological origin and blood plasma which includes proteins, peptides, nucleic acids, hormones, whole cells, viral particles and vaccines. This review focuses on various types of naturally occurring polymers which has been considered for the delivery of biopharmaceuticals. An attention is made on the chemical and physical properties of polymers in the form of its compiled data.

Key-Words: Biopharmaceutical, Natural polymer, Drug delivery

Introduction

The term "Biopharmaceutical" may be defined as the product which is obtained from the blood and blood plasma or genetically altered recombinant of fungi and bacteria. Biopharmaceutical products or drug have the unique qualities which was derived and manufactured from traditional opposed drug product [1]. Biopharmaceuticals are protein based drug which may represent the group of products that include insulin, growth hormone, vaccines, interferon, erythropoietin, interleukin, DNA, gene, hormones, monoclonal antibody, enzymes, protein and peptides etc [2]. Many plant derived biopharmaceuticals are also used for some vaccination and treatment of animal and human being diseases [1]. Biopharmaceuticals are the fastest growing field in the medicine which represent new generation of medicinal products. Approx 200 biopharmaceutical products in the world are marketed and about 300 in clinical trials. 50% of biopharmaceuticals products are approved [3].

Natural polysaccharide has enzymatic degradation behaviour and good biocompatibility [4]. The pharmaceutical application for natural polymer is attractive over the synthetic polymer due to non-toxic, biodegradable, biocompatible, economical, easy availability. Natural polysaccharides are naturally occurring and abundant renewable source.

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The plant resources are cultivated in substantial manner, that it provides continuous supply of raw material [5]. Plant derived polymer are suitable for the formulation of pharmaceutical production the form of nanoparticles, microsphere, microparticles, implants, films, blends, matrix and viscous liquid. The different type of natural polysaccharide used in the pharmaceutical application i.e. chitosan, alginate, dextran, starch, gelatin, pectin, guar gum, xanthan gum, arabic gum, cellulose, insulin and carrageenans etc [6].

The drug delivery system has to select for optimizing bioavailability of drug compound for controlled release of drug from formulation to assure the chemical and physical stability of the system. Delivery of biopharmaceuticals product via oral, nasal, transdermal, ocular has been developed for more effective and easy to administer in the body [7]. The different polymers are available, which is used in the drug delivery system. Biodegradable polymers are more pronounced in the design of controlled release system. The variety of biodegradable formulation were introduced to deliver biopharmaceutical products by controlled release system such as microcapsules, liposome's, gel beads, microsphere, nanoparticles and hydrogels [8].

The natural polysaccharide is used as carrier for controlled released of the drug and bioactive protein due to it biodegradable property and they administered via various route in the body without any surgical

removal [9]. This review provides information about various natural polymers which have been reported for delivery of biopharmaceutical in form of various formulations through several routes. Concentration is also provided on the basics of natural polymer.

Natural polysaccharides for delivery of biopharmaceuticals

Chitosan: - It is a natural polysaccharide obtained from the deacetylated derivative of natural occurring chitin. The polymer is linear consisting of randomly distributed β-(1-4)-linked with unbranched polymer of D-glucosamine (deacetylated unit) and N-acetyl –D-glucosamine (acetylated unit) composed of mucopolysaccharide and amino sugar [10,11,12]. Chitin on hydrolysis of acetamide group gives chitosan [13]. Chitosan is most widely used in medical and pharmaceutical industrial field due to the unique chemical and physical property of natural polymer. The natural polysaccharide which is most abundant next to cellulose is chitin [10], which is the exoskeleton of crustaceans such as shrimps and crab shells, etc. in the structural element [14].

Chitosan is soluble in acidic media i.e. organic acid (acetic acid) and inorganic acid (hydrochloric acid) while, chitin is insoluble in aqueous medium [14]. Due to presence of amino group in the D-glucosamine chitosan is soluble in acidic condition [13,15]. The molecular weight of chitosan depends on the solubility, viscosity, elasticity and tears strength [15]. The structural unit of chitosan and chitin are given in figure 1.

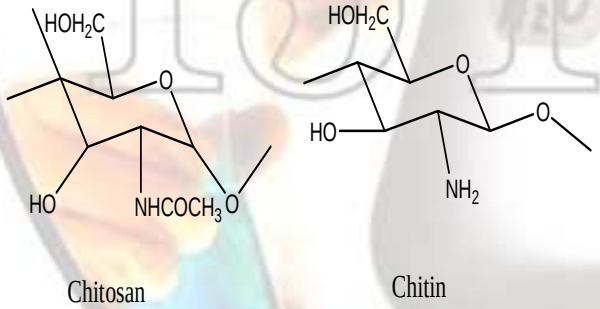


Figure 1: Structural unit of chitosan and chitin

Chitosan is renewable source and chemically inert, non-toxic, biodegradable, biocompatible, inexpensive and easily available and it provide pharmaceutical, medical and food application [10,12]. The derivatives of chitosan are N-trimethylene chloride chitosan, chitosan ester and chitosan conjugation for the delivery of biopharmaceutical or therapeutic product. Chitosan has excellent enhancing viscosity agent in acidic environment and it shows characteristic like pseudo plastic material [15].

The chitosan has a cationic, hydrophilic and crystalline polymer which represents the gelation characteristics and film forming ability [16]. Due to it biocompatibility, biodegradable and non-toxic effect of chitosan is suitable for pharmaceutical excipient in nasal, oral, transdermal, implant and ocular drug delivery [14]. The formation of various type of formulation using chitosan in different drug delivery system are nanoparticles, microsphere, blends, microparticles, bioadhesion and mucoadhesion of chitosan natural polymer [17].

Chitosan is natural polysaccharide which has been demonstrate for the delivery of various biopharmaceutical such as insulin [16,18,19], genetic material [20], DNA [21], vaccines [22,23], human growth hormone [24], condensed plasmid DNA [25]. Chaudhury A. et.al demonstrated development of nanoparticles by biodegradable polysaccharide chitosan for the carrier of insulin delivery and controlled release of protein. It also enhanced mucoadhesion, permeation and sustained release of therapeutic agent. These suggest that chitosan based nanoparticles has a good potential for oral delivery of insulin [16].

Pectin: - Pectin was first demonstrated in 1820s for making jams and jellies. Pectin is mainly extracted from pulp of the fruit [26]. It can isolated from the cell wall of the plant and also present within secondary cell of xylem and fibre of woody tissue [5]. Pectin is a complex polymer which contain linear polysaccharide of 1,4- linked α-D galacturonic acid residues. Galacturonic acid is a good source of neutral sugar like glucose, rhamanose, xylose, arabinose and galactose. Pectin composition is vary according to their botanical sources of different fruits or cell wall [5, 27]. The structure of galacturonic acid units is given in figure 2.

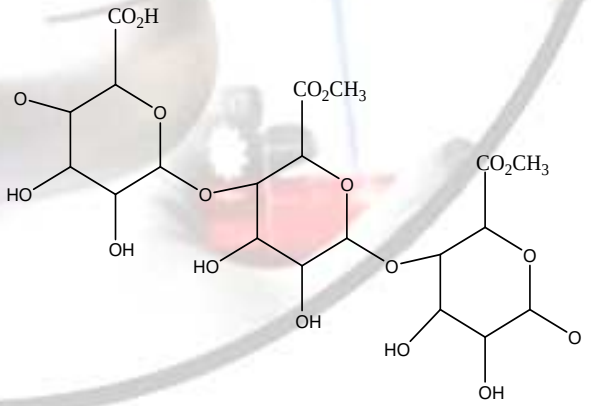


Figure 2: Structure of galacturonic acid units

The most widely used application of pectin was thickening, gelling and stabilizing agent in the

production of pharmaceutical and medical product. It is most versatile polysaccharide which is used as excipients design various dosages form for the delivery of different biopharmaceutical products in form of micro particles, matrix type transdermal patches, hydrogels etc [5].

Pectin has been studied for effective delivery of insulin [28], gene delivery [29]. Mark T. et.al developed aminated pectin hydrogel beads for oral administration of insulin. Pectin hydrogel insulin patches were investigated to deliver the insulin through skin. They used four rat for investigation, measure basal blood glucose concentration and applied patches before oral glucose tolerance test administration. After some hour rats were sacrificed and blood plasma was collected to measure the release of insulin through skin by the ultra sensitive insulin ELISA. They found that hydrogel insulin patches has greater insulin dissolution. Result shows, pectin hydrogel patches has effective in type I diabetes mellitus for transdermal delivery of insulin [28].

Starch: - Starch consists of polysaccharide carbohydrate as discrete granules in the mature grains of cereals like wheat, tubers of potato, rice, maize, etc [30]. Starch is the main carbohydrate of plant storage organ. Starch is a naturally occurring polymer which contain chain of D- glucose units joined together by glycosidic bonds [10,31] obtained from carbohydrate mainly in seeds and underground organs. There are various types of starch species which is obtained from plant source namely potato, arrowroot, corn, cassava, sorghum, wheat, sweet potato, rice, sago, potato canna (Aust. Arrowroot). Starch is mainly composed of two polymer i.e. amylose and amylopectin. Amylose is linear polymer with unbranched [5,30] or small amount of branching [31] consisting (1-4)-α-D-glucan. Amylopectin is branched polymer which contains (1-4)-D-glucan and approximately 4% (1-6)-α-D linkage both. The main constituent of starch is amylopectin, has 80%, while 20% of amylose contain by starch [30]. Amylose has molecular weight in the range of 10^5 to 10^6 g/mol and amylopectin has 1000 times' greater molecular weight in the range of 10^7 to 10^9 g/mol [31]. Amylose is water soluble and gives unstable solution. Chemical structure of starch i.e. Amylose and Amylopectin are given in figure 3.

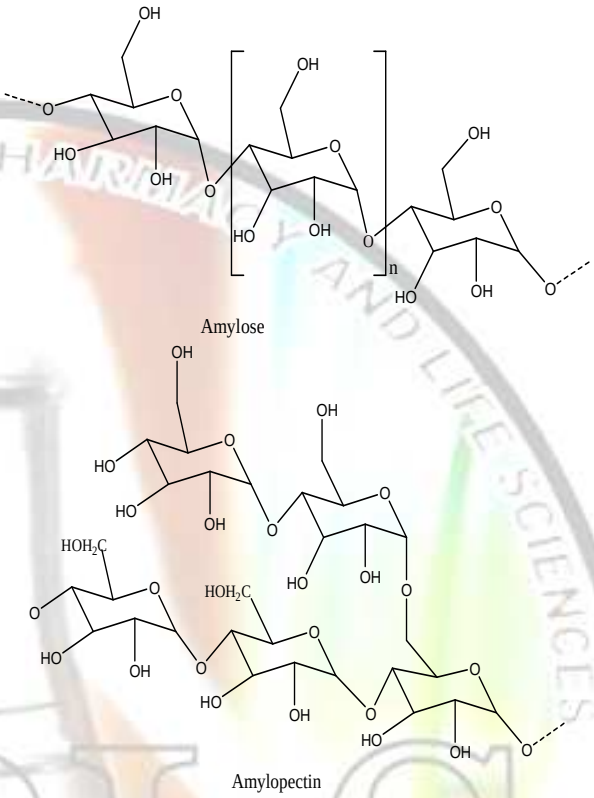


Figure 3: Chemical structure of starch i.e. (1) Amylose and (2) Amylopectin

Starch is unsuitable for controlled release of biopharmaceutical products delivery system due to of swelling properties of granules and rapid enzymatic degradation. To overcome this situation, modified starch derivatives are available which affect these phenomena and causes hybridization, in which actual physical and chemical modification of native starch occurs [32]. Derivative of starch are also available to prevent enzymatic degradation, it has more resistant and cross-linking properties [5].

Starch is a polysaccharide which has been reported for the delivery of biopharmaceutical product. Starch was used for the formation of nanoparticles in which insulin was incorporated. Nanoparticles showed mucoadhesive properties, hence it has greater affinity for trans-nasal delivery of insulin and that provide larger surface area to reach highly blood vessels absorption area of nasal having higher concentration gradient. The starch nanoparticles showed that peak plasma level of insulin was more pharmacodynamically effective as compared to other formulations. Insulin loaded starch nanoparticles has controlled release rate and higher surface area to effective delivery of insulin through trans-nasal mucoadhesive carrier [33].

Dextran:-Dextran is obtained from sucrose by the catalytic fermentation i.e. enzyme transglucosylase present in *Leuconostoc mesenteroides* [21]. For production of dextran various bacteria are used such as *Lactobacillus, Leuconostoc, Streptococcus* and *Saccharomyces* [34].

Dextran consists of an α-D-1,6-glucose-linked glucan with the attachment of side chain at the position 1-3 linkage of the backbone glucose units [35]. The molecular weight of dextran has according to their clinical utility i.e. 40,000, 70,000 and 75,000 [30]. The structure of dextran is given in figure 4.

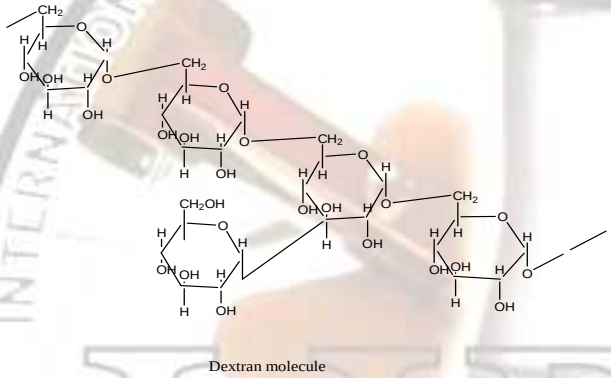


Figure 4: Structure of Dextran

Dextran is a natural polysaccharide which is non-toxic, biodegradable, biocompatible, and serologically neutral for prolonged effect and eliminated completely [30]. Due to this reason, dextran has wide application in pharmaceutical field i.e. for the delivery of biopharmaceutical products.

Dextran has been studied and found suitable for the delivery of interferon-alpha. First FDA approved biopharmaceutical Interferon-α has bio therapeutic cytokine. Encapsulated interferon-α (hIFN-α) within dextran microsphere shows effective result by increase plasma neopterin levels in rats and evaluation of biological activities in cellular and animal system. They suggest that biodegradable dextran microsphere was suitable vehicle for the delivery of interferon-α (IFN-α) [36].

Schizophyllan:-Schizophyllan obtained from the fungus *Schizophyllum commune*, having a neutral extracellular polysaccharide which produced a glucose unit consist of 1,3-β-D-linked with 1,6-β-D-glucosyl side groups. Molecular weight of native Schizophyllan is 10^6 Dalton [37,38]. The primary structure of schizophyllan is given in figure 5.

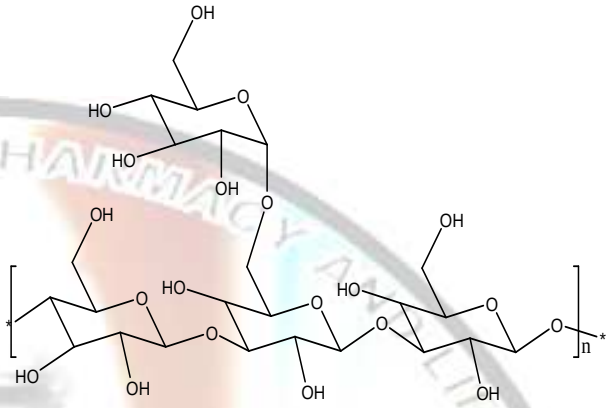


Figure 5: Primary structure of Schizophyllan

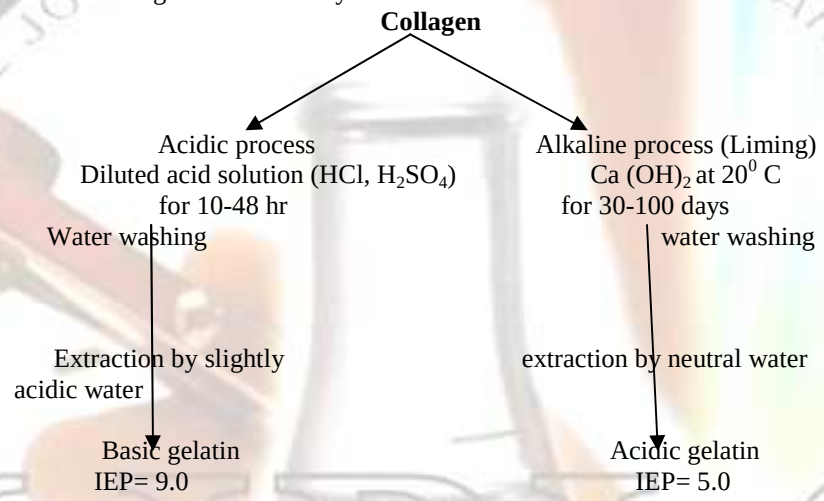
Schizophyllan (SPG) is a natural biodegradable polymer which was used as carrier of biopharmaceutical products due to its environment acceptability. Immunotherapeutic agent like CpG DNA activates innate immune system of vertebrate through toll-like receptor 9 (TLR-9). Schizophyllan used as effective carrier for the delivery of CpG DNA due to complex formation of SPG-CpG DNA, this initiate nuclease resistant of the bound DNA. Chemically modified complexes of SPG and CpG DNA which have phosphodiester backbone, cytokines secretion increases about 4 to 15 fold. Large amount of IL-12 production occurred when CpG DNA complex intraperitoneally injected as compared to uncomplexed CpG DNA and it was found that SPG complex suitable for CpG DNA therapy [39].

Gelatin: - Gelatin, obtained from natural source of animals like bones, cartilage, tendons, skin and ligaments. Gelatin is heterogeneous mixture of protein constitute high molecular weight from treating animal tissues or hydrolysis of collagen. It is not found in nature but derived from enzymatic degradation of collagen protein. Gelatin is transparent, slightly yellow or colourless in appearance and brittle in nature, it is also tasteless and odourless [30]. Gelatin in the presence of moisture may be degraded due to microbial attack and in the presence of air, it is stable. It is soluble in hot water but insoluble in cold water [40]. The two type of gelatin are mainly produced i.e. Type A gelatine and Type B gelatin. Type A gelatin (acid) have isoelectric point between pH 7 and 9 and Type B gelatin (basic) have isoelectric point between pH 4.7 and 5 [41]. Procedure for the preparation of gelatin A and B type from collagen are given in figure 6.

Figure 6: Preparation of Type A and Type B Gelatin

Gelatin is naturally occurring biocompatible polymer and used in the delivery of biopharmaceuticals like growth hormones, vaccines, etc. Human growth

hormone is most important component that it help in the repair of cartilage and bone healing process. The delivery of growth factor such as hGH in the body through the specific carrier i.e. in the form of microsphere and coating on titanium screws. Gelatin microsphere or coated on titanium implants was loaded with growth factors such as hGH which was dispersed polymatrix. Water has important role for the release of growth factor due to it biodegradation activity and soluble in water. Gelatin microsphere released therapeutic doses of growth factor to a specific site, and after delivery of hGH, gelatin was degraded. It shows that biodegradable gelatin is effective for the delivery of growth factor and improved controlled release of drug [8].



Alginate: - Alginate is obtained from the brown seaweed and marine algae like *Macrocystis pyrifera, Laminaria hyperborean, Ascophyllumnodosum,* and *Ecklonia* [5,30,40]. Alginates are most abundant in nature and it is found in both fresh (wet) and dry matters. Alginates or alginic acid is a linear natural polysaccharide, unbranched based on two different monomeric units, β-D-mannuronic acid and α-L-guluronic acid linked with β-1,4-glycosidic linkages. It is cream coloured powder and soluble in water to form viscous fluid which has gelling properties i.e. mixture of polyuronic acid [42]. The chemical structure of alginates is given in figure 7.

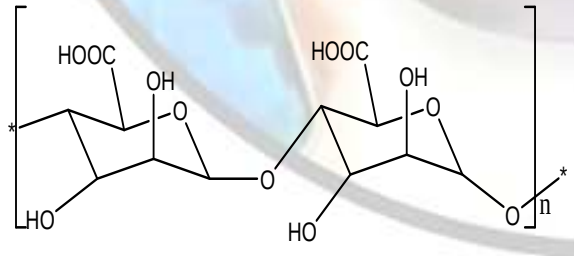


Figure 7: Chemical structure of alginate

Molecular weight of alginates varies from 20 to 600 kDa. Alginates are used as stabilizer, binder, disintegrant, suspending agent, gelling and film forming agents. Alginates are widely used in the industry due to it gelling properties with polyvalent ions such as sodium, calcium and aluminium which allows gel formation to prepare matrices, micro particles, nanoparticles, blends, pellets, microsphere and films [5].

Alginate has wide application in the biomedical and biopharmaceuticals for the controlled delivery system such as growth hormones [43,44,45], vaccines [46,47], insulin [48], human factor IX [49]. Alginate is natural biodegradable polysaccharide which is used for the delivery of somatic gene therapy by making the formulation of microcapsule. The universal recombinant cells which are immunologically protection is required and it is encapsulated in alginate microcapsule which prevent from graft rejection. They performed experiment on rodents and human diseases to prove that use of alginate microcapsules in the delivery system. Genetically modified canine kidney cells were incorporated into alginate-poly-l-lysine-alginate microcapsules of the diameters of 500 micron and implanted into brain. Somatic gene products are measured throughout 21 days periodically in the plasma and cerebrospinal fluid after the delivery of product. Barsoum SC. et.al suggest that use of

biocompatible microcapsule-cell recombinant combination are less invasive and more accurate neurosurgical procedure for the CNSs of human beings and animals [43].

Conclusion

The present review concluded that the utilization of natural polysaccharide in the form of formulation may be used for effective delivery of various biopharmaceutical products at the targeted site with enhanced tolerability for the treatment of variety of diseases. Natural polysaccharides have traditionally included in the formulation as inert material to assist the manufacturing process, they are increasingly included in dosage form to fulfil their effective drug delivery due to many new drugs have unfavourable pharmacokinetic and physiochemical properties. Polymer obtained from natural sources such as chitosan, alginate, dextran, starch, pectin, etc has shows excellent potential as carrier of biopharmaceuticals in the form of microsphere, nanoparticles, cross-linked hydrogels, beads and granules. The semi-synthetic polymers are more extensively used in the formulation for conventional dosage form and it has investigated for novel drug delivery system. Plants provide attractive and renewable source for the material that utilised in biopharmaceutical product for the effective delivery of drug.

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Table 1:-Different natural polysaccharide used for the delivery of biopharmaceuticals

Natural polysaccharide	Formulations	Route of administration	Biopharmaceuticals	Purpose	Reference
Chitosan	Nanoparticles	Oral	Insulin	Controlled drug release	16
	Chitosan-phytic acid capsules	Oral	Insulin	Sustained control of blood glucose level	18
	Nanoparticles	-	Therapeutic agents(genetic material)	*in vitro* and *in vivo* gene delivery	20
	Microsphere	Nasal	Insulin	Bioavailability of insulin	19
	Polyelectrolyte complexes	-	DNA	Gene delivery	21
	Micro particle	Mucosal	mucosal vaccine of BHV-1 was incorporated	the mucosal immunization against BHV-1.	22
	Microsphere	parenteral	Vaccines of tetanus toxoid	Sustained release of tetanus toxoid to maintain the antibody titre for prolonged	23

				period of non-injectable	
	Blends and granules	Intranasal administration	recombinant human growth hormone	hGH for the treatment of growth hormone deficiency.	24
	Nanoparticles	Oral	condensed plasmid DNA	promoting gene expression and also enhanced drug uptake	25
Alginate	Microcapsules	Intraventicular implantation	Recombinant gene product (hGH or canine alpha-iduronidase)	Treatment of neurodegenerative genetic disorders and less invasive and more accurate neurosurgical procedure for the CNSs	43,44
	Beads		Vascular growth hormone	Slow release of protein models of growth factors which are cytrochrome c, methemoglobin and myoglobin	45
	Microsphere	Oral	Vaccines	Increased delayed-type hypersensitivity, a cell mediated immune response, shows positive result to the vaccine	46
	Microparticles	Oral	*Lactococcus garvieae* vaccine	To improve the duration and protection of rainbow trout against lactcoccosis	47
	Microsphere within hyaluronic acid (HA) hydrogels	Implanted directly into cartilage defect site	Transforming growth factor-beta (TGF-β3)	Sustained local delivery of TGF-β3 and to promote chondrogenesis of mesenchymal stem cells(MSCs)	50
	Microsphere	Oral	Insulin	To prevent degradation of protein in GIT and improved the absorption in the intestinal tract.	48
	Microcapsules	Implanted	Human factor IX	Allogeneic gene therapy of Hemophilia B	49
Dextran	Microsphere		Interferon-alpha (IFN-α)	suitable vehicle for the delivery of interferon-α (IFN-α)	36
Starch	Nanoparticles	Trans- nasal	Insulin	controlled release rate and higher surface	33

				area to effective delivery of insulin	
Pectin	Hydrogel insulin patches	Transdermal	Insulin	to prevent chronic complication and daily requirement of insulin therapy	28
	Modified pectin (Pectin-NH2-Q)		DNA	Gene delivery	29
Schizophyllan	Complex between SPG and CpG DNA	Intraperitoneal	CpG DNA	Immunotherapeutic agent	39
Gelatin	Microsphere or coated on titanium implants	Implant	Human growth hormone	Controlled released and non-invasive method for hGH delivery	8
	Hydrogel microspheres	Parenteral	Erythropoietin	Sustain release and improvement of blood perfusion to the ischemic limb	51
Chitosan/Dextran sulphate	Nanoparticles	Oral	Insulin	Mainly protect the insulin from degradation in GI tract and enhance intestinal absorption of insulin to maintain blood glucose level	4
	Nanoparticles	Oral	Human growth hormone	Treatment of hGH deficiency and controlled release at high pH	12
Alginate/Chitosan	Nanoparticles	Oral	Insulin	Improvement of oral absorption and oral bioactivity	52
Alginate-Chitosan	Microparticles	Oral	Bovine serum albumin (BSA) vaccine	Protect protein (BSA)from degradation in acidic medium and increase the stability of antigen	53
	Microsphere	Oral	Insulin	Sustained release of insulin and stable at GI pH medium	54
Chitosan –gum Arabic	Nanoparticles	Oral	Insulin	Lower the dose of drug and improve patient compliance	55
Locust bean gum	Mucoadhesive buccal tablets	Buccal oral cavity	Insulin	To overcome the poor permeability and ineffectiveness when administered orally and improve the bioavailability of insulin	56

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