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Review

Microparticles, microcapsules and microspheres: A review of recent developments and prospects for oral delivery of insulin

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ABSTRACT

Diabetes mellitus is a chronic metabolic health disease affecting the homeostasis of blood sugar levels. However, subcutaneous injection of insulin can lead to patient non-compliance, discomfort, pain and local infection. Submicron sized drug delivery systems have gained attention in oral delivery of insulin for diabetes treatment. In most of the recent literature, the terms "microparticles" and "nanoparticle" refer to particles where the dimensions of the particle are measured in micrometers and nanometers respectively. For instance, insulin-loaded particles are defined as microparticles with size larger than 1 μm by most of the research groups. The size difference between nanoparticles and microparticles proffers numerous effects on the drug loading efficiency, aggregation, permeability across the biological membranes, cell entry and tissue retention. For instance, microparticulate drug delivery systems have demonstrated a number of advantages including protective effect against enzymatic degradation, enhancement of peptide stability, site-specific and controlled drug release. Compared to nanoparticulate drug delivery systems, microparticulate formulations can facilitate oral absorption of insulin by paracellular, transcellular and lymphatic routes. In this article, we review the current status of microparticles, microcapsules and microspheres for oral administration of insulin. A number of novel techniques including layer-by-layer coating, self-polymerisation of shell, nanocomposite microparticulate drug delivery system seem to be promising for enhancing the oral bioavailability of insulin. This review draws several conclusions for future directions and challenges to be addressed for optimising the properties of microparticulate drug formulations and enhancing their hypoglycaemic effects.

1. Introduction

Diabetes mellitus is a chronic metabolic disease characterised by either an insufficiency in insulin production as a result of pancreatic islet cells destruction, or insensitivity of host cells to the endogenous insulin (Pillay and Makgoba, 1991). In developed countries, diabetes mellitus is one of the major causes of mortality (King et al., 1998). The goal of diabetes treatment is to reduce the rate of disease progression, and prevent its life-threatening complications. Insulin is extensively used to manage the blood sugar level (BSL) in a substantial proportion of type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) patients. In the case of T2DM, administration of oral antidiabetic drugs and insulin replacement therapy are the main approaches to control BSL and minimise long-term complications. On the other hand, only subcutaneous insulin injection and surgical implantation of β-Langerhans cells exist for T1DM patients, which suffer from patient non-compliance due to their invasive nature and side-effects (Korsgren and Nilsson, 2009). As a result, alternative simple and painless routes for insulin administration is crucial to overall diabetes management.

Insulin is a 5800 Da peptide hormone consisting of 51 amino acids (Matsuura et al., 1993). The insulin monomer is composed of two polypeptide chains, an A-chain of 21 amino acids and B-chain of 30 amino acids, which are connected by two disulfide bonds. The structure of insulin varies between monomers, dimers, tetramers, and hexamers in solution under the influence of ions and solvent media (Vanea et al., 2014). Additionally, insulin is prone to fibril formation in acidic pH, elevated temperatures, organic chemicals and vibration (Hong and Fink, 2005). Apart from the above physical instability, oral insulin administration faces various physiological challenges such as chemical and enzymatic degradation in the gastrointestinal (GI) tract, intrinsic poor oral absorption, and rapid systemic clearance, resulting in low bioavailability and insufficient therapeutic effect (Khafagy el et al., 2007).

Consequently, insulin has been administered by multiple daily subcutaneous injections, which lead to a number of shortcomings, for example patient discomfort, pain, trauma, non-compliance, local

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infection, stress, and needle phobia (Wong et al., 2016). In the meantime, exogenous insulin exerts its effect in peripheral tissue without undergoing metabolism in the liver, hence it does not present identical pharmacokinetics as endogenous insulin (Arbit, 2004). Unlike subcutaneous injection of insulin, oral insulin undergoes hepatic metabolism, thus reducing gluconeogenesis in the liver. It also improves patient's compliance, comfort and acceptability (Renukuntla et al., 2013). Therefore, oral delivery of insulin is a convenient route especially for chronic disease. In order to endure the physiological difficulties, a number of approaches have been established to attain an oral insulin delivery system, including permeation enhancer, enteric coatings, enzyme inhibitors, chemical modifications, polymeric carriers, liposomes, nanoparticles and microparticles (Wong et al., 2016, 2017a).

Alternative insulin administration routes (Wong et al., 2016), physiological function of insulin, and strategies such as nanoparticulate drug delivery system for oral insulin delivery have been previous reviewed. The dividing line between nanoparticles and microparticle is not well defined, with some sources considering 1000 nm insulin-loaded particles to be nanoparticles (Wong et al., 2017b). In most of the recent literature, the terms "microparticles" and "nanoparticle" refer to particles where the dimensions of the particle are measured in micrometers and nanometers respectively. For instance, insulin-loaded particles are defined as microparticles with size larger than 1 μm by most of the research group. The size difference between nanoparticles and microparticles entails numerous effects on the drug loading efficiency, aggregation, permeability across the biological membranes, cell entry and tissue retention. In this review, we will introduce the status quo for oral insulin-loaded microparticles, followed by discussing features of natural polymeric microparticles, synthetic polymeric microparticles, inorganic microparticles, colon-targeting microparticles, hydrogel-based microparticles, insulin-loaded microcapsules, and insulin-loaded microspheres. Lastly, the future prospects and direction for development of oral insulin-loaded microparticulate formulations will be discussed.

2. Microparticulate delivery system for oral insulin administration

Over the years, microparticulate drug delivery systems composed of biopolymers have been explored to deliver insulin in a site-specific and controlled manner (Uddin et al., 2009). Microparticles, when formulated with appropriate excipients and polymers, are promising encapsulation systems for protecting the liable protein/peptide *in vitro* and *in vivo* degradation, enhancing its stability, providing an increased surface to volume ratio for peptide release and GI absorption (Singh et al., 2010), reducing adverse effects, and hence an improvement in bioavailability (Onal and Zihnioglu, 2002; Builders et al., 2008a). The main difficulties (Meinel et al., 2001) that impede the development of oral insulin-loaded microparticulate drug delivery systems are illustrated in Fig. 1. A few factors, including drug delivery system component, size, zeta potential, drug loading efficiency, encapsulation efficiency, release kinetics, and peptide bioactivity, have to be taken into consideration to develop a clinically successful oral peptide drug (Ye et al., 2010; Jin et al., 2012). Particle size is an indicator of the overall oral absorption of microparticles in the GI tract, entry to the systemic circulation, and insulin serum concentration. The intestinal absorption mechanism of insulin-loaded microparticles, ranging from 1 to 10 μm, is associated with cell internalisation through enterocytes and gut-associated lymphoid tissue (Peyer's patches) that avoid the first pass metabolism (Desai et al., 1996; Eldridge et al., 1989), or paracellular absorption through intestinal cells (Norris et al., 1998). Zeta potential can have an effect on both the stability and aggregation of insulin-loaded microparticles. In the meantime, some studies showed that microparticulate delivery system that exhibited uncontrolled high burst effect can lead to hypoglycaemic and detrimental immunological response (Hinds et al., 2005). In order to preserve three-dimensional structure and bioactivity of peptide drug, exposure to high temperatures, rigorous mechanical shear force or organic chemical in drug preparation must be minimised (van de Weert et al., 2000).

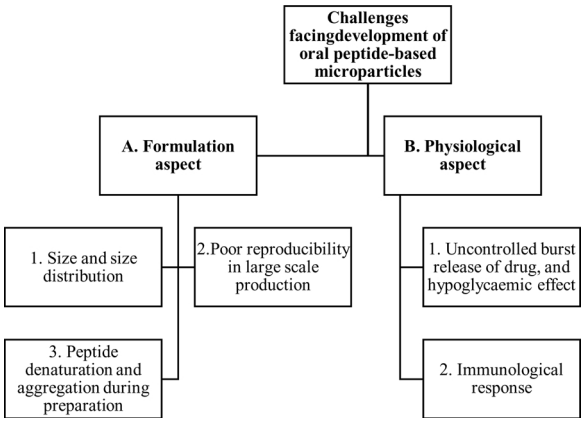

Fig. 1. Challenges facing development of microparticles for oral delivery of insulin. The difficulties in the development of clinically successful oral peptide-based microparticulate drug delivery systems are illustrated.

2.1. Insulin-loaded natural polymeric microparticles

Microparticles make frim natural polymers, due to their biodegradability, biocompatibility and stability in the GI tract, have been used to deliver a variety of therapeutic drugs to human. The polymeric microparticulate formulation, such as chitosan, dextran, alginate, poly (D, L-lactide-*co*-glycolide) (PLGA), could protect insulin against chemical and enzymatic degradation, enhance oral absorption and control insulin release kinetics. Table 1 lists the physical characteristics, *in vitro* and *in vivo* testing of insulin-loaded microparticle formulations.

2.1.1. Chitosan-based insulin-loaded microparticles

Chitosan is a positively charged, biocompatible, non-toxic, biodegradable, and mucoadhesive polymeric polysaccharide that can be prepared by the hydrolysis of chitin from crabs or shrimps (Prabaharan and Mano, 2005; Lehr et al., 1992). It comprises glucosamine and *N*-acetyl-glucosamine units (Yao et al., 1995; Illum, 1998). Chitosan can enhance paracellular drug absorption by opening epithelial tight junctions transiently and reversibly (Dodane et al., 1999). When insulin is microencapsulated in chitosan, the use of chemicals can lead to crosslinking between proteins and the loss of their bioactivity. Several methods, for instance, ionotropic gelation technique (Amidi et al., 2006) and self-assembly method (Min et al., 2008), have been developed to enhance drug stability. Ionotropic gelation technique involves an ionic interaction between cationic chitosan/chitosan derivatives and anionic insulin or low molecular weight crosslinker, such as tripolyphosphate and cyclodextrin (Merkus et al., 1999), whereas self-assembly method conjugates hydrophobic side moieties with chitosan.

Chitosan derivatives including hydrophilic (*N*-succinylated chitosan, glycochitosan), thiolated and hydrophobic chitosan (*N*-acylated chitosan, lauryl chitosan) have been investigated in a number of drug formulations (Yamaguchi et al., 1981; Roldo et al., 2004; Le Tien et al., 2003). Lauryl succinyl chitosan, which is a chitosan derivative consisting of both hydrophobic and hydrophilic groups, was used to formulate insulin-loaded microparticles (Rekha and Sharma, 2009). It was reported that the modified chitosan could exhibit calcium binding capacity, disrupt the tight junction, protect insulin from GI enzymatic degradation, and reduce BSL significantly. A multifunctional microparticulate platform, trimethyl chitosan-poly (ethylene glycol) and dimethacrylate-methacrylic acid (TMC-PEGDMA-MAA), was designed to deliver three therapeutic proteins including insulin, interferon beta and erythropoietin (Kondiah et al., 2017). These peptides are widely used to

Table 1
Insulin-loaded polymeric, inorganic and colon-targeting microparticles.

Carrier	Method of synthesis	Components	Physical analysis	Entrapment efficiency	Drug loading	*In vitro* insulin release	Animal model	Dose	*In vivo* observation	Ref/Year
Chitosan	Free radical polymerisation	Trimethyl chitosan + Poly (ethylene glycol) (MW 4000 g/mol) + dimethacrylate methacrylic acid + tablet	4.7 μm in gastric pH, 1.2 μm in intestinal pH	58.78%	–	pH 1.2 (3.2% in 2 h), pH 6.8 (83% in 24 h)	Alloxan-induced diabetic New Zealand White rabbits	Oral: 100 IU/kg SC: 10 IU/kg	54.19% reduction in BSL after 4 h; normal histological finding, no signs of inflammation or ulceration	(Kondiah et al., 2017)
	Ionotropic gelation	Lauryl succinyl chitosan + TPP + human insulin	315 nm–1.09 μm, negative zeta potential	48.1%	–	pH 1.2 (8.5% in 2 h), pH 7.4 (59.8% in 8 h), SGF with pepsin (90% undegraded), SIF with trypsin (86% undegraded), SIF with chymotrypsin (82% undegraded)	Streptozotocin-induced diabetic adult male Wistar rats	Oral: 60 IU/kg	BSL reduction for 6 h	(Rekha and Sharma, 2009)
Chitosan/ dextran	Layer-by-layer assembly	Dextran sulfate + 85% deacetylated chitosan + Bowman birk inhibitor + human recombinant insulin + insulin aspart	3–9 μm, 32 mV	62–62%	–	pH 1.1 (< 5% in 2 h), followed by pH 6 (< 5% in 2 h), followed by pH 7.4 (90% in 4 h)	Streptozotocin-induced diabetic male Wistar rats (250–350 g)	Oral: 100 IU/kg	Maximum reduction of BSL in 1 h; 38% reduction in BSL after 1 h	(Balabushevich et al., 2013)
		Dextran sulfate + 85% deacetylated chitosan + aprotinin/soybean Bowman inhibitor/Kunitz soybean trypsin inhibitor + recombinant human insulin + insulin aspart	6 μm, 30 mV	60%	–	Microparticles remained stable for 2 hr at pH 1.1 and pH 6	–	–	–	(Pechenkin et al., 2013)
		Dextran sulfate (500 kDa) + 75–85% deacetylated chitosan + recombinant human insulin zinc salt	3–12 μm, irregular-shaped particles	65%	50%	pH 7.4 (85% in 1 h), pH 7.1 and pH 7.8 with pancreatic juice (40% of insulin avoided degradation)	Chinchilla male rabbits (2.5–3.5 kg); Streptozotocin-induced diabetic male Wistar rats (250–350 g)	Rabbit: Oral (4 IU/kg), SC (4 IU/kg) Rats: Oral (25 IU/kg), SC (2.5 IU/kg)	Rabbit: 40% reduction in BSL in 1hr Rats: 50% reduction in BSL, 10 hr of hypoglycaemic effect; pharmacological availability is 10.7%	(Pechenkin et al., 2011)
		Dextran sulfate + 85% deacetylated chitosan + insulin aspart OR insulin lispro Or human insulin	5–6 μm, 28–36 mV	64–67%	53–59%	Protected 40% human insulin and 23% insulin aspart, insulin lispro was not protect in pancreatic juice containing trypsin and chymotrypsin	–	–	–	(Balabushevich et al., 2011)
Resistant starch/ glycoprotein	Extrusion-spheronisation	Native corn starch + concanavalin A + insulin	500 μm	–	–	–	Streptozotocin-induced diabetic rats	Oral: 60, 80, 100 IU/kg SC: 35 IU/kg (glargine insulin injection)	BSL declined to low level from 10 to 54 h after low- and medium-dose; high-dose showed prolonged hypoglycaemic effect from 8 to 60 h; exhibited hypoglycaemic effect for 44-52 h	(Situ et al., 2015)
Resistant starch	Extrusion-spheronisation	High amylose corn starch + insulin	Round shape	–	–	Cumulative release of 25.31% within		Oral: 15, 25, 35 IU/	25IU/kg: hypoglycaemic effect	(Situ et al., 2014)

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Table 1 (*continued*)

Carrier	Method of synthesis	Components	Physical analysis	Entrapment efficiency	Drug loading	*In vitro* insulin release	Animal model	Dose	*In vivo* observation	Ref/Year
						the first 8 h, and 80.66% in 30 h	Streptozotocin induced diabetic rats	kg SC: 25 IU/kg (glargine insulin injection	and high level of plasma insulin in 6 h; 47.8% reduction in BSL; Pharmacological availability is 88.2%	
β-cyclodextrin	Spray drying	β-cyclodextrin + insulin + hydroxypropyl methylcellulose acetate succinate + ethyl cellulose + insulin + enteric coated size 9 capsules	0.8 μm, 3.57 mV, uniform surface morphology	94.9%	–	No significant release in SGF (pH 1.2) in 2 h, followed by 50% in SIF (pH 6.8) in 8 h	Streptozotocin-induced Sprague-Dawley male diabetic rats (100–150 g, 4–6 weeks old, 300 mg/dL BSL)	Oral: 4 IU/kg SC: 0.4 IU/kg	60% BSL reduction after 12 h	(D'Souza et al., 2015)
Silica	Sol-gel process *via* spray drying and freeze drying	Zinc + silica + bovine pancreas insulin	2.5–35 μm, 12.2–21.3 mV, spherical, smooth surface with a large particles size distribution	–	–	pH 1.2 (25–41% in 2 h), pH 8.2 (12–19% in 72 h)	–	–	–	(Vanea et al., 2014)
Alginate/whey proteins	Extrusion/cold gelation process	Alginate + denatured whey proteins + calcium ions + insulin solution (umuline rapide)	1.583 mm, spherical	85%	13.1%	pH 1.2 (40% in 15 min, followed by 80% in 1 h), pH 6.8 (~60% in 15 min), pH 7.4 with trypsin and chymotrypsin (inhibit 52 and 66% of respective enzyme activity)	–	–	–	(Deat-Laine et al., 2012)
Alginate	Coacervation, diffusion filling	Sodium alginate + mucin + insulin + hard gelatin capsules	260–680 μm, round, smooth	88%	–	Insulin release from the formulation within 10 min in the dissolution media	Alloxan-induced diabetic rabbits (1.8–2.5 kg, 120 mg/dl)	Oral: 50 IU/kg	Maximum BSL reduction 5 h after oral administration	(Builders et al., 2008c)
	Ionotropic gelation	Sodium alginate (~250 cP and ~3500 CP) + poly(acrylic acid) (450 kDa) + cysteine hydrochloride + calcium chloride dihydrate + insulin	400–600 μm, spherical	–	15–65%	pH 6.8 (controlled release of insulin over 3 h)	–	–	–	(Greimel et al., 2007)
	Emulsification/ internal gelation	Sodium alginate + span 801 + calcium carbonate + recombinant human insulin (insulin Actrapid)	4–135 μm, spherical	80%	–	pH 1.2 (100% at 5 min)	–	–	–	(Reis et al., 2007)
Alginate/ WGA	Piezoelectric ejection process, ionotropic gelation	Alginate + WGA + calcium chloride + insulin	1–20 μm	–	–	–	Streptozotocin-induced diabetic Sprague-Dawley rats (190–230 g, 280–3380 mg.dl)	Oral: 50 IU/kg	60% reduction in BSL in 4 h	(Kim et al., 2005)
PLGA	Flow focusing	PLGA (50:50, 12 kDa) + recombinant human insulin	1.23 μm, spherical and smooth surface	98.95%	4.74%	pH 7.4 (biphasic profile, initial burst release, followed by a much slower release, release was complete after 10 h)	–	–	–	(Cozar-Bernal et al., 2011)
	Multiple emulsion-solvent evaporation	PLGA + Eudragit L30D + polyvinyl alcohol + recombinant human insulin	37 μm	40%	10 μg/mg	pH 1.5 (17.5% in 30 mins), pH 7.4 (32.9% in 30 mins)	Alloxan-induced female outbreed Wistar rats	Oral: 25 IU/kg	62.7% reduction in BSL in 2 h; Effect continue up to 24 h; peak reduction in 2 h	(Naha et al., 2008)

(*continued on next page*)

Table 1 (*continued*)

Carrier	Method of synthesis	Components	Physical analysis	Entrapment efficiency	Drug loading	*In vitro* insulin release	Animal model	Dose	*In vivo* observation	Ref/Year
							(200–250 g, 400–500 mg/dL)			
	Water-in-oil-in-water solvent evaporation (double emulsion)	PLGA + magnetite nanocrystals + insulin (Humunlin R)	4.6–7.2 μm, spherical	68–79%	–	–	Balb/c mice	Oral: 100 IU/kg IV: 2 IU/kg	43.5% reduction in BSL in the presence of external magnetic field for 20 h; pharmacological availability is 0.87–2.77%	(Cheng et al., 2006)
Hyaluronan	–	Hyaluronic bioadhesive liposomes + bovine pancreas insulin OR human recombinant insulin	10–30 μm	64–100%	–	More than 60% of insulin was protected in proteolytic environment	Streptozotocin-induced diabetic ten weeks old male ICR mice	Oral: 250 IU/kg SC: 1.5 IU/kg	11% BSL reduction for gagomeric insulin; 27% BSL reduction for coated insulin fibrils	(Dekel et al., 2010)

Key: BSL: blood sugar level; MW: molecular weight; PLGA: poly(D, L-lactide-co-glycolide); Ref: reference; SC: subcutaneous; SGF: simulated gastric fluid; SIF: simulated intestinal fluid; TPP: tripolyphosphate; WGA: wheat germ agglutinin.

treat diabetes, multiple sclerosis, and anaemia for chronic kidney disease and cancer chemotherapy. When the microparticles were formulated as tablets, the oral formulation had a promising protective effect and drug release profile in the small intestine, in which only 3.2% insulin was released in simulated gastric fluid (SGF), and 83% was released in simulated intestinal fluid (SIF) within 4 h. The formulation also demonstrated satisfactory hypoglycaemic effect with 54% reduction in BSL.

2.1.2. Dextran-based insulin-loaded microparticles

Layer-by-layer coating could encapsulate insoluble insulin-polyanion complexes to improve drug entrapment efficiency and loading efficiency (Pechenkin et al., 2011; Balabushevich et al., 2013; Ariga et al., 2014; Ariga et al., 2016). However, layer-by-layer coating is complex and time-consuming. Polyelectrolyte microparticles were prepared by the formation of insulin-dextran sulfate microaggregates, followed by alternate deposition of oppositely charged chitosan and dextran sulfate for four cycles (Pechenkin et al., 2011; Tong et al., 2012). Chitosan and dextran polymers were coated to the complexes to offer additional electrostatic interaction, protect encapsulated insulin from acidic environment, facilitate mucoadhesiveness, and improve gastrointestinal permeability (Balabushevich et al., 2013). Compared to native insulin powder, it was reported that the resistance of encapsulated insulin to SIF was enhanced (Pechenkin et al., 2011). When insulin and its analogues were encapsulated in polyelectrolyte microparticles, insulin aspart and lispro were more liable to proteolytic enzymes as compared to human insulin (Balabushevich et al., 2011). Polyelectrolyte insulin-loaded microparticles could exert blood sugar reduction in both diabetic rabbit and rat models with 10.7% oral bioavailability. However, around 60% of insulin was degraded *in vitro*. Therefore, protease inhibitor was incorporated into the matrix to protect insulin from proteolytic degradation (Balabushevich et al., 2013). It was concluded that Bowman-Birk inhibitor demonstrated better protection against trypsin, chymotrypsin and elastase degradation than other protein protease inhibitors (aprotinin and Kunitz soybean trypsin inhibitor) (Pechenkin et al., 2013). When both insulin aspart (monomer) and human insulin (hexamer) were microencapsulated in the drug delivery system, it could potentially reduce self-association and penetrate the intestinal epithelial tight junction rapidly (Sonaje et al., 2010).

2.1.3. Alginate-based insulin-loaded microparticles

Alginate is an anionic, biodegradable, biocompatible and hydrophilic polysaccharide, which is an ideal material to form a matrix for microencapsulation of therapeutic drugs and cells (Gombotz and Wee, 1998). It is made up of a mixture of glucuronic and mannuronic acid units. Multivalent cations, such as calcium, can trigger gelation by forming a crosslink between ions and the glucuronic acid units of alginate. It was found that the viscosity of alginate did not affect the drug loading of microparticle formulations (Greimel et al., 2007). Emulsification is a traditional approach to disperse insulin in the aqueous phase (alginate solution), followed by triggering internal gelation with crosslinking divalent cations (Reis et al., 2007). The drawback of alginate is associated with the drug release profile. Even though the secondary structure of insulin was maintained in the alginate matrix, the natural polymer could not suppress insulin release in gastric pH (100% was released within 5 min). Meanwhile, insulin-loaded alginate microparticles disintegrated rapidly in SIF as a result of calcium removal by the intestinal medium (Chan and Heng, 2002).

In order to achieve the required drug release profile, ionotropic gelation technique was employed to prepare novel thiomer microparticles for oral delivery of insulin (Greimel et al., 2007). The microparticles were comprised of alginate-cysteine and poly(acrylic acid)-cysteine, which formed both intra-chain (within microparticles) and inter-chain disulfide bonds (between microparticles), and hence improved the stability and cohesiveness of the microparticles in

physiological pH. The immobilised thiolated components of microparticles could also enhance 2–140-fold mucoadhesiveness in the GI tract by forming disulfide bonds with cysteine-rich mucus glycoproteins (Bernkop-Schnurch, 2005). Compared to unmodified polymers, the thiolated microparticles demonstrated a lower particle size, higher drug loading, and presented a more favourable disintegration profile (Greimel et al., 2007).

Several nonspecific (mucin) or specific (wheat germ agglutinin) mucoadhesive materials have been studied to interact with surface ligands at thr epithelial layer (Ponchel and Irache, 1998). Mucins are glycoproteins that possess both cohesive and viscous features (Mortazavi et al., 1993). They are the major building blocks of mucus in the GI tract (Bloomfield, 1983). Natural polymers can form a drug encapsulation matrix with the hydrophobic groups of mucin (Builders et al., 2008b). One of the studies used temperature-controlled solvent-induced coacervation and enteric film coating process to formulate insulin-loaded mucin-sodium alginate microparticles (Builders et al., 2008c). After 5 h of oral administration, animal studies showed that microparticles could reduce BSL. However, the microparticles had large size distribution (260–860 μm), and almost 40% insulin was released in SGF (pH 2.2). Another study employed piezoelectric ejection to prepare insulin-loaded alginate-wheat germ agglutinin (WGA) microparticles (Kim et al., 2005). Nontoxic lectins, such as WGA, can specifically form a covalent bond with the mucus layer *via* the *N*-acetyl glucosamine and sialic acid residues of the GI tract (Ertl et al., 2000). Thereby, lectin can prolong the microparticles residence time and potentially reduce the dosing frequency. When insulin-loaded microparticles were administered to diabetic rats orally, the GI absorption of insulin increased and the BSL reduced by 60% (Kim et al., 2005).

2.1.4. PLGA-based insulin-loaded microparticles

PLGA has been widely used as a therapeutic microcarrier in the formulation due to its biocompatible and biodegradable characteristics (Holgado et al., 2008). However, hydrophobic PLGA is not ideal to encapsulate hydrophilic insulin due to poor stability and loading efficiency (Mundargi et al., 2008). A novel flow focusing technique was used to formulate insulin-loaded PLGA microparticles (Cozar-Bernal et al., 2011). Compared to conventional double emulsion evaporation technique, the novel approach could produce microparticles with narrower size distributions, greater hydrophobicity, higher drug loading efficiency, slower controlled release profile, and larger production scale (Cozar-Bernal et al., 2011; Holgado et al., 2009). It was hypothesised that the application of external magnetic field can facilitate the localisation of therapeutic peptide drugs in the GI tract (Cheng et al., 2006). In the presence of circumferential magnetic force, magnetite-PLGA microparticles had a prolonged transit time in the intestine of mice, and revealed a substantial improvement in hypoglycaemic effect. Eudragit L30D is an acid resistant enteric coating polymer, which is insoluble in acidic buffer (below pH 5.5), but readily disintegrates in alkaline pH (Naha et al., 2008). Compared to uncoated PLGA microparticles (31.62%), Eudragit L30D-coated insulin-loaded microparticles could achieve controlled drug release and stabilise peptide drug, with 17.5% insulin release at acidic pH after 30 min (Naha et al., 2008). After oral administration of microparticles, the formulation could result in 62.7% reduction in BSL in 2 h.

2.1.5. Other insulin-loaded polymeric microparticles

β-Cyclodextrin is non-toxic, biodegradable, biocompatible and does not provoke immune response (Daoud-Mahammed et al., 2008). Cyclodextrin and their derivatives are also known to enhance drug stabilisation (Oh et al., 1994) and solubilisation (Otagiri et al., 1983), promote drug absorption, enhance insulin loading efficiency in the polymeric matrix, and positively influence the drug release profile (Sajeesh et al., 2010a). β-Cyclodextrin can form an inclusion complex with the hydrophobic cavity of insulin molecules, which stabilise the structure of insulin and improve its GI absorption (Irie and Uekama, 1999; Shao et al., 1994). Spray drying can be used to produce β-cyclodextrin microparticles and protect orally delivered insulin from chemical and enzymatic degradation in the SGF (D'Souza et al., 2015). It was indicated that β-cyclodextrin microparticles could ensure minimal insulin release in the SGF, promote translocation of glucose transporters, and produce a hypoglycaemic effect (D'Souza et al., 2015). One of the studies suggested that fibrillated insulin, when coated by phospholipid and lipid-hyaluronan conjugates, or encapsulated within a gagomer, is beneficial for diabetes treatment (Dekel et al., 2010). The gagomer is composed of exterior hyaluronan, and interior lipid clusters and hydrophilic regions. In the study, it was found that insulin fibrils could provide stable blood sugar reduction over several hours (Dekel et al., 2010).

3. Insulin-loaded inorganic microparticles

Microencapsulation of peptide into the matrix of inorganic amorphous silica is beneficial due to its inert, stable and biocompatible features (Vanea et al., 2014; Oh et al., 1994; Jin and Brennan, 2002). Insulin-loaded zinc-silica microparticles (Table 1) could be prepared by using sol-gel process (freeze drying and spray drying) (Vanea et al., 2014). In spray drying, the fluid within the sol-gel solution is evaporated to yield silica microparticles (Jin and Brennan, 2002). The nanopores (< 10 nm) formed on silica shell (< 10 nm) could provide a stable barrier around protein molecules and stabilize formulations (Macmillan et al., 2009). The microparticle matrix could prevent insulin aggregation and denaturation, whereas zinc oxide could preserve the secondary structure of peptide during preparation (Johnson et al., 1997). Nevertheless, the formulation released less than 20% of insulin in SIF after 72 h, which hindered the insulin absorption rate in the GI tract.

4. Colon-targeting bioadhesive microparticles

Oral colon-targeting drug delivery system has several favourable characteristics over small intestine, including a lengthy residence time, lower mucus turnover, reduced activity of proteolytic enzymes and better sensitivity to permeation enhancers (Maroni et al., 2012). Such a delivery system can be classified into bacterial-degradable, time-dependent, pH-responsive, pressure-sensitive, and mucoadhesive biomaterials (Xin Hua, 1994; Bernkop-Schnurch, 1998). Colon targeting studies have been carried out for polypeptides such as insulin, calcitonin, and metenkaphalin (Patel et al., 2007). However, oral colon-specific delivery is not ideal for absorption of systemically-acting drugs. Consequently, studies attempted to co-administer enzyme inhibitors and absorption enhancers with peptide drug in microparticulate drug delivery systems.

Among a variety of biopolymers, resistant starch is the most successful bacteria-degradable polysaccharide for oral colonic delivery (McConnell et al., 2008). A resistant starch film can be modified by temperature, enzyme, pressure and retrogradation, resulting in an improved resistance against acidic pH (Situ et al., 2014; Chen et al., 2007) and intestinal pancreatic amylase degradation (Englyst et al., 1996). In the colon, it is readily fermented by bacteria and amylolytic enzymes for drug release (Cummings et al., 1996). It was revealed that resistant starch film-coated microparticles could deliver insulin to the large intestine, release drug in a controlled manner, exhibit a steady reduction in BSL, and maintain hypoglycaemic effect for 14–22 h (Situ et al., 2014). However, the mucoadhesive property of resistant starch is not specific, thereby ligand conjugation is essential to achieve specific adhesion. A novel oral colon-targeting, insulin-loaded starch-glycoprotein microparticulate drug delivery system was developed to enable colonic cell recognition (Situ et al., 2015). After oral administration to type 2 diabetes rat model, the microparticles coated with concanavalin A were capable to exhibit prolonged hypoglycaemic effect for 44–52 h, which implicated that the dosing frequency can be potentially reduced.

Table 2
Insulin-loaded hydrogel microparticles.

Carrier	Method of synthesis	Components	Physical analysis	Entrapment efficiency	Drug loading	*In vitro* insulin release	Animal model	Dose	*In vivo* observation	Ref/ Year
P((MAA-co-NVP)-g-PEG)	UV-initiated free-radical polymerisation	Poly(methacrylic acid) + N-vinyl pyrrolidone + poly (ethylene glycol) + insulin	30–45 μm, irregular morphology, smooth and rough regions on surface	58.8–61.1%	–	–	–	–	–	(Steichen et al., 2017)
P(MAA-co-NVP)-chitosan	Ionic gelation	Polymethacrylic acid + N-vinyl pyrrolidone + EDMA + 85% deacetylated chitosan (270 kDa) + recombinant human insulin	–	80–88%	–	pH 1.2 (less than 20%), pH 7.4 (50% in 1 h, 90% in 3 h)	–	–	–	(Sajeesh and Sharma, 2011)
P(MAA-co-NVP)	UV-initiated free radical polymerisation	Polymethacrylic acid + N-vinyl pyrrolidone + EGDMA OR TEGDMA + bovine pancreatic insulin	75 μm	85–95%	11–12%	pH 3 (absence of release), pH 7 (rapid release)	–	–	–	(Carr and Peppas, 2010)
P(VCL-co-MAA)	Free radical polymerisation	Methacrylic acid + N-vinylprolactam + TEMED + human recombinant insulin	50–100 μm, irregular shapes, rough surface	52%	–	pH 1.2 (no release in 2 h), pH 7.4 (100% in 6 h)	Alloxan-induced male Wistar diabetic rats	Oral: 20 IU/kg; SC: 5 IU/kg	65% reduction in BSL in 3 h, increased to the control value within the next 5 h	(Mundargi et al., 2011a)
PMAA	Gamma radiation-induced copolymerisation	Methacrylic acid + DMAEMA + insulin	Porous, 3D interconnected microstructure	45–85%	–	pH 1.5 (30% in 2 h), pH 7.2 (80% in 1 h, all release in 2 h)	–	–	–	(Abou Taleb, 2013)
	Free radical polymerization	Methacrylic acid + β-cyclodextrin + purified porcine insulin	1–3 μm, highly irregular	–	–	Release for more than 7 h	–	–	–	(Victor and Sharma, 2002)
P(MAA-g-PEG)	Free radical UV polymerization	Polymethacrylic acid + polyethylene glycol (100 kDa) + PEGDMA + insulin-transferrin conjugate	–	–	–	–	–	–	–	(Shofner et al., 2010)
Polymethacrylic acid and chitosan	Ionic gelation, templated assisted polymerisation	Polymethacrylic acid + 85% deacetylated chitosan (270 kDa) + polyethylene glycol (20 kDa) + methyl-β-cyclodextrin (MS 1.8, MW 1313) + human insulin	–	82–90%	–	pH 1.2 (10% in 2 h), pH 7.4 (70–90% in 3 h)	Streptozotocin-induced diabetes male Wistar rats (200–250 g, > 250 mg/dL BSL)	Oral: 50 IU/kg; SC: 1 IU/kg	15-30% reduction in BSL in 2 h; pharmacological bioavailability is 1.8–1.95%	(Sajeesh et al., 2010a)
		Thiol functionalised polymethacrylic acid + polyethylene glycol (20 kDa) + chitosan (270 kDa) + human insulin	1.9 μm	79–85%	–	pH 1.2 (25% in 2 h), pH 7.4 (90% in 3 h)	Streptozotocin-induced diabetes male Wistar rats (200–250 g)	Oral: 50 IU/kg; SC: 1 IU/kg	40% reduction in BSL in 2 h; pharmacological bioavailability is 2.45%	(Sajeesh et al., 2010b)
P(MAA-g-PEG) WGA	UV-initiated free radical solution polymerisation	Poly(methacrylic acid) + poly(ethylene glycol) + WGA (36 kDa) + bovine pancreatic insulin	–	74%	5.03%	pH 3.2 (10% in 1 h), followed by pH 7 (70% in 1 h)	–	–	–	(Wood et al., 2008)
P(MAA-g-PEG))	UV-initiated free radical solution polymerisation	Poly(methacrylic acid) + poly(ethylene glycol) + TEGDMA + DMPA + crystalline recombinant human insulin + gelatin capsule	53 μm	86.4–88.4%	–	–	Streptozotocin induced diabetes male Wistar rats (180–200 g, 319 mg/dL), Goto-Kakizaki rats (149 mg/dL)	Oral: 25 IU/kg; SC: 1 IU/kg	30% reduction in BSL in 3 h; pharmacological bioavailability is 9.5%	(Morishita et al., 2006)
		Poly(methacrylic acid) + poly(ethylene glycol) + TEGDMA	–	72.8%	–	–	–	–	–	(Besheer et al., 2006)
		Poly(methacrylic acid) + poly(ethylene glycol) + TEGDMA + bovine insulin	150–212 μm	32.7–98.4%	–	pH 7.4 (39.7–56.61%), maximum insulin was released	–	–	–	(Lopez and Peppas, 2004)

(*continued on next page*)

Table 2 (*continued*)

Carrier	Method of synthesis	Components	Physical analysis	Entrapment efficiency	Drug loading	*In vitro* insulin release	Animal model	Dose	*In vivo* observation	Ref/ Year
						within 60 and 80 mins				
		Methacrylic acid + PEGMA + TEGDMA + DMPA + crystalline human insulin	–	–	–	–	Male Sprague Dawley rats (180–200 g)	Oral: 25 IU/kg; SC: 0.5–2 IU/kg	60% reduction in BSL in 1 h; pharmacological bioavailability is 12.8%	(Morishita et al., 2004)
		Methacrylic acid + PEGMA + TEGDMA + bovine insulin OR human recombinant insulin	43 μm	95%	–	pH 1.2 and pH 5 (no release), pH 7.4 (rapid release)	Male Sprague-Dawley rats	Oral: 25 IU/kg	20–60% reduction in BSL in 1 h; pharmacological bioavailability is 4.6–7.4%	(Nakamura et al., 2004)
		Poly(methacrylic acid) + poly(ethylene glycol) + TEGDMA + DMPA + insulin	–	–	–	–	–	–	–	(Ichikawa and Peppas, 2003)
		Poly(methacrylic acid) + poly(ethylene glycol) + TEGDMA + DMPA + crystalline porcine insulin	100–150 μm	87.4%	–	pH 1.2 (less than 12.5% in 2 h), followed by pH 6.8 (100% in 3 h)	–	–	–	(Morishita et al., 2002)
		Poly(methacrylic-g-ethylene glycol) + crystalline porcine insulin + gelatin capsules	–	94%	–	–	Streptozotocin induced diabetic Wistar rats (200 g)	Oral: 25, 50 IU/kg; SC: 0.25, 0.5, 1 IU/kg	40% reduction in BSL; strong dose independent hypoglycaemic effects within 2 h; hypoglycaemic effect was up to 8 h; pharmacological bioavailability is 4.22%	(Lowman et al., 1999)
PMAA-alginate	Free radical polymerisation	Poly(methacrylic acid) + sodium alginate	20 μm, highly irregular	–	–	pH 1.2 (30% in 2 h), pH 7.4 (90% in 1 h)	–	–	–	(Sajeesh and Sharma, 2004)
Bacterial cellulose-g-poly(acrylic acid)	Electron beam radiation	Bacterial cellulose + poly(acrylic acid) + recombinant human insulin	50–100 μm, irregular	66.1–84.9%	7.6–9.6%	pH 1.2 (10% in 2 h), followed by pH 6.8 (60–90% in 5 h)	Streptozotocin-induced diabetes male Wistar rats (275–325 g, > 16.7 mmol/L BSL)	Oral: 50 IU/kg; SC: 5 IU/kg	No significant pathological changes (inflammation, necrosis, or ulceration), 49% reduction in BSL in 4 h; pharmacological bioavailability is 6.98–7.45%	(Ahmad et al., 2016)
Alginate/whey protein	Extrusion/cold gelation technique	Sodium alginate + whey protein + calcium ion + insulin	1.3 μm	84.3–98.7%	10%	pH 1.2 and 6.8 (complete release over 2 h), pH 7.4 containing trypsin and chymotrypsin (84% was protected)	Adult male Wistar rats (280–320 g) Male albino rabbits (2.8–3.2 g)	Oral: 70 IU/kg; SC: 6 IU/kg	No significant difference in glycaemic control after 120 mins; pharmacological bioavailability is 8.8%	

(*continued on next page*)

Table 2 (*continued*)

Carrier	Method of synthesis	Components		Physical analysis	Entrapment efficiency	Drug loading	*In vitro* insulin release	Animal model	Dose	*In vivo* observation	Ref/ Year
											(Deat-Laine et al., 2013)

Key: BSL: blood sugar level; DMPA: dimethoxy propyl acetophenone; EDMA: ethylene glycol dimethacrylate; EGDMA: ethylene glycol dimethylacrylate; MW: molecular weight; PEGDMA: polyethylene glycol dimethacrylate; PMAA: poly(methacylic acid); Ref: reference; SC: subcutaneous; TEMED: tetramethylethylenediamine; TEGDMA: triethylene glycol dimethacrylate; WGA: wheat germ agglutinin.

5. Hydrogel-based insulin-loaded microparticles

Hydrogels, which consist of stimuli-responsive materials that response to physiological environmental triggers (pH, temperature, ions), are of great interest for oral administration of insulin (Sharpe et al., 2014). Table 2 illustrates the physical characteristics, *in vitro* and *in vivo* testing of insulin-loaded hydrogel-based microparticle formulations. Hydrogels are biocompatible, tunable, hydrophilic, and three-dimensional polymeric networks. They can be classified based on the nature of polymer, preparation method, cross-linking reaction, response to environmental stimuli, and physical structure (Peppas et al., 2000). Hydrogel-based microparticles can deliver peptide drug in a site-specific and controlled manner (Sharpe et al., 2014). The drug delivery rate is significantly dependent on the swelling properties of polymers (Andreopoulos, 1989). When the polymeric hydrogel is in contact with fluid, the water molecules penetrate and diffuse into the hydrophilic matrix, leading to the relaxation of polymer structure and chains in the hydrogel network (Karadag et al., 2004). The copolymer composition can also influence the drug release kinetics due to a shift in pH-responsiveness and swelling profile (Abou Taleb, 2013). Fig. 2 presents the properties of a successful oral hydrogel-based microparticulate formulation (Madsen and Peppas, 1999).

5.1. Alginate/whey insulin-loaded hydrogel microparticles

Whey proteins are natural polymers with valuable nutritional contents and flexible physical states such as foam, emulsion and gel. Encapsulation matrix formed by whey proteins and alginates have been investigated for delivery of drug compounds (Hebrard et al., 2013) and live microorganisms (Guerin et al., 2003). A pre-heating step can warrant complete whey protein denaturation, chain polymerisation, and allow gel formation *via* cold gelation technique and calcium ion addition (Hongsprabhas and Barbut, 1997). Microparticles can be formulated when insulin forms covalent bonds and steric hindrance with both whey proteins and alginate (Deat-Laine et al., 2012). The study revealed that alginate/whey protein microparticles could effectively protect insulin against enzymatic hydrolysis and proteolysis. A follow-up evaluation was carried out to investigate the efficacy of alginate/whey protein microparticles for oral administration of insulin (Deat-Laine et al., 2013). The insulin-loaded alginate/whey protein microparticles had high drug entrapment efficiency (98%), excellent *in vitro* matrix swelling behaviour and *in vivo* mucoadhesiveness to duodenum. However, insulin was released rapidly from the matrix in both SGF and SIF, which will require microparticle coating or enteric coated capsule in future studies to formulate a successful oral delivery vehicle. Despite fast release in SIF, *in vitro* experiments reveal that the microcarrier could protect insulin against enzymatic degradation, and promote insulin absorption across duodenal membranes.

5.2. Poly(methacylic acid) (PMAA)-based insulin-loaded hydrogel microparticles

Among hydrogel-based delivery systems, PMAA offers favourable properties for oral insulin delivery, including pH-sensitive swelling behaviour, prolonged GI residence time, proteolytic inhibition by its calcium chelating property, and the ability to promote reversible paracellular absorption (Sajeesh et al., 2010a, 2010b). In the radiation process, there are no harmful radical initiators, solvents, crosslinking agents, and separation agents involved for preparation of PMAA, therefore the method is economic, simple and environmentally friendly. One of the studies investigated PMAA microparticles for encapsulation of insulin β-cyclodextrin complex (Victor and Sharma, 2002). The study revealed that an increase in swelling degree of hydrogels was associated with a smaller amount of crosslinking agent in the formulation. In the meantime, a reduction of particle size was related to an increase in drug loading. Another study encapsulated methyl-β-cyclodextrin/insulin

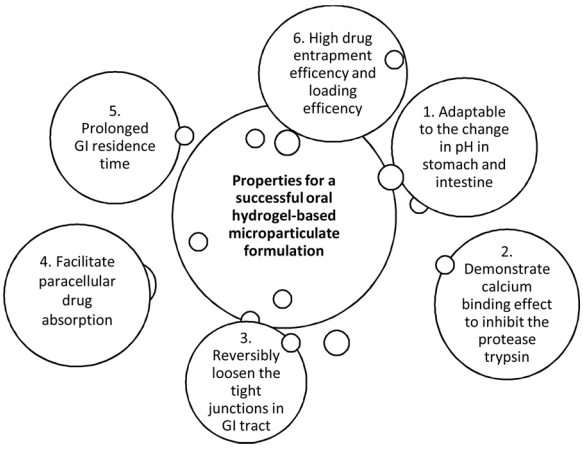

Fig. 2. Properties for a successful oral hydrogel-based microparticulate formulation. The ideal characteristics of hydrogel-based microparticulate formulation are illustrated.

complex into the matrix of PMAA-chitosan-poly(ethylene glycol) (PEG) hydrogel microparticles (Sajeesh et al., 2010a). However, it was reported that the formulation had around 2% relative pharmacological bioavailability after oral delivery of insulin-loaded microparticles.

Complexation hydrogel microparticles comprised of insulin and PEG-grafted PMAA (P(MAA-g-PEG)) were also designed (Morishita et al., 2006, 2002; Wood et al., 2008). The hydrogel microparticles were synthesised by free radical solution polymerisation, forming physical hydrogen bonds between methacrylic acid and PEG. The oral drug delivery system improved the stability of peptide against enzymatic degradation, and improved the residence time of drug in Caco-2 cells (Wood et al., 2008). Firstly, the PEG-grafted PMAA hydrogel microparticles had strong adhesion to GI tract for controlled drug release (Lowman et al., 1999). Secondly, the microparticles exhibited no significant cytotoxicity to Caco-2 cells, and reversibly reduced calcium ion-dependent trans-epithelial resistance by 55% after 2.5 h of incubation (Ichikawa and Peppas, 2003). Thirdly, it was reported that PEG chain length and particle size had no significant effect on insulin loading (Lopez and Peppas, 2004). However, the size of microparticles could influence insulin absorption, in which the particles with smaller size had a more rapid burst-release, stronger bioadhesive competence and induced higher insulin absorption from the ileum (Morishita et al., 2004). Lastly, the overall hypoglycaemic effect of the complexation hydrogel microparticles was demonstrated in diabetic rats with an oral bioavailability of 12.8% (Morishita et al., 2004).

However, there are limitations to the P(MAA-g-PEG) complexation hydrogel microparticles. Several studies reported that the oral drug delivery system did not entirely retain insulin in acidic pH (Wood et al., 2008; Besheer et al., 2006), and insulin was released abruptly in alkaline pH (Nakamura et al., 2004). At physiological pH, insulin prefers to partition into PEG moieties (Moriyama et al., 1999). In order to achieve an optimal oral peptide drug delivery system, alternative materials will be required to encapsulate and protect insulin effectively. The release kinetics of spin-labelled insulin and the microviscosity of medium can be examined by a flow system called electron spin resonance spectroscopy (Besheer et al., 2006). Similarly, burst release of insulin was observed from both P(MAA-g-PEG)-WGA and PMAA-alginate microparticles (Wood et al., 2008; Sajeesh and Sharma, 2004). For insulin-loaded PMAA-alginate microparticles, there was around 30% of insulin release in 2 h in SGF, and 90% of loaded drug was released within 60 min in SIF (burst release) (Sajeesh and Sharma, 2004). A study evaluated the drug loading of the above oral hydrogel microparticulate platform, and utilised insulin, theophylline, vancomycin and fluorescein-isothiocyanate-labelled dextrans as model drugs (Morishita et al., 2002). It was suggested that the nature and shape of peptide molecule could influence the entrapment efficiency of the hydrogel microparticles. In T1DM and T2DM rat models, the drug delivery system could suppress the postprandial rise in BSL following 3 times a day of oral administration of hydrogel microparticles, and demonstrated up to 9.5% relative pharmacological bioavailability (Morishita et al., 2006).

Surface modification, such as thiolation, was suggested to be a promising approach to improve the bioadhesion of drug delivery systems (Sajeesh et al., 2010c). When cysteine was grafted to the P(MAA-g-PEG)-chitosan hydrogel microparticles, an enhancement in polymer-mucus interaction and paracellular absorption was achieved (Sajeesh et al., 2010b). However, it should be noted that the protease inhibition capacity was reduced, and hence the oral bioavailability was low (2.45%). Apart from conjugation of cysteine to hydrogel microparticles, transferrin-modified insulin molecules can be used to develop an oral drug targeting delivery system (Shofner et al., 2010). Transferrin is a glycoprotein involved in iron transport and its receptor is extensively distributed on GI epithelial cells. In Caco-2/HT29-MTX co-culture cells, when insulin-transferrin conjugate was microencapsulated in P(MAA-g-PEG) particles, the transcellular uptake of insulin increased by fourteen times as compared to pure insulin molecules. This study explained that the diffusion of large insulin-transferrin complexes in the mucus (HT29-MTX cells) should not be disregarded (Shofner et al., 2010). Therefore, the use of Caco-2/HT29-MTX cells could provide a more precise representation for the diffusion of insulin-loaded hydrogel microparticles in the GI tract.

5.2.1. Poly(methacylic acid-co-N-vinyl pyrrolidone) (P(MAA-co-NVP))-based insulin-loaded hydrogel microparticles

P(MAA-*co*-NVP) hydrogel microparticles consist of a monomer mixture of methacrylic acid and *N*-vinyl pyrrolidone (NVP). When 1% EGDMA was used as a cross-linker, the entrapment efficiency and drug loading efficiency was 85% and 10% respectively (Carr and Peppas, 2010). At acidic pH, no insulin release was detected from the formulation, which indicated that a higher drug absorption will be present in the GI tract. The study also showed that 5 mg/mL of hydrogel microparticles had minor effect on cell viability. However, the formulation had no significant effect on transepithelial resistance and transportation across Caco-2 cells. It was suggested that active drug absorption, such as insulin-transferrin conjugates, will be required for the formulation to deliver oral insulin effectively. Another study applied ionic gelation technique to improve the physiochemical properties of P(MAA-*co*-NVP)-chitosan hydrogel microparticles (Sajeesh and Sharma, 2011). An incorporation of a hydrophilic non-ionic NVP segment reduced the concentration of insulin release in SGF, prolonged the residence time of formulation, and enhanced the absorption of microparticles in the GI tract. However, P(MAA-*co*-NVP)-chitosan hydrogel microparticles were less effective in facilitating paracellular absorption when compared to PMAA-chitosan microparticles (Sajeesh and Sharma, 2011). A novel cross- linked teropolymer, consisting of PMAA, NVP and PEG, was designed to improve the encapsulation of insulin in the pores of the hydrogel matrix (Steichen et al., 2017). The hydrogel microparticles possessed irregular morphology, which was hypothesised to improve the mucoadhesiveness of formulation in the GI tract due to an increase in surface area.

5.2.2. Poly(N-vinylcaprolactam-co-methacrylic acid) (P(PVCL-co-MAA))-based insulin-loaded hydrogel microparticles

Poly(*N*-vinylcaprolactam) (PVCL) is a biopolymer that displays a low critical solution temperature (~31.5 °C) (Lau and Wu, 1999). The pH-sensitive P(PVCL-*co*-MAA) hydrogel microparticles can be prepared by free radical polymerisation to load insulin (Mundargi et al., 2011a). The hydrogel microparticles were produced by forming hydrogen bonds between PVCL and MAA, which retain insulin in the hydrogel network at acidic pH. Even though the formulation reduced 50% of BSL in diabetic rats, freeze-drying was recommended to eliminate moisture from the microparticles and improve insulin encapsulation efficiency (52%).

5.3. Poly(acrylic acid) (PAA)-based insulin-loaded hydrogel microparticles

Toxic crosslinking agents and chemical initiators can damage the biological active peptide (Hennink and van Nostrum, 2012). Electron beam irradiation can be employed to graft PAA onto bacterial cellulose without utilising any initiator or cross-linking agents (Amin et al., 2012; Ahmad et al., 2014). Bacterial cellulose-g-PAA has high water holding capacity, biocompatibility and protein loading compatibility (Muller et al., 2014). This hydrogel delivery system possesses both thermo- and pH-responsive peptide release behaviour *in vitro* (Amin et al., 2012). When bovine serum albumin was encapsulated into hydrogel microparticles, only a minimal (10%) of model protein was released in SGF (Ahmad et al., 2014). The hydrogel microparticles could also maintain the structural stability of loaded peptide, facilitated the transportation of peptide across intestinal mucosa, and exhibited excellent cytocompatibility. A recent study prepared BC-g-PAA hydrogel microparticles by crushing and grinding the purified the bacterial cellulose-g-PAA hydrogel sheet (Ahmad et al., 2016). The formulation demonstrated irregular morphology and highly porous structure for enhanced mucoadhesion and insulin entrapment. In *ex vivo* intestinal tissues, it was reported that the adhesion of hydrogel microparticles increased from the duodenum to colon. Bacterial cellulose-g-poly(acrylic acid) is a potential biomaterial to enhance the hypoglycaemic effect and oral bioavailability of insulin.

6. Insulin-loaded microcapsules

A microcapsule is composed of interior core (active ingredient) and exterior shell (polymer or wax). The core of microcapsules can withhold either liquid or solid. Microcapsules have been extensively applied for drug delivery, food and agriculture industry (He et al., 2009; Poe et al., 2007). There are several methodologies to prepare microcapsules such as self-assembly, W/O/W double emulsion, complex coacervation, polymerisation, and LBL assembly. Spray drying is often involved in the production of microcapsules, but hot air can lead to instability and denaturation of peptide. Table 3 illustrates the physical characteristics, *in vitro* testing and *in vivo* observation of microcapsule formulations for oral delivery of insulin.

6.1. Natural polymeric-based insulin-loaded microcapsules

Whey protein isolate, carboxymethyl cellulose and sodium alginate are potential natural polymers to protect insulin in the GI tract. In one study, W/O/W double emulsion, complex coacervation and spray drying were conducted to prepare microcapsules (Furtado et al., 2008). The drug release kinetic, peptide biological activity and stability of the formulation varied with different methods of microcapsule preparation. It was reported that microcapsules only exhibited high solubility at alkali pH (pH 7), which released insulin in the small intestine. The formulation does not need organic solvents during preparation, and the integrity and biological activity of spray dried insulin-loaded microcapsules were maintained (Furtado et al., 2008).

6.2. Synthetic polymeric-based insulin-loaded microcapsules

Polylactide, being composed of aliphatic polyester, is a biocompatible polymer. In physiological and bacterial-existing environment, polylactide can breakdown to lactic acid. Polylactide was used to prepare insulin-loaded microcapsules by a two-step method of emulsion (Ma et al., 2000). In the study, the microcapsules were capable of withstanding enzymatic degradation and alleviating the BSL for 12 h in diabetic rats. It was suggested that the reduction in BSL was positively correlated to the dose of microcapsules. However, the absorption rate of oral formulation in the GI tract was varied.

Another pH-sensitive synthetic polymer, PLGA, was used to prepare insulin-loaded nanoparticles, followed by incorporation into microcapsules using solvent diffusion evaporation (Sun et al., 2015; Sun et al., 2016). In brief, insulin formed a complex with sodium oleate by hydrophobic ion pairing, and the complex was simultaneously loaded into the matrix of PLGA nanoparticles. Lastly, nanoparticles can be encapsulated into Eudragit® FS 30D by organic spray-drying method. It was reported that the hydrophobic insulin complex had an increased in entrapment efficiency to 94.6% as compared to free insulin (Sun et al., 2010). Eudragit® FS 30D is a pH-sensitive enteric material, which demonstrates biphasic drug release properties, including a reduction in initial burst release in alkali pH and subsequent prolonged insulin release from the nanoparticle matrix (Kshirsagar et al., 2009).

Sodium deoxycholate is an amphiphilic bile acid that has both hydrophobic and hydrophilic domains. It is synthesised in the liver and undergoes hepatic recirculation (Ferrebee and Dawson, 2015). Sodium deoxycholate can promote paracellular absorption of insulin by loosening the tight junction and transcellular absorption *via* enterocytes in the GI tract. In the meantime, bile acid can promote reabsorption of insulin-loaded particles from the GI tract. One of the studies adopted a similar strategy (nanocomposite microcapsule), but insulin-sodium deoxycholate complex was microencapsulated by pH-sensitive hydroxypropyl methyl cellulose phthalate (Sun et al., 2016). For spray drying, low temperature could be used in conjunction with organic solvent, which have positive effects on the size of particles, encapsulation effect, stability and release kinetics of the formulation. These studies confirmed that the insulin-loaded nanocomposite microcapsules could exert hypoglycaemic effect and improve relative bioavailability (Sun et al., 2015).

6.3. Inorganic insulin-loaded microcapsules

Dopamine, a simple catecholamine and neurotransmitter, can self-polymerise in an alakaline buffer and attach to polymers (Lee et al., 2007), carbon nanotubes (Fei et al., 2008) and magnetic nanoparticles (Si and Yang, 2011). Polydopamine (PDA)-coated microcapsules were constructed by a co-precipitation method for oral insulin delivery (Li et al., 2017). The insulin-loaded microcapsules were first prepared by simple salting out method as reported (Qi et al., 2009), followed by self-polymerisation of dopamine onto $MnCO_3$ microparticles to form a shell. Compared to LBL assembly, self-polymerisation is a facile, simple, single-step, low-cost, and green approach. The shape, homogeneity and strength of shell of microcapsules can be adjusted by dopamine concentration. It was found that the PDA shell-coated microcapsules released insulin in a pH-dependent behaviour. The insulin-loaded microcapsules could also maintain its intact morphology after long-term storage (60 days).

7. Insulin-loaded microspheres

Microspheres are characterised as uniform dispersion of drug in the matrix of polymers. Insulin is usually dissolved in the polymeric solution before precision particle fabrication processing into microspheres. They can be classified into three main categories including natural polymeric, synthetic polymeric and enteric polymeric-based spheres. Microspheres with large size (Jani et al., 1992) can be obtained from spray drying (Coppi et al., 2001) and coacervation technique (Mi et al., 2002), whereas emulsification (Vandenberg and De La Noue, 2001) can produce smaller microspheres. However, the biological activity of insulin can be deactivated by high shear force in the emulsion. These methods also produce insulin-loaded microspheres with large polydispersity index result in unwanted side-effects and poor reproducibility (Wang et al., 2005). Table 4 illustrates the physical characteristics, *in vitro* and *in vivo* testing of insulin-loaded microsphere formulations.

Table 3
Insulin-loaded microcapsules.

Carrier	Method of synthesis	Components	Physical analysis	Entrapment efficiency	*In vitro* insulin release	Animal model	Dose	*In vivo* observation	Ref/ Year
–	Co-precipitation	Dopamine hydrochloride + bovine pancreas insulin	4.5 μm, sphere	–	pH 5.4 (50% in 2 h), pH 7.4 (sustained release from the beginning to 40 h; 100% release)	–	–	–	(Li et al., 2017)
PLGA	Emulsion solvent diffusion method, hydrophobic ion pairing	PLGA + polyvinyl alcohol + hydroxypropylmethyl cellulose + insulin-sodium deoxycholate	1–5 μm, −29.2 mV, pdi 0.072, monodisperse spherical, smooth surface	94.2%	pH 1.2 (20.3% in 2 h), followed by pH 6.8 (55.8% in 6 h), followed by pH 7.4 (in 24 h)	Streptozocin–induced diabetic male Wistar rats (180–220 g, 12–13 weeks)	Oral: 20 IU/kg SC: 1.5 IU/ kg	29.2% reduction in BSL within 2 h; 36.8% reduction in 4 h	(Sun et al., 2016)
	Emulsion solvent diffusion method, spray-drying method	PLGA + polyvinyl alcohol + porcine insulin-sodium oleate complex + Eudragit FS 30D	−32.6 mV, monodisperse sphere	94.6%; drug loading (10%)	pH 1.2 (22.3% in 2 h), followed by pH 6.8 (37.7% in 6 h), followed by pH 7.4 (90.2% in 24 h)	Streptozocin–induced male Wistar rats (180–220 g, 12–13 weeks old)	Oral: 20 IU/kg SC: 1 IU/ kg	32.03% reduction in BSL in 2hr and 38.47% reduction in 24 h; pharmacological bioavailability is 15.6%	(Sun et al., 2015)
Sodium alginate/ carboxymethylcellulose	W/O/W double emulsion, complex coacervation, spray drying	Whey protein isolate + sodium carboxymethylcellulose OR sodium alginate + human insulin DNA recombinant solution	13–20 μm	88.7–98%	–	–	–	–	(Cardenas-Bailon et al., 2015)
Polylactide	Two-step method of emulsion and solvent extraction	Polylactide + methylene chloride + span 80 + crystalline porcine zinc insulin	2–5 μm, spherical, smooth surface	93%	pH 7.4 (65–74% over 6–8 h)	Alloxan-induced diabetic Male Wistar rats (180–300 g)	Oral: 40 IU/kg	39.7% reduction in BSL in 1–3 h	(Ma et al., 2000)

Key: BSL: blood sugar level; PLGA: poly(lactic-co-glycolic acid); Ref: reference; SC: subcutaneous.

7.1. Natural polymeric-based microspheres

7.1.1. Chitosan-based insulin-loaded microsphere

Chitosan-based microspheres with the size of 7.2 μm can be transported across the GI tract to the blood circulation (Wei et al., 2008). Poly(acrylic acid)-coated chitosan microspheres were developed for oral administration of 5-fluorouracil and insulin (Ramdas et al., 1999). This study stated that the sphere morphology and drug release were associated with the concentration of crosslinking agents. The insulin-loaded microspheres also exhibited mucoadhesiveness and had prolonged drug release for 8 days. Further study demonstrated that high molecular weight chitosan microspheres increased contact of encapsulated insulin with duodenum and jejunum in rats (Shimoda et al., 2001). However, insulin absorption was not enhanced by chitosan microspheres and required optimisation. Protease inhibitor (bacitracin) (Radlowski et al., 2005; Jose et al., 2013) and absorption enhancer (sodium taurocholate) (Meaney and O'Driscoll, 1999) were formulated in chitosan microspheres. These strategies protected 49.1% of insulin from trypsin degradation and prolonged the drug release for up to 12 h.

A few modified chitosan-based microspheres were prepared by emulsion phase separation. Firstly, chitosan phthalate microspheres could retain and protect insulin in the drug delivery system in SGF (Ubaidulla et al., 2007a). Compared to insulin solution, the relative oral pharmacological bioavailability of insulin-loaded chitosan phthalate microspheres increased 4 times (Ubaidulla et al., 2007b). Secondly, insulin-loaded chitosan succinate microspheres could reduce BSL for a prolonged period significantly, and increase oral pharmacological bioavailability (16%) in diabetic rats (Ubaidulla et al., 2007c). In order to optimise the formulation and preparation conditions, a Box-Behnken design was employed to prepare microspheres (Ubaidulla et al., 2009). This design took a few factors such as concentration of polymers, crosslinker and stirring speed into consideration. It was reported that the insulin entrapment efficiency and loading efficiency reduced with higher concentration of crosslinking agents. Apart from Box-Behnken design, Taguchi orthogonal method can enhance the efficiency of microsphere preparation by eliminating unnecessary experiments (Jose et al., 2012). The effect of each variable including polymer concentration, stirring time, crosslinking agents on the formulation can be evaluated.

7.1.2. Alginate-based microspheres

In the presence of calcium ions, alginate forms a gel (Strand et al., 2000; Gacesa, 1988) but encounters limitations such as insulin leakage from the matrix (Liu and Krishnan, 1999). The alginate microspheres prepared by ionotropic gelation could reduce insulin release in SGF after the addition of calcium ions (Martins et al., 2007). The alginate microspheres had less than 6% of insulin release in SGF within 2 h, and almost 90% was released in SIF after 2 h. On the other hand, insulin loading efficiency can be enhanced by β-cyclodextrin and emulsion-based technique with optimised solvents and preparation time (Jerry et al., 2001). After oral administration of insulin-loaded microspheres, the formulation produced a dose-dependent hypoglycaemic effect in diabetic rats. To overcome limitations such as poor drug release property (Silva et al., 2006) and broad size distribution (Hari et al., 1996), pH-sensitive the alginate-chitosan microspheres were prepared by membrane emulsion technique (Zhang et al., 2011). This technique form an electrostatic interaction between chitosan and alginate, reduce the size of pores, tighten the polydispersity index of microspheres, and lastly minimise insulin leakage during preparation (Huguet et al., 1996; Sezer and Akbuga, 1999). After oral administration of insulin-loaded microspheres, the BSL of diabetic rats was reduced and maintained for 60 h (Zhang et al., 2011).

7.2. Synthetic polymeric-based insulin-loaded microsphere

Fluorescent microscopy can be used to observe microspheres, consisting of PLGA, polyanhydride, and poly[*p*-(carboxyethylformamido)-benzoic anhydride] (PCEFB), in the absence of fluorescent dyes (Li et al., 2004). It was illustrated that PLGA/PCEFB microspheres were bioadhesive and transport across epithelia cells within 1.5 h, followed by substantial uptake by Peyer's patches (Li et al., 2004). Small microspheres can be fabricated by phase inversion nanoencapsulation with the use of poly(fumaric-co-sebacic) anhydride. Poly(fumaric-co-sebacic) anhydride is made up of a mixture of fumaric acid and PLGA (Furtado et al., 2008). It was reported that such microspheres adhered strongly to the GI tract. In biodistribution study, majority of insulin-loaded microspheres were distributed in GI cells, Peyer's patches, spleen and liver (Mathiowitz et al., 1997). Most importantly, the insulin-loaded microsphere formulation could suppress BSL in both T1DM rats and dogs with 5.5% and 23.3% of oral bioavailability respectively. Membrane emulsification process can also produce insulin-loaded PLGA microspheres with uniform size, high encapsulation efficiency, and retained peptide bioactivity (Ma, 2014). In the study, PLGA microspheres with smaller size released insulin at a faster rate due to larger surface area. However, burst release of insulin was observed from the formulation (Uchida et al., 1997), which will require the addition of additives (Manoharan and Singh, 2009), optimiaation of microsphere formation time, and a reduction in droplet size of emulsion (Qi et al., 2014).

7.3. Enteric polymer-based microsphere

Eudragit L100 (Morishita et al., 1993) and Eudragit S100 (Jain et al., 2005) were used to prepare insulin-loaded enteric microspheres. These anionic polymers are synthesised from a blend of MAA and MAA-methyl ester. They can withstand enzymatic degradation and allow insulin release at alkali pH. Compared to solvent evaporation method, a w/o/w emulsion solvent evaporation technique could encapsulate insulin in the matrix of hydroxypropylmethylcellulose acetate succinate with better drug loading efficiency (Nagareya et al., 1998). The use of an enzyme inhibitor (Jelvehgari et al., 2011) and permeation enhancer (Zhao et al., 2011) enhanced hypoglycaemic effect of drug formulation. In the presence of aprotinin (enzyme inhibitor) and sodium glycocholate (absorption enhancer), insulin-loaded microspheres demonstrated significant BSL lowering effect over 3 h of oral administration (Gowthamarajan et al., 2003).

Similar findings were reported for Eudragit S100 microspheres (Jain et al., 2005), Eudragit L100 microspheres (Jain et al., 2006) and polylactic acid microspheres (Uchida et al., 1997), in which a small amount of internal aqueous phase is favourable for insulin entrapment efficiency, formulation stability against enzyme degradation, drug release profile and *in vivo* therapeutic effect (Jain et al., 2005). However, the drug loading was low for both Eudragit S100 and Eudragit S100 microspheres. Trimethyl-chitosan can be used to facilitate insulin absorption by modulating the tight junction openings (van der Merwe et al., 2004). A study investigated the synergistic absorption-enhancing effect of Eudragit L100 and trimethyl-chitosan (Marais et al., 2013). Compared to control group, the tested microspheres lead to a 10-fold improvement in insulin absorption.

Eudragit RL is a water-insoluble, positively-charged, mucoadhesive, pH-independent (Zhang et al., 2012) and non-biodegradable polymer (Sahoo et al., 2009). Owing to its water insolubility, drug can be released in a controlled and sustained manner from the polymeric matrix. Appropriate selection of excipients can optimise the pharmacological bioavailability and stability of formulation (Zhao and Augsburger, 2005). When emulsification coacervation was used to prepare insulin-loaded Eudragit RL microspheres, magnesium stearate was incorporated as a lubricant to optimise the size of emulsion droplets (Meza et al., 2015; Liu et al., 2006). Another study used an enteric polymer, hydroxypropyl methylcellulose phthalate, incorporating sodium *N*-(8-[2-hydroxybenzoyl] amino) (SNAC) to prepare insulin-loaded microspheres (Qi and Ping, 2004). SNAC is an absorption

Table 4
Insulin-loaded microspheres.

Carrier	Method of synthesis	Components	Physical analysis	Entrapment efficiency	Drug loading	*In vitro* insulin release	Animal model	Dose	*In vivo* observation	Ref/ Year
Eudragit S100	Water-in-oil-in-oil double emulsion solvent evaporation, solvent evaporation	Eudragit S100 + polyvinyl alcohol + Human insulin + Aprotinin	57.42 μm, spherical	76.84%	–	pH 1.2 (3.25% in 2 h), pH 7.4 (50.41% in 2 h, 88.78 in 8 h)	–	–	–	(Agrawal et al., 2017)
	Water-in-oil-in-oil double emulsion solvent evaporation	Eudragit S100 + aprotinin + - recombinant insulin	222.4 μm, sphere	77.36%	4.65%	–	Streptozocin-induced diabetic adult male Wistar rats (240–260 g, 300 mg/ dl)	Oral: 20 IU/kg	30% reduction in BSL in 2 h; pharmacological bioavailability is 2.98%	(Jelvehgari et al., 2011)
		Polysorbate 20 + polyvinyl alcohol/ polyvinyl pyrrolidone + porcine insulin	32.51 μm	81.8%	0.43%	pH 1 (2.5% in 2 h), pH 7.4 (burst release of 22% in 1 h, with an additional 28% in next 5 h)	Male albino rabbits (2.3–2.7 kg)	Oral: 6.6I U/kg	24% BSL reduction; 76% reduction in 2 h and effect continuing up to 6 h	(Jain et al., 2005)
Eudragit S100/ Eudragit L100	Double emulsion-solvent evaporation technique	Eudragit S100 OR Eudragit L100 + human recombinant insulin + tablet	1–50 μm, sphere	33–64%	–	pH 1.2 (almost no insulin was released), pH 7.4 (100% in 5 h)	Alloxan-induced diabetic male Wistar rats (250 g, 300 mg/ dL)	Oral: 20 IU/kg	42% reduction in BSL; hypoglycaemic effect lasted for 300 min	(Mundargi et al., 2011b)
	Solvent diffusion technique	Eudragit S100 OR Eudragit L 100 + aprotinin + sodium glycocholate + bovine insulin + gelatin	24.6–61 μm	35.7–77.6%	–	pH 6.5 (99.3% in 4 h)	Alloxan-induced diabetic rats (180–250 g)	Oral: 50 IU/kg	Prolonged hypoglycaemic effect for 3 h	(Gowthamarajan et al., 2003)
	–	Eudragit L100 and S100 + aprotinin + - insulin	180–500 μm	65.8–80.2%	–	pH 6 (30–70% in 3 h), pH 7.5 (more than 90% in 60 min)	Male Wistar rats (180–220 g)	Oral: 50 IU/kg	80–180% BSL reduction in formulation containing enzyme inhibitors; pharmacological bioavailability is 1.2–3.6%	(Morishita et al., 1993)
Eudragit RL 100	Oil-in-oil emulsion-coacervation	Eudragit RL 100 + magnesium stearate + span 60 + insulin + gelatin capsule	14.2 –19.8 μm, spherical, brownish	74.55–75.9%	–	pH 7.2 (66.2–73.4% insulin release in 3 h)	–	–	–	(Kenechukwu and Momoh, 2016)
	Oil-in-oil emulsion-coacervation	Eudragit RL 100 + magnesium stearate + span 60 + insulin + hard gelatin capsules	30.5–42.7 μm, spherical, brownish	77.8–79.74%	–	pH 7.2 (68.2%–79.4%)	Alloxan-induced diabetic rats (180–280 g)	Oral: 50 IU/kg SC: 5 IU/kg	Prominent anti-hyperglycaemic effect from 2–4 h and effect was up to 12 h	(Momoh et al., 2015)
PLA/ PLGA	Membrane emulsion (direct membrane emulsification)	PLA OR PLGA + chitosan + - PVA + SDS	Submicron −100 μm	–	–	–	–	–	–	(Ma, 2014)
Poly(ester amide)	Solution polycondensation reaction, solid-in-oil-in-oil, modified isoelectric point precipitation	Poly(ester amide) + lysine OR leucine OR arginine + porcine insulin	13.4–16.7 μm	55.8–82%	3.12–4.6%	pH 1.2 (16–40% in 2 h), followed by pH 6.8 (30–82.1% in 6 h)	Streptozotocin-induced diabetic male Wistar rats (200–240 g)	Oral: 50 IU/kg SC: 5 IU/kg	43.6% reduction in BSL in 4.5 h; hypoglycaemic effect maintained for 10 h; pharmacological	(He et al., 2013)

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Table 4 (*continued*)

Carrier	Method of synthesis	Components	Physical analysis	Entrapment efficiency	Drug loading	*In vitro* insulin release	Animal model	Dose	*In vivo* observation	Ref/ Year
									bioavailability is 5.89%	
	Solid-in-oil-in-oil	Poly(ester amide) + lysine + leucine + span 83 + porcine insulin	8.6 μm	–	–	pH 1.2 (less than 5% in 2 h), pH 6.8 (85% in 6 h)	Streptozotocin-induced diabetic male Wistar rats (200–240 g)	Oral: 60 IU/kg SC: 3 IU/kg	50.6% reduction in BSL in 5 h; Hypoglycaemic effect continued up to 8 h; pharmacological bioavailability is 4.44%	(He et al., 2012)
Eudragit L100	Water-in-oil emulsion evaporation	Eudragit L100 + N-trimethylchitosan chloride + Tween 80 + recombinant human insulin	135.7–157.3 μm, spherical, smooth	–	27.9–52.4%	pH 7.4 (mean dissolution time is 34.5–42.6 min)	–	–	–	(Marais et al., 2013)
	Water-in-oil-in water emulsion-solvent evaporation	Polysorbate 20 + PVA OR PVP	59.11 μm, spherical to elliptical	84.5%	0.45%	pH 1 (7% in 2 h), pH 7.4 (burst release of 21% in 1 h, with additional 35% release in the next 5 h)	–	–	–	(Jain et al., 2006)
Chitosan	Emulsion cross-linking	Chitosan + bacitracin + sodium taurocholate + glutaraldehyde + insulin	32.6 μm	–	–	pH 2 with trypsin (protect 49.13% insulin), pH 7.4 (burst release in the first 3 h, and then a controlled release in the following 5–6 h)	–	–	–	(Jose et al., 2013)
	Emulsion cross-linking method	Chitosan + glutaraldehyde + Span 80 + insulin	29.5 μm	71.6%	–	–	Alloxan-induced diabetic male Wistar albino rats	Oral: 20 IU/kg SC: 2 IU/kg	The rate of BSL reduction was slow and reached maximum within 5 h; 25% BSL reduction and the hypoglycaemic effect was maintained for a period of 3-12 h; pharmacological bioavailability is 15.8%	(Jose et al., 2012)
	Dry-in-oil	82% deacetylated chitosan (500–800 kDa)	20 μm, spherical	–	5.5%	–	Wistar rats (240–270 g)	Oral: 50 IU/kg	60% insulin was released at 30 mins, and more than 70% at 2 h; BSL reduction was little	(Shimoda et al., 2001)
Chitosan phthalate/ Chitosan succinate	Emulsion phase separation	Chitosan phthalate OR chitosan succinate + glutaraldehyde + span 80 + porcine insulin	30–35 μm, 10–12 mV	–	74–78%	–	Streptozotocin-induced diabetic adult Wistar male albino rats (230–250 g, 280–380 mg/dL)	Oral: 20 IU/kg SC: 2 IU/kg	40.42%–41.08% reduction in BSL; hypoglycaemic effect was maintained for more than 16 h; pharmacological bioavailability is 16.24–18.66%	(Ubaidulla et al., 2009)
Chitosan phthalate				62%	88%					

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Table 4 (*continued*)

Carrier	Method of synthesis	Components	Physical analysis	Entrapment efficiency	Drug loading	*In vitro* insulin release	Animal model	Dose	*In vivo* observation	Ref/ Year
	Emulsion cross-linking	Chitosan phthalate + span 80 + glutaraldehyde + porcine insulin	13.14 μm, 10 mV, spherical, smooth			pH 2 (less than 20%), pH 7.4 (100% in 10 h)	Streptozotocin-induced diabetic adult Wistar male albino rats (230–250 g, 280–380 mg/dL)	Oral: 20 IU/kg SC: 2 IU/kg	49.46% BSL reduction at 6 h and BSL maintained over a prolonged period between 6 and 24 h; pharmacological bioavailability is 18.66%	(Ubaidulla et al., 2007b)
	Emulsion phase separation, passive absorption	Chitosan phthalate + glutaraldehyde + span 80 + porcine insulin	59.11 μm, 13 mV, spherical, rough and regular shape	62%	88%	pH 2 (27% in 24 h), pH 7.4 (98% in 24 h), protect 88% and 86% of insulin in pepsin and trypsin degradation respectively	–	–	–	(Ubaidulla et al., 2007a)
Chitosan succinate	Emulsion phase separation, passive absorption	Chitosan succinate + glutaraldehyde + span 80 + insulin	49 μm, 10 mV, sphere	80%	62%	pH 2 (less than 15%), pH 7.4 (100% in 12 h), protect 83.34% and 80.62% of insulin in peptic and trypsin degradation respectively	Streptozotocin-induced diabetic male adult Wistar albino rats (230–250 g, 280–380 mg/dL)	Oral: 20 IU/kg SC: 2 IU/kg	45% BSL reduction at 6 h; Hypoglycaemic effect was observed between 6 and 24 h; pharmacological bioavailability is 16%	(Ubaidulla et al., 2007c)
Chitosan/ alginate polyacrylic acid	–	Sodium alginate + 85% deacetylated chitosan + polyacrylic acid + glutaraldehyde	–	–	–	No release in the simulated gastric fluid for 4 h; burst release was observed within 24 h, followed by a slower terminal phase lasting about 6 days	Male albino rats	–	A thin gel was formed on the mucosa of the gastrointestinal tract after 16 h	(Ramdas et al., 1999)
Alginate	Impinging aerosols method	Sodium alginate + insulin	32.9 μm	48%	50%	pH 1.2 (40% in 2 h), pH 7.4 (90% in 8 h)	–	–	–	(Hariyadi et al., 2012)
	Ionotropic gelation	Sodium alginate + chitosan + dextran sulphate + human zinc-insulin	–	–	–	pH 1.2 (5–8% in 2 h), pH 6.8 (67–90% in 24 h)	–	–	–	(Martins et al., 2007)
	Emulsification/ internal gelation	Sodium alginate + cellulose acetate phthalate + Eudragit L100 + sodium carboxymethylcellulose + polyphosphate + dextran sulfate + cellulose sulfate + calcium carbonate + chitosan coating + Actrapid insulin	65–106 μm, discrete, spherical	14-100%	–	pH 1.2 (80% in 2 h), follow by pH 6.8 (a complete and fast dissolution of microspheres occurred in 4 h for all uncoated formulations)	–	–	–	(Silva et al., 2006)
		Sodium alginate + β-	–	–	7.36–28.37-%	–	Diabetic albino rats (250–300 gm)	Oral: 8 IU/kg		(Jerry et al., 2001)

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Table 4 (*continued*)

Carrier	Method of synthesis	Components	Physical analysis	Entrapment efficiency	Drug loading	*In vitro* insulin release	Animal model	Dose	*In vivo* observation	Ref/ Year
	Emulsion-based process, remote loading process	cyclodextrin + bovine insulin							36% reduction in serum glucose within 3 h	
Alginate/Chitosan	Membrane emulsion technique	Sodium alginate + chitosan + calcium ion + insulin	7.5 μm, −16.7 mV, spherical	–	56.7%	pH 1.2 (5% in 2 h), followed by pH 6.8 (32% in 4 h)	Streptozocin-induced diabetic male Sprague-Dawley rats	Oral: 100 IU/kg	44% reduction in BSL at 12 h; hypoglycaemic effect maintained for 60 h	(Zhang et al., 2011)
Poly(fumaric-co-sebacic)	Melt polycondensation, precipitation method, phase inversion nanoencapsulation	Poly(fumaric-co-sebacic) + PEG + Span 85 + bovine zinc insulin	1.2–5.9 μm	–	50%	–	Diabetes prone male rats, female beagle dogs	Rats (Oral: 75–250 IU/kg)Dogs (Oral: 50 IU/kg; SC: 2 IU/kg)	Rats: BSL reduction from 153% to 143% gradual decrease in BSL over time; pharmacological bioavailability is 5.5%–23.3%	(Furtado et al., 2008)
PLGA/PCEFB	Modified sater-in-oil-in-water emulsion solvent evaporation	PLGA + luminescent polyanhydride + poly[p-(carboxyethylformamido)- benzoic anhydride] + PVA- + insulin	1.95 μm, sphere, rough surface, rigid cross section	48%	–	pH 7.4 (28% in 3 hr, followed by 70% cumulative release by 4 days)	Female diabetic Sprague-Dawley rats	Oral: 28 IU/kg	BSL reduction to low level in 1.5 h, reached subcritical levels at 4 h, and then began to return to normal at 6 h; pharmacological bioavailability is 15.9%	(Li et al., 2004)
N-(8-[2-hydroxybenzoyl] amino) caprylate + hydroxypropyl methylcellulose phthalate	Water-in-oil-in-water and oil-in oil emulsion solvent evaporation	N-(8-[2-hydroxybenzoyl] amino) caprylate + hydroxypropyl methylcellulose phthalate + PVA + -porcine insulin	30–500 μm, sphere	30–70%	3–7%	pH 1.2 (20% released in 2 h), pH 1.2 with pepsin (20–60%% digested in 1 h), pH 6.8 (drug release time was 10–75 min)				

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Table 4 (*continued*)

Carrier	Method of synthesis	Components	Physical analysis	Entrapment efficiency	Drug loading	*In vitro* insulin release	Animal model	Dose	*In vivo* observation	Ref/ Year
Male Sprague-Dawley rats (180–220 g)	Oral: 100 IU/kg	BSL (53%) reduction was remarkable an maintain the hypoglycaemic effect for 4 h	(Qi and Ping, 2004)							
–	Water-in-oil-in-water emulsion solvent evaporation	Lauric acid + coat with hydroxypropylmethyl cellulose acetate succinate + polyvinyl alcohol + bovine insulin	30.8 μm	90%	–	pH 1.2 (no release), pH 6.8 (fast release)	Normal male Wistar eats (8 weeks age, 170–180 g)	Oral: 50 IU/kg	BSL became minimal at 0.5 hr and gradually rose to normal	(Nagareya et al., 1998)
PLA	Water-in-oil-in-water emulsion solvent evaporation	PLA + polyvinyl alcohol + bovine insulin	15–25 μm	95%	9.5%	Exhibited burst release in initial followed by additional slow release phase	Normal rats	SC: 4 IU/kg	The serum glucose level became minimum level at 3 h and gradually rose up to normal level	(Uchida et al., 1997)

Key: BSL: blood sugar level; PLA: poly(lactic acid); PLGA: poly(lactic-co-glycolic acid); Ref: reference; SC: subcutaneous.

enhancer that can improve oral bioavailability of human growth hormone (Mlynek et al., 2000) and heparin (Brayden et al., 1997). In cellular viability assay, SNAC did not present any toxicities (Wu and Robinson, 1999). Similarly, SNAC microspheres could protect insulin against enzymatic degradation and improve its GI absorption (Qi and Ping, 2004). However, hydroxypropyl methylcellulose phthalate is soluble below pH 5, and hence insulin can be susceptible to enzyme degradation. It was also reported that the microspheres had only weak hypoglycaemic effect when given orally with SNAC.

7.4. Poly(ester amide) (PEA)-based microsphere

The physical properties of PEA, such as charge density, pH-dependent behaviour, and hydrophobicity/hydrophilicity, can be adjusted by varying the building blocks and type of fatty diols, amino acids, and diacids (He et al., 2012; Deng et al., 2009; Pang and Chu, 2010). The amino acid groups (leucine) can improve the hydrophobicity of PEA microspheres (Ding et al., 2010). In the GI tract, PEAs with amino acid building blocks can breakdown by enzymatic erosion and biodegradation for insulin release and absorption (Paredes et al., 1998). Novel arginine-based PEA microspheres have also been synthesized (He et al., 2013). Their amino acid groups (lysine and leucine) protected insulin from physiological degradation, whereas arginine enhanced the GI absorption of insulin by introducing hydrophobicity. Overall, the relative oral bioavailability of drug formulation was 5.89% in diabetic rats, suggesting the interaction between microspheres and the GI tract, and the promotion of insulin absorption. However, further investigation will be required to clarify the permeation-enhancing effect and absorption mechanism of microspheres.

8. Conclusion

Diabetes is a chronic epidemic metabolic health condition affecting an extensive number of people especially in developed countries. Microvascular complications include retinopathy, nephropathy, neuropathy and diabetic foot disorders. Subcutaneous injection of insulin remains the conventional pharmacotherapy for T1DM and T2DM treatment. The reasons for patient non-compliance to insulin injection are attributed to issues such as discomfort, pain and local infection. On the other hand, oral administration of insulin formulation can improve patient acceptability and closely mimic the pharmacokinetics of endogenous insulin. Approaches towards oral administration of insulin have centred on using sub-micron sized pH-sensitive, biodegradable and biocompatible carriers, for instance, nanoparticles, microparticles and liposomes. A number of novel techniques including layer-by-layer coating, self-polymerisation of shell, and nanocomposite microparticulate drug delivery system seems to be promising for enhancing the oral bioavailability of insulin. Although there have been successes reported with microparticulate drug delivery systems for oral administration of insulin, future research needs to consider the size, nature of the polymer, zeta potential, vehicle, and coating of the formulation. Last but not least, there are a few challenges, such as broad size distribution, poor reproducibility, uncontrolled initial burst release, excessive hypoglycaemic effect and immunological response, which have to be addressed. These will optimise the physiochemical and pharmacokinetic properties of drug carrier, and most importantly, facilitate oral absorption and therapeutic effect of insulin in diabetes treatment.

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

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References

Abou Taleb, M.F., 2013. Radiation synthesis of multifunctional polymeric hydrogels for oral delivery of insulin. Int. J. Biol. Macromol. 62, 341–347.

Agrawal, G.R., Wakte, P., Shelke, S., 2017. Formulation, physicochemical characterization and in vitro evaluation of human insulin-loaded microspheres as potential oral carrier. Prog. Biomater. 6 (September (3)), 125–136.

Ahmad, N., Amin, M.C.I.M., Mahali, S.M., Ismail, I., Chuang, V.T.G., 2014. Biocompatible and mucoadhesive bacterial cellulose-g-poly(acrylic acid) hydrogels for oral protein delivery. Mol. Pharm. 11, 4130–4142.

Ahmad, N., Mohd Amin, M.C., Ismail, I., Buang, F., 2016. Enhancement of oral insulin bioavailability: in vitro and in vivo assessment of nanoporous stimuli-responsive hydrogel microparticles. Expert Opin. Drug. Deliv. 13, 621–632.

Amidi, M., Romeijn, S.G., Borchard, G., Junginger, H.E., Hennink, W.E., Jiskoot, W., 2006. Preparation and characterization of protein-loaded N-trimethyl chitosan nanoparticles as nasal delivery system. J. Control. Release 111, 107–116.

Amin, M.C.I.M., Ahmad, N., Halib, N., Ahmad, I., 2012. Synthesis and characterization of thermo- and pH-responsive bacterial cellulose/acrylic acid hydrogels for drug delivery. Carbohydr. Polym. 88, 465–473.

Andreopoulos, A.G., 1989. Preparation and swelling of polymeric hydrogels. J. Appl. Polym. Sci. 37, 2121–2129.

Arbit, E., 2004. The physiological rationale for oral insulin administration. Diabetes Technol. Ther. 6, 510–517.

Ariga, K., Yamauchi, Y., Rydzek, G., Ji, Q.M., Yonamine, Y., Wu, K.C.W., Hill, J.P., 2014. Layer-by-layer nanoarchitectonics: invention, innovation, and evolution. Chem. Lett. 43, 36–68.

Ariga, K., Li, J.B., Fei, J.B., Ji, Q.M., Hill, J.P., 2016. Nanoarchitectonics for dynamic functional materials from atomic-/molecular-level manipulation to macroscopic action. Adv. Mater. 28, 1251–1286.

Balabushevich, N.G., Pechenkin, M.A., Zorov, I.N., Shibanova, E.D., Shibanova, N.I., 2011. Mucoadhesive polyelectrolyte microparticles containing recombinant human insulin and its analogs aspart and lispro. Biochemistry (Mosc.) 76, 327–331.

Balabushevich, N.G., Pechenkin, M.A., Shibanova, E.D., Volodkin, D.V., Mikhalchik, E.V., 2013. Multifunctional polyelectrolyte microparticles for oral insulin delivery. Macromol. Biosci. 13, 1379–1388.

Bernkop-Schnurch, A., 1998. The use of inhibitory agents to overcome the enzymatic barrier to perorally administered therapeutic peptides and proteins. J. Control. Release 52, 1–16.

Bernkop-Schnurch, A., 2005. Thiomers: a new generation of mucoadhesive polymers. Adv. Drug Deliv. Rev. 57, 1569–1582.

Besheer, A., Wood, K.M., Peppas, N.A., Mader, K., 2006. Loading and mobility of spin-labeled insulin in physiologically responsive complexation hydrogels intended for oral administration. J. Control. Release 111, 73–80.

Bloomfield, V.A., 1983. Hydrodynamic properties of mucous glycoproteins. Biopolymers 22, 2141–2154.

Brayden, D., Creed, E., O'Connell, A., Leipold, H., Agarwal, R., Leone-Bay, A., 1997. Heparin absorption across the intestine: effects of sodium *N*-[8-(2-hydroxybenzoyl) amino]caprylate in rat in situ intestinal instillations and in Caco-2 monolayers. Pharm. Res. 14, 1772–1779.

Builders, P.F., Kunle, O.O., Okpaku, L.C., Builders, M.I., Attama, A.A., Adikwu, M.U., 2008a. Preparation and evaluation of mucinated sodium alginate microparticles for oral delivery of insulin. Eur. J. Pharm. Biopharm. 70, 777–783.

Builders, P.F., Kunle, O.O., Adikwu, M.U., 2008b. Preparation and characterization of mucinated agarose: a mucin-agarose physical crosslink. Int. J. Pharm. 356, 174–180.

Builders, P.F., Kunle, O.O., Okpaku, L.C., Builders, M.O., Attama, A.A., Adikwu, M.U., 2008c. Preparation and evaluation of mucinated sodium alginate microparticles for oral delivery of insulin. Eur. J. Pharm. Biopharm. 70, 777–783.

Cardenas-Bailon, F., Osorio-Revilla, G., Gallardo, T., Velazquez, 2015. Microencapsulation of insulin using a W/O/W double emulsion followed by complex coacervation to provide protection in the gastrointestinal tract. J. Microencapsul. 32, 308–316.

Carr, D.A., Peppas, N.A., 2010. Assessment of poly(methacrylic acid-co-N-vinyl pyrrolidone) as a carrier for the oral delivery of therapeutic proteins using Caco-2 and HT29-MTX cell lines. J. Biomed. Mater. Res. 92, 504–512.

Chan, L.W., Heng, P.W.S., 2002. Effects of aldehydes and methods of cross-linking on properties of calcium alginate microspheres prepared by emulsification. Biomaterials 23, 1319–1326.

Chen, L., Li, X., Li, L., Guo, S., 2007. Acetylated starch-based biodegradable materials with potential biomedical applications as drug delivery systems. Curr. Appl. Phys. 7, 90–93.

Cheng, J., Teply, B.A., Jeong, S.Y., Yim, C.H., Ho, D., Sherifi, I., Jon, S., Farokhzad, O.C., Khademhosseini, A., Langer, R.S., 2006. Magnetically responsive polymeric microparticles for oral delivery of protein drugs. Pharm. Res. 23, 557–564.

Coppi, G., Iannuccelli, V., Leo, E., Bernabei, M.T., Cameroni, R., 2001. Chitosan-alginate microparticles as a protein carrier. Drug Dev. Ind. Pharm. 27, 393–400.

Cozar-Bernal, M.J., Holgado, M.A., Arias, J.L., Munoz-Rubio, I., Martin-Banderas, L., Alvarez-Fuentes, J., Fernandez-Arevalo, M., 2011. Insulin-loaded PLGA microparticles: flow focusing versus double emulsion/solvent evaporation. J. Microencapsul. 28, 430–441.

Cummings, J.H., Beatty, E.R., Kingman, S.M., Bingham, S.A., Englyst, H.N., 1996. Digestion and physiological properties of resistant starch in the human large bowel. Br. J. Nutr. 75, 733–747.

Daoud-Mahammed, S., Grossiord, J.L., Bergua, T., Amiel, C., Couvreur, P., Gref, R., 2008. Self-assembling cyclodextrin based hydrogels for the sustained delivery of hydrophobic drugs. J. Biomed. Mater. Res. 86, 736–748.

Deat-Laine, E., Hoffart, V., Cardot, J.M., Subirade, M., Beyssac, E., 2012. Development and in vitro characterization of insulin loaded whey protein and alginate microparticles. Int. J. Pharm. 439, 136–144.

Deat-Laine, E., Hoffart, V., Garrait, G., Jarrige, J.F., Cardot, J.M., Subirade, M., Beyssac, E., 2013. Efficacy of mucoadhesive hydrogel microparticles of whey protein and alginate for oral insulin delivery. Pharm. Res. 30, 721–734.

Dekel, Y., Glucksam, Y., Margalit, R., 2010. Novel fibrillar insulin formulations for oral administration: formulation and in vivo studies in diabetic mice. J. Control. Release 143, 128–135.

Deng, M.X., Wu, J., Reinhart-King, C.A., Chu, C.C., 2009. Synthesis and characterization of biodegradable poly(ester amide)s with pendant amine functional groups and in vitro cellular response. Biomacromolecules 10, 3037–3047.

Desai, M.P., Labhasetwar, V., Amidon, G.L., Levy, R.J., 1996. Gastrointestinal uptake of biodegradable microparticles: effect of particle size. Pharm. Res. 13, 1838–1845.

Ding, H., Inoue, S., Ljubimov, A.V., Patil, R., Portilla-Arias, J., Hu, J.W., Konda, B., Wawrowsky, K.A., Fujita, M., Karabalin, N., Sasakie, T., Black, K.L., Holler, E., Ljubimova, J.Y., 2010. Inhibition of brain tumor growth by intravenous poly (beta-L-malic acid) nanobioconjugate with pH-dependent drug release. Proc. Natl. Acad. Sci. U. S. A. 107, 18143–18148.

Dodane, V., Khan, M.A., Merwin, J.R., 1999. Effect of chitosan on epithelial permeability and structure. Int. J. Pharm. 182, 21–32.

D'Souza, B., Bhowmik, T., Uddin, M.N., Oettinger, C., D'Souza, M., 2015. Development of beta-cyclodextrin-based sustained release microparticles for oral insulin delivery. Drug Dev. Ind. Pharm. 41, 1288–1293.

Eldridge, J.H., Meulbroek, J.A., Staas, J.K., Tice, T.R., Gilley, R.M., 1989. Vaccine-containing biodegradable microspheres specifically enter the gut-associated lymphoid tissue following oral administration and induce a disseminated mucosal immune response. Adv. Exp. Med. Biol. 251, 191–202.

Englyst, H.N., Kingman, S.M., Hudson, G.J., Cummings, J.H., 1996. Measurement of resistant starch in vitro and in vivo. Br. J. Nutr. 75, 749–755.

Ertl, B., Heigl, F., Wirth, M., Gabor, F., 2000. Lectin-mediated bioadhesion: preparation, stability and Caco-2 binding of wheat germ agglutinin-functionalized poly(D,L-lactic-co-glycolic acid)-microspheres. J. Drug Target. 8, 173–184.

Fei, B., Qian, B.T., Yang, Z.Y., Wang, R.H., Liu, W.C., Mak, C.L., Xin, J.H., 2008. Coating carbon nanotubes by spontaneous oxidative polymerization of dopamine. Carbon 46, 1795–1797.

Ferrebee, C.B., Dawson, P.A., 2015. Metabolic effects of intestinal absorption and enterohepatic cycling of bile acids. Acta Pharm. Sin. B 5, 129–134.

Furtado, S., Abramson, D., Burrill, R., Olivier, G., Gourd, C., Bubbers, E., Mathiowitz, E., 2008. Oral delivery of insulin loaded poly(fumaric-co-sebacic) anhydride microspheres. Int. J. Pharm. 347, 149–155.

Gacesa, P., 1988. Alginates. Carbohydr. Polym. 8, 161–182.

Gombotz, W.R., Wee, S.F., 1998. Protein release from alginate matrices. Adv. Drug Deliv. Rev. 31, 267–285.

Gowthamarajan, K., Kulkarni, T.G., Rajan, D.S., Suresh, B., 2003. Microspheres as oral delivery system for insulin. Indian J. Pharm. Sci. 65, 176–179.

Greimel, A., Werle, M., Bernkop-Schnurch, A., 2007. Oral peptide delivery: in-vitro evaluation of thiolated alginate/poly(acrylic acid) microparticles. J. Pharm. Pharmacol. 59, 1191–1198.

Guerin, D., Vuillemard, J.C., Subirade, M., 2003. Protection of bifidobacteria encapsulated in polysaccharide-protein gel beads against gastric juice and bile. J. Food Prot. 66, 2076–2084.

Hari, P.R., Chandy, T., Sharma, C.P., 1996. Chitosan/calcium-alginate beads for oral delivery of insulin. J. Appl. Polym. Sci. 59, 1795–1801.

Hariyadi, D.M., Wang, Y.W., Lin, S.C.Y., Bostrom, T., Bhandari, B., Coombes, A.G.A., 2012. Novel alginate gel microspheres produced by impinging aerosols for oral delivery of proteins. J. Microencapsul. 29, 250–261.

He, Q., Cui, Y., Li, J.B., 2009. Molecular assembly and application of biomimetic microcapsules. Chem. Soc. Rev. 38, 2292–2303.

He, P., Tang, Z.H., Lin, L., Deng, M.X., Pang, X., Zhuang, X.L., Chen, X.S., 2012. Novel biodegradable and pH-sensitive poly(ester amide) microspheres for oral insulin delivery. Macromol. Biosci. 12, 547–556.

He, P., Liu, H.Y., Tang, Z.H., Deng, M.X., Yang, Y., Pang, X., Chen, X.S., 2013. Poly(ester amide) blend microspheres for oral insulin delivery. Int. J. Pharm. 455, 259–266.

Hebrard, G., Hoffart, V., Cardot, J.M., Subirade, M., Beyssac, E., 2013. Development and characterization of coated-microparticles based on whey protein/alginate using the encapsulator device. Drug Dev. Ind. Pharm. 39, 128–137.

Hennink, W.E., van Nostrum, C.F., 2012. Novel crosslinking methods to design hydrogels. Adv. Drug Deliv. Rev. 64, 223–236.

Hinds, K.D., Campbell, K.M., Holland, K.M., Lewis, D.H., Piché, C.A., Schmidt, P.G., 2005. PEGylated insulin in PLGA microparticles. In vivo and in vitro analysis. J. Control. Release 104, 447–460.

Holgado, M.A., Arias, J.L., Cozar, M.J., Alvarez-Fuentes, J., Ganan-Calvo, A.M., Fernandez-Arevalo, M., 2008. Synthesis of lidocaine-loaded PLGA microparticles by flow focusing – effects on drug loading and release properties. Int. J. Pharm. 358, 27–35.

Holgado, M.A., Cozar-Bernal, M.J., Salas, S., Arias, J.L., Alvarez-Fuentes, J., Fernandez-Arevalo, M., 2009. Protein-loaded PLGA microparticles engineered by flow focusing: physicochemical characterization and protein detection by reversed-phase HPLC. Int. J. Pharm. 380, 147–154.

Hong, D.P., Fink, A.L., 2005. Independent heterologous fibrillation of insulin and its B-chain peptide. Biochemistry 44, 16701–16709.

Hongsprabhas, P., Barbut, S., 1997. Ca2 + -induced cold gelation of whey protein isolate: effect of two-stage gelation. Food Res. Int. 30, 523–527.
Huguet, M.L., Neufeld, R.J., Dellacherie, E., 1996. Calcium alginate beads coated with polycationic polymers: comparison of chitosan and DEAE-dextran. Process Biochem. 31, 347–353.
Ichikawa, H., Peppas, N.A., 2003. Novel complexation hydrogels for oral peptide delivery: in vitro evaluation of their cytocompatibility and insulin-transport enhancing effects using Caco-2 cell monolayers. J. Biomed. Mater. Res. 67, 609–617.
Illum, L., 1998. Chitosan and its use as a pharmaceutical excipient. Pharm. Res. 15, 1326–1331.
Irie, T., Uekama, K., 1999. Cyclodextrins in peptide and protein delivery. Adv. Drug Deliv. Rev. 36, 101–123.
Jain, D., Panda, A.K., Majumdar, D.K., 2005. Eudragit S100 entrapped insulin microspheres for oral delivery. AAPS PharmSciTech 6.
Jain, D., Majumdar, D.K., Panda, A.K., 2006. Insulin loaded Eudragit L100 microspheres for oral delivery: preliminary in vitro studies. J. Biomater. Appl. 21, 195–211.
Jani, P.U., Mccarthy, D.E., Florence, A.T., 1992. Nanosphere and microsphere uptake via peyer patches – observation of the rate of uptake in the rat after a single oral dose. Int. J. Pharm. 86, 239–246.
Jelvehgari, M., Milani, P.Z., Siahi-Shadbad, M.R., Monajjemzadeh, F., Nokhodchi, A., Azari, Z., Valizadeh, H., 2011. In vitro and in vivo evaluation of insulin microspheres containing protease inhibitor. Drug Res. 61, 14–22.
Jerry, N., Anitha, Y., Sharma, C.P., Sony, P., 2001. In vivo absorption studies of insulin from an oral delivery system. Drug Deliv. 8, 19–23.
Jin, W., Brennan, J.D., 2002. Properties and applications of proteins encapsulated within sol-gel derived materials. Anal. Chim. Acta 461, 1–36.
Jin, Y., Song, Y., Zhu, X., Zhou, D., Chen, C., Zhang, Z., Huang, Y., 2012. Goblet cell-targeting nanoparticles for oral insulin delivery and the influence of mucus on insulin transport. Biomaterials 33, 1573–1582.
Johnson, O.L., Jaworowicz, W., Cleland, J.L., Bailey, L., Charnis, M., Duenas, E., Wu, C.C., Shepard, D., Magil, S., Last, T., Jones, A.J.S., Putney, S.D., 1997. The stabilization and encapsulation of human growth hormone into biodegradable microspheres. Pharm. Res. 14, 730–735.
Jose, S., Fangueiro, J.F., Smitha, J., Cinu, T.A., Chacko, A.J., Premaletha, K., Souto, E.B., 2012. Cross-linked chitosan microspheres for oral delivery of insulin: Taguchi design and in vivo testing. Colloid Surf. B 92, 175–179.
Jose, S., Fangueiro, J.F., Smitha, J., Cinu, T.A., Chacko, A.J., Premaletha, K., Souto, E.B., 2013. Predictive modeling of insulin release profile from cross-linked chitosan microspheres. Eur. J. Med. Chem. 60, 249–253.
Karadag, E., Saraydin, D., Guven, O., 2004. Water absorbency studies of gamma-radiation crosslinked poly(acrylamide-co-2,3-dihydroxybutanedioic acid) hydrogels. Nucl. Instr. Meth. Phys. Res. B 225, 489–496.
Kenechukwu, F.C., Momoh, M.A., 2016. Formulation, characterization and evaluation of the effect of polymer concentration on the release behavior of insulin-loaded Eudragit ((R))-entrapped mucoadhesive microspheres. Int. J. Pharm. Investig. 6, 69–77.
Khafagy el, S., Morishita, M., Onuki, Y., Takayama, K., 2007. Current challenges in non-invasive insulin delivery systems: a comparative review. Adv. Drug Deliv. Rev. 59, 1521–1546.
Kim, B.Y., Jeong, J.H., Park, K., Kim, J.D., 2005. Bioadhesive interaction and hypoglycemic effect of insulin-loaded lectin-microparticle conjugates in oral insulin delivery system. J. Control. Release 102, 525–538.
King, H., Aubert, R.E., Herman, W.H., 1998. Global burden of diabetes, 1995–2025 – prevalence, numerical estimates, and projections. Diabetes Care 21, 1414–1431.
Kondiah, P.P., Choonara, Y.E., Tomar, L.K., Tyagi, C., Kumar, P., du Toit, L.C., Marimuthu, T., Modi, G., Pillay, V., 2017. Development of a gastric absorptive, immediate responsive, oral protein-loaded versatile polymeric delivery system. AAPS PharmSciTech 1, 1–15.
Korsgren, O., Nilsson, B., 2009. Improving islet transplantation: a road map for a widespread application for the cure of persons with type I diabetes. Curr. Opin. Organ. Tran. 14, 683–687.
Kshirsagar, S.J., Bhalekar, M.R., Umap, R.R., 2009. In vitro in vivo comparison of two pH sensitive eudragit polymers for colon specific drug delivery. J. Pharm. Sci. Res. 1, 61–70.
Lau, A.C.W., Wu, C., 1999. Thermally sensitive and biocompatible poly(N-vinylcaprolactam): synthesis and characterization of high molar mass linear chains. Macromolecules 32, 581–584.
Le Tien, C., Lacroix, M., Ispas-Szabo, P., Mateescu, M.A., 2003. N-acylated chitosan: hydrophobic matrices for controlled drug release. J. Control. Release 93, 1–13.
Lee, H., Dellatore, S.M., Miller, W.M., Messersmith, P.B., 2007. Mussel-inspired surface chemistry for multifunctional coatings. Science 318, 426–430.
Lehr, C.M., Bouwstra, J.A., Schacht, E.H., Junginger, H.E., 1992. In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. Int. J. Pharm. 78, 43–48.
Li, Y., Jiang, H.L., Jin, J.F., Zhu, K.J., 2004. Bioadhesive fluorescent microspheres as visible carriers for local delivery of drugs. II: uptake of insulin-loaded PCEFB/PLGA microspheres by the gastrointestinal tract. Drug Deliv. 11, 335–340.
Li, H., Jia, Y., Feng, X., Li, J., 2017. Facile fabrication of robust polydopamine microcapsules for insulin delivery. J. Colloid Interface Sci. 487, 12–19.
Liu, P., Krishnan, T.R., 1999. Alginate-pectin-poly-L-lysine particulate as a potential controlled release formulation. J. Pharm. Pharmacol. 51, 141–149.
Liu, R., Huang, S.S., Wan, Y.H., Ma, G.H., Su, Z.G., 2006. Preparation of insulin-loaded PLA/PLGA microcapsules by a novel membrane emulsification method and its release in vitro. Colloid Surf. B 51, 30–38.
Lopez, J.E., Peppas, N.A., 2004. Effect of poly (ethylene glycol) molecular weight and microparticle size on oral insulin delivery from P(MAA-g-EG) microparticles. Drug Dev. Ind. Pharm. 30, 497–504.
Lowman, A.M., Morishita, M., Kajita, M., Nagai, T., Peppas, N.A., 1999. Oral delivery of insulin using pH-responsive complexation gels. J. Pharm. Sci. 88, 933–937.
Ma, G., 2014. Microencapsulation of protein drugs for drug delivery: strategy, preparation, and applications. J. Control. Release 193, 324–340.
Ma, X.Y., Pan, G.M., Lu, Z., Hu, J.S., Bei, J.Z., Jia, J.H., Wang, S.G., 2000. Preliminary study of oral polylactide microcapsulated insulin in vitro and in vivo. Diabetes Obes. Metab. 2, 243–250.
Macmillan, A.M., Panek, D., McGuinness, C.D., Pickup, J.C., Graham, D., Smith, W.E., Birch, D.J.S., Karolin, J., 2009. Improved biocompatibility of protein encapsulation in sol-gel materials. J. Sol-Gel Sci. Technol. 49, 380–384.
Madsen, F., Peppas, N.A., 1999. Complexation graft copolymer networks: swelling properties, calcium binding and proteolytic enzyme inhibition. Biomaterials 20, 1701–1708.
Manoharan, C., Singh, J., 2009. Insulin loaded PLGA microspheres: effect of zinc salts on encapsulation, release, and stability. J. Pharm. Sci. 98, 529–542.
Marais, E., Hamman, J., du Plessis, L., Lemmer, R., Steenekamp, J., 2013. Eudragit (R) L100/N-trimethylchitosan chloride microspheres for oral insulin delivery. Molecules 18, 6734–6747.
Maroni, A., Zema, L., Del Curto, M.D., Foppoli, A., Gazzaniga, A., 2012. Oral colon delivery of insulin with the aid of functional adjuvants. Adv. Drug Deliv. Rev. 64, 540–556.
Martins, S., Sarmento, B., Souto, E.B., Ferreira, D.C., 2007. Insulin-loaded alginate microspheres for oral delivery – effect of polysaccharide reinforcement on physicochemical properties and release profile. Carbohydr. Polym. 69, 725–731.
Mathiowitz, E., Jacob, J.S., Jong, Y.S., Carino, G.P., Chickering, D.E., Chaturvedi, P., Santos, C.A., Vijayaraghavan, K., Montgomery, S., Bassett, M., Morrell, C., 1997. Biologically erodable microspheres as potential oral drug delivery systems. Nature 386, 410–414.
Matsuura, J., Powers, M.E., Manning, M.C., Shefter, E., 1993. Structure and stability of insulin dissolved in 1-octanol. J. Am. Chem. Soc. 115, 1261–1264.
McConnell, E.L., Short, M.D., Basit, A.W., 2008. An in vivo comparison of intestinal pH and bacteria as physiological trigger mechanisms for colonic targeting in man. J. Control. Release 130, 154–160.
Meaney, C., O'Driscoll, C., 1999. Mucus as a barrier to the permeability of hydrophilic and lipophilic compounds in the absence and presence of sodium taurocholate micellar systems using cell culture models. Eur. J. Pharm. Sci. 8, 167–175.
Meinel, L., Illi, O.E., Zapf, J., Malfanti, M., Peter Merkle, H., Gander, B., 2001. Stabilizing insulin-like growth factor-I in poly(D,L-lactide-co-glycolide) microspheres. J. Control. Release 70, 193–202.
Merkus, F.W.H.M., Verhoef, J.C., Marttin, E., Romeijn, S.G., van der Kuy, P.H.M., Hermens, W.A.J.J., Schipper, N.G.M., 1999. Cyclodextrins in nasal drug delivery. Adv. Drug Deliv. Rev. 36, 41–57.
Meza, B.E., Peralta, J.M., Zorrilla, S.E., 2015. Rheological properties of a commercial food glaze material and their effect on the film thickness obtained by dip coating. J. Food Process Eng. 38, 510–516.
Mi, F.L., Sung, H.W., Shyu, S.S., 2002. Drug release from chitosan-alginate complex beads reinforced by a naturally occurring cross-linking agent. Carbohydr. Polym. 48, 61–72.
Min, K.H., Park, K., Kim, Y.S., Bae, S.M., Lee, S., Jo, H.G., Park, R.W., Kim, I.S., Jeong, S.Y., Kim, K., Kwon, I.C., 2008. Hydrophobically modified glycol chitosan nanoparticles-encapsulated camptothecin enhance the drug stability and tumor targeting in cancer therapy. J. Control. Release 127, 208–218.
Mlynek, G.M., Calvo, L.J., Robinson, J.R., 2000. Carrier-enhanced human growth hormone absorption across isolated rabbit intestinal tissue. Int. J. Pharm. 197, 13–21.
Momoh, M.A., Kenechukwu, F.C., Nnamani, P.O., Uetiti, J.C., 2015. Influence of magnesium stearate on the physicochemical and pharmacodynamic characteristics of insulin-loaded Eudragit entrapped mucoadhesive microspheres. Drug Deliv. 22, 837–848.
Morishita, I., Morishita, M., Takayama, K., Machida, Y., Nagai, T., 1993. Enteral insulin delivery by microspheres in 3 different formulations using Eudragit-L100 and Eudragit-S100. Int. J. Pharm. 91, 29–37.
Morishita, M., Lowman, A.M., Takayama, K., Nagai, T., Peppas, N.A., 2002. Elucidation of the mechanism of incorporation of insulin in controlled release systems based on complexation polymers. J. Control. Release 81, 25–32.
Morishita, M., Goto, T., Peppas, N.A., Joseph, J.I., Torjman, M.C., Munsick, C., Nakamura, K., Yamagata, T., Takayama, K., Lowman, A.M., 2004. Mucosal insulin delivery systems based on complexation polymer hydrogels: effect of particle size on insulin enteral absorption. J. Control. Release 97, 115–124.
Morishita, M., Goto, T., Nakamura, K., Lowman, A.M., Takayama, K., Peppas, N.A., 2006. Novel oral insulin delivery systems based on complexation polymer hydrogels: single and multiple administration studies in type 1 and 2 diabetic rats. J. Control. Release 110, 587–594.
Moriyama, K., Ooya, T., Yui, N., 1999. Hyaluronic acid grafted with poly(ethylene glycol) as a novel peptide formulation. J. Control. Release 59, 77–86.
Mortazavi, S.A., Carpenter, B.G., Smart, J.D., 1993. A comparative-study on the role played by mucus glycoproteins in the rheological behavior of the mucoadhesive mucosal interface. Int. J. Pharm. 94, 195–201.
Muller, A., Wesarg, F., Hessler, N., Muller, F.A., Kralisch, D., Fischer, D., 2014. Loading of bacterial nanocellulose hydrogels with proteins using a high-speed technique. Carbohydr. Polym. 106, 410–413.
Mundargi, R.C., Babu, V.R., Rangaswamy, V., Patel, P., Aminabhavi, T.M., 2008. Nano/micro technologies for delivering macromolecular therapeutics using poly(D,L-lactide-co-glycolide) and its derivatives. J. Control. Release 125, 193–209.
Mundargi, R.C., Rangaswamy, V., Aminabhavi, T.M., 2011a. Poly(N-vinylcaprolactam-co-methacrylic acid) hydrogel microparticles for oral insulin delivery. J. Microencapsul. 28, 384–394.
Mundargi, R.C., Rangaswamy, V., Aminabhavi, T.M., 2011b. pH-Sensitive oral insulin

delivery systems using Eudragit microspheres. Drug Dev. Ind. Pharm. 37, 977–985.
Nagareya, N., Uchida, T., Matsuyama, K., 1998. Preparation and characterization of enteric microspheres containing bovine insulin by a w/o/w emulsion solvent evaporation method. Chem. Pharm. Bull. (Tokyo) 46, 1613–1617.
Naha, P.C., Kanchan, V., Manna, P.K., Panda, A.K., 2008. Improved bioavailability of orally delivered insulin using Eudragit-L30D coated PLGA microparticles. J. Microencapsul. 25, 248–256.
Nakamura, K., Murray, R.J., Joseph, J.I., Peppas, N.A., Morishita, M., Lowman, A.M., 2004. Oral insulin delivery using P(MAA-g-EG) hydrogels: effects of network morphology on insulin delivery characteristics. J. Control. Release 95, 589–599.
Norris, D.A., Puri, N., Sinko, P.J., 1998. The effect of physical barriers and properties on the oral absorption of particulates. Adv. Drug Deliv. Rev. 34, 135–154.
Oh, I.J., Song, H.M., Lee, K.C., 1994. Effect of 2-hydroxypropyl-β-cyclodextrin on the stability of prostaglandin E2 in solution. Int. J. Pharm. 106, 135–140.
Onal, S., Zihnioglu, F., 2002. Encapsulation of insulin in chitosan-coated alginate beads: oral therapeutic peptide delivery. Artif. Cells Blood Substit. Immobil. Biotechnol. 30, 229–237.
Otagiri, M., Imai, T., Matsuo, N., Uekama, K., 1983. Improvements to some pharmaceutical properties of flurbiprofen by beta-cyclodextrin and gamma-cyclodextrin complexations. Acta Pharm. Suec. 20, 1–10.
Pang, X., Chu, C.C., 2010. Synthesis, characterization and biodegradation of functionalized amino acid-based poly(ester amide)s. Biomaterials 31, 3745–3754.
Paredes, N., Rodriguez-Galan, A., Puiggali, J., 1998. Synthesis and characterization of a family of biodegradable poly(ester amide)s derived from glycine. J. Polym. Sci. Pol. Chem. 36, 1271–1282.
Patel, M., Shah, T., Amin, A., 2007. Therapeutic opportunities in colon-specific drug-delivery systems. Crit. Rev. Ther. Drug Carr. Syst. 24, 147–202.
Pechenkin, M.A., Balabushevich, N.G., Zorov, I.N., Staroseltseva, L.K., Mikhalchik, E.V., Izumrudov, V.A., Larionova, N.I., 2011. Design in vitro and in vivo characterization of chitosan-dextran sulfate microparticles for oral delivery of insulin. J. Bioequiv. Availab. 3, 244–250.
Pechenkin, M.A., Balabushevich, N.G., Zorov, I.N., Izumrudov, V.A., Klyachko, N.L., Kabanov, A.V., Larionova, N.I., 2013. Use of protease inhibitors in composite polyelectrolyte microparticles in order to increase the bioavailability of perorally administered encapsulated proteins. Pharm. Chem. J. 47, 62–69.
Peppas, N.A., Bures, P., Leobandung, W., Ichikawa, H., 2000. Hydrogels in pharmaceutical formulations. Eur. J. Pharm. Biopharm. 50, 27–46.
Pillay, T.S., Makgoba, M.W., 1991. Molecular mechanisms of insulin resistance. S. Afr. Med. J. 79, 607–613.
Poe, S.L., Kobaslija, M., McQuade, D.T., 2007. Mechanism and application of a microcapsule enabled multicatalyst reaction. J. Am. Chem. Soc. 129, 9216–9221.
Ponchel, G., Irache, J.M., 1998. Specific and non-specific bioadhesive particulate systems for oral delivery to the gastrointestinal tract. Adv. Drug Deliv. Rev. 34, 191–219.
Prabaharan, M., Mano, J.F., 2005. Chitosan-based particles as controlled drug delivery systems. Drug Deliv. 12, 41–57.
Qi, R., Ping, Q.N., 2004. Gastrointestinal absorption enhancement of insulin by administration of enteric microspheres and SNAC to rats. J. Microencapsul. 21, 37–45.
Qi, W., Yan, X.H., Fei, J.B., Wang, A.H., Cui, Y., Li, J.B., 2009. Triggered release of insulin from glucose-sensitive enzyme multilayer shells. Biomaterials 30, 2799–2806.
Qi, F., Wu, J., Hao, D.X., Yang, T.Y., Ren, Y., Ma, G.H., Su, Z.G., 2014. Comparative studies on the influences of primary emulsion preparation on properties of uniform-sized exenatide-loaded PLGA microspheres. Pharm. Res. 31, 1566–1574.
Radlowski, M., Bartkowiak, S., Winiarczyk, K., Kalinowski, A., 2005. Differential influence of bacitracin on plant proteolytic enzyme activities. Biochim. Biophys. Acta 1722, 1–5.
Ramdas, M., Dileep, K.J., Anitha, Y., Paul, W., Sharma, C.P., 1999. Alginate encapsulated bioadhesive chitosan microspheres for intestinal drug delivery. J. Biomater. Appl. 13, 290–296.
Reis, C.P., Ribeiro, A.J., Neufeld, R.J., Veiga, F., 2007. Alginate microparticles as novel carrier for oral insulin delivery. Biotechnol. Bioeng. 96, 977–989.
Rekha, M.R., Sharma, C.P., 2009. Synthesis and evaluation of lauryl succinyl chitosan particles towards oral insulin delivery and absorption. J. Control. Release 135, 144–151.
Renukuntla, J., Vadlapudi, A.D., Patel, A., Boddu, S.H., Mitra, A.K., 2013. Approaches for enhancing oral bioavailability of peptides and proteins. Int. J. Pharm. 447, 75–93.
Roldo, M., Hornof, M., Caliceti, P., Bernkop-Schnurch, A., 2004. Mucoadhesive thiolated chitosans as platforms for oral controlled drug delivery: synthesis and in vitro evaluation. Eur. J. Pharm. Biopharm. 57, 115–121.
Sahoo, J., Murthy, P.N., Biswal, S., Manik, 2009. Formulation of sustained-release dosage form of verapamil hydrochloride by solid dispersion technique using Eudragit RLPO or KollidonA (R) SR. AAPS PharmSciTech 10, 27–33.
Sajeesh, S., Sharma, C.P., 2004. Poly methacrylic acid-alginate semi-IPN microparticles for oral delivery of insulin: a preliminary investigation. J. Biomater. Appl. 19, 35–45.
Sajeesh, S., Sharma, C.P., 2011. Mucoadhesive hydrogel microparticles based on poly (methacrylic acid-vinyl pyrrolidone)-chitosan for oral drug delivery. Drug Deliv. 18, 227–235.
Sajeesh, S., Bouchemal, K., Marsaud, V., Vauthier, C., Sharma, C.P., 2010a. Cyclodextrin complexed insulin encapsulated hydrogel microparticles: an oral delivery system for insulin. J. Control. Release 147, 377–384.
Sajeesh, S., Vauthier, C., Gueutin, C., Ponchel, G., Sharma, C.P., 2010b. Thiol functionalized polymethacrylic acid-based hydrogel microparticles for oral insulin delivery. Acta Biomater. 6, 3072–3080.
Sajeesh, S., Bouchemal, K., Sharma, C.P., Vauthier, C., 2010c. Surface-functionalized polymethacrylic acid based hydrogel microparticles for oral drug delivery. Eur. J. Pharm. Biopharm. 74, 209–218.
Sezer, A.D., Akbuga, J., 1999. Release characteristics of chitosan treated alginate beads: II. Sustained release of a low molecular drug from chitosan treated alginate beads. J. Microencapsul. 16, 687–696.
Shao, Z.H., Li, Y.P., Chermak, T., Mitra, A.K., 1994. Cyclodextrins as mucosal absorption promoters of insulin. II. Effects of beta-cyclodextrin derivatives on alpha-chymotryptic degradation and enteral absorption of insulin in rats. Pharm. Res. 11, 1174–1179.
Sharpe, L.A., Daily, A.M., Horava, S.D., Peppas, N.A., 2014. Therapeutic applications of hydrogels in oral drug delivery. Expert Opin. Drug Deliv. 11, 901–915.
Shimoda, J., Onishi, H., Machida, Y., 2001. Bioadhesive characteristics of chitosan microspheres to the mucosa of rat small intestine. Drug Dev. Ind. Pharm. 27, 567–576.
Shofner, J.P., Phillips, M.A., Peppas, N.A., 2010. Cellular evaluation of synthesized insulin/transferrin bioconjugates for oral insulin delivery using intelligent complexation hydrogels. Macromol. Biosci. 10, 299–306.
Si, J.Y., Yang, H., 2011. Preparation and characterization of bio-compatible Fe3O4@ Polydopamine spheres with core/shell nanostructure. Mater. Chem. Phys. 128, 519–524.
Silva, C.M., Ribeiro, A.J., Ferreira, D., Veiga, F., 2006. Insulin encapsulation in reinforced alginate microspheres prepared by internal gelation. Eur. J. Pharm. Sci. 29, 148–159.
Singh, M.N., Hemant, K.S., Ram, M., Shivakumar, H.G., 2010. Microencapsulation: a promising technique for controlled drug delivery. Res. Pharm. Sci. 5, 65–77.
Situ, W., Chen, L., Wang, X.Y., Li, X.X., 2014. Resistant starch film-coated microparticles for an oral colon-specific polypeptide delivery system and its release behaviors. J. Agric. Food Chem. 62, 3599–3609.
Situ, W.B., Li, X.X., Liu, J., Chen, L., 2015. Preparation and characterization of glycoprotein-resistant starch complex as a coating material for oral bioadhesive microparticles for colon-targeted polypeptide delivery. J. Agric. Food Chem. 63, 4138–4147.
Sonaje, K., Lin, K.J., Wey, S.P., Lin, C.K., Yeh, T.H., Nguyen, H.N., Hsua, C.W., Yen, T.C., Juang, J.H., Sung, H.W., 2010. Biodistribution, pharmacodynamics and pharmacokinetics of insulin analogues in a rat model: oral delivery using pH-responsive nanoparticles vs. subcutaneous injection. Biomaterials 31, 6849–6858.
Steichen, S., O'Connor, C., Peppas, N.A., 2017. Development of a P((MAA-co-NVP)-g-EG) hydrogel platform for oral protein delivery: effects of hydrogel composition on environmental response and protein partitioning. Macromol. Biosci. 17, 1–15.
Strand, B.L., Morch, Y.A., Skjak-Braek, G., 2000. Alginate as immobilization matrix for cells. Trends Biotechnol. 12, 223–233.
Sun, S.P., Liang, N., Piao, H.Z., Yamamoto, H., Kawashima, Y., Cui, F.D., 2010. Insulin-S.O (sodium oleate) complex-loaded PLGA nanoparticles: formulation, characterization and in vivo evaluation. J. Microencapsul. 27, 471–478.
Sun, S., Liang, N., Yamamoto, H., Kawashima, Y., Cui, F.D., Yan, P.F., 2015. pH-Sensitive poly(lactide-co-glycolide) nanoparticle composite microcapsules for oral delivery of insulin. Int. J. Nanomed. 10, 3489–3498.
Sun, S., Liang, N., Gong, X., An, W., Kawashima, Y., Cui, F., Yan, P., 2016. Multifunctional composite microcapsules for oral delivery of insulin. Int. J. Mol. Sci. 18, 1–13.
Tong, W.J., Song, X.X., Gao, C.Y., 2012. Layer-by-layer assembly of microcapsules and their biomedical applications. Chem. Soc. Rev. 41, 6103–6124.
Ubaidulla, U., Sultana, Y., Ahmed, F.J., Khar, R.K., Panda, A.K., 2007a. Chitosan phthalate microspheres for oral delivery of insulin: preparation, characterization, and in vitro evaluation. Drug Deliv. 14, 19–23.
Ubaidulla, U., Khar, R.K., Ahmed, F.J., Panda, A.K., 2007b. Development and in-vivo evaluation of insulin-loaded chitosan phthalate microspheres for oral delivery. J. Pharm. Pharmacol. 59, 1345–1351.
Ubaidulla, U., Khar, R.K., Ahmad, F.J., Sultana, Y., Panda, A.K., 2007c. Development and characterization of chitosan succinate microspheres for the improved oral bioavailability of insulin. J. Pharm. Sci. 96, 3010–3023.
Ubaidulla, U., Khar, R.K., Ahmad, F.J., Tripathi, P., 2009. Optimization of chitosan succinate and chitosan phthalate microspheres for oral delivery of insulin using response surface methodology. Pharm. Dev. Technol. 14, 96–105.
Uchida, T., Nagareya, N., Sakakibara, S., Konishi, Y., Nakai, A., Nishikata, M., Matsuyama, K., Yoshida, K., 1997. Preparation and characterization of polylactic acid microspheres containing bovine insulin by a w/o/w emulsion solvent evaporation method. Chem. Pharm. Bull. (Tokyo) 45, 1539–1543.
Uddin, A.N., Bejugam, N.K., Gayakwad, S.G., Akther, P., D'Souza, M.J., 2009. Oral delivery of gastro-resistant microencapsulated typhoid vaccine. J. Drug Target. 17, 553–560.
van de Weert, M., Hennink, W.E., Jiskoot, W., 2000. Protein instability in poly(lactic-co-glycolic acid) microparticles. Pharm. Res. 17, 1159–1167.
van der Merwe, S.M., Verhoef, J.C., Verheijden, J.H.M., Kotze, A.F., Junginger, H.E., 2004. Trimethylated chitosan as polymeric absorption enhancer for improved peroral delivery of peptide drugs. Eur. J. Pharm. Biopharm. 58, 225–235.
Vandenberg, G.W., De La Noue, J., 2001. Evaluation of protein release from chitosan-alginate microcapsules produced using external or internal gelation. J. Microencapsul. 18, 433–441.
Vanea, E., Moraru, C., Vulpoi, A., Cavalu, S., Simon, V., 2014. Freeze-dried and spray-dried zinc-containing silica microparticles entrapping insulin. J. Biomater. Appl. 28, 1190–1199.
Victor, S.P., Sharma, C.P., 2002. Stimuli sensitive polymethacrylic acid microparticles (PMAA) – Oral insulin delivery. J. Biomater. Appl. 17, 125–134.
Wang, L.Y., Ma, G.H., Su, Z.G., 2005. Preparation of uniform sized chitosan microspheres by membrane emulsification technique and application as a carrier of protein drug. J. Control. Release 106, 62–75.
Wei, W., Wang, L.Y., Yuan, L., Yang, X.D., Su, Z.G., Ma, G.H., 2008. Bioprocess of uniform-sized crosslinked chitosan microspheres in rats following oral administration. Eur. J. Pharm. Biopharm. 69, 878–886.
Wong, C.Y., Martinez, J., Dass, C.R., 2016. Oral delivery of insulin for treatment of diabetes: status quo, challenges and opportunities. J. Pharm. Pharmacol. 68, 1093–1108.

Wong, C.Y., Martinez, J., Carnagarin, R., Dass, C.R., 2017a. In-vitro evaluation of enteric coated insulin tablets containing absorption enhancer and enzyme inhibitor. J. Pharm. Pharmacol. 69, 285–294.
Wong, C.Y., Al-Salami, H., Dass, C.R., 2017b. Potential of insulin nanoparticle formulations for oral delivery and diabetes treatment. J. Control. Release 264, 247–275.
Wood, K.M., Stone, G.M., Peppas, N.A., 2008. Wheat germ agglutinin functionalized complexation hydrogels for oral insulin delivery. Biomacromolecules 9, 1293–1298.
Wu, S.J., Robinson, J.R., 1999. Transport of human growth hormone across Caco-2 cells with novel delivery agents: evidence for P-glycoprotein involvement. J. Control. Release 62, 171–177.
Xin Hua, Z., 1994. Overcoming enzymatic and absorption barriers to non-parenterally administered protein and peptide drugs. J. Control. Release 29, 239–252.
Yamaguchi, R., Arai, Y., Itoh, T., Hirano, S., 1981. Preparation of partially N-succinylated chitosans and their cross-linked gels. Carbohydr. Res. 88, 172–175.
Yao, K.D., Peng, T., Yin, Y.J., Xu, M.X., Goosen, M.F.A., 1995. Microcapsules/microspheres related to chitosan. J. Macromol. Sci. Polym. Rev. 35, 155–180.
Ye, M.L., Kim, S., Park, K., 2010. Issues in long-term protein delivery using biodegradable microparticles. J. Control. Release 146, 241–260.
Zhang, Y.L., Wei, W., Lv, P.P., Wang, L.Y., Ma, G.H., 2011. Preparation and evaluation of alginate-chitosan microspheres for oral delivery of insulin. Eur. J. Pharm. Biopharm. 77, 11–19.
Zhang, Y., Wu, X.R., Meng, L.K., Zhang, Y., Ai, R.T., Qi, N., He, H.B., Xu, H., Tang, X., 2012. Thiolated Eudragit nanoparticles for oral insulin delivery: preparation, characterization and in vivo evaluation. Int. J. Pharm. 436, 341–350.
Zhao, N., Augsburger, L.L., 2005. The influence of swelling capacity of superdisintegrants in different pH media on the dissolution of hydrochlorothiazide from directly compressed tablets. AAPS PharmSciTech 6, 120–126.
Zhao, L., Ding, J.X., He, P., Xiao, C.S., Tang, Z.H., Zhuang, X.L., Chen, X.S., 2011. An efficient pH sensitive oral insulin delivery system enhanced by deoxycholic acid. J. Control. Release 152, 184–186.