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Title: The impact of natural and synthetic polymers in formulating micro and nanoparticles for anti-diabetic drugs

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Abstract

Diabetes mellitus is one of the long known chronic diseases, today over 400 million people are diagnosed with diabetes. Yet curing diabetes is a challenge. Over the decades, the approaches of treating diabetes mellitus have evolved and polymeric materials have played an integral part in developing and manufacturing anti-diabetic medications. However, injection of insulin remains the conventional therapy for the treatment of diabetes. Oral administration is generally the most preferred route; yet, physiological barriers lead to a challenge for the formulation development for oral delivery of antidiabetic peptide and protein drugs. This present review focuses on the role of different types of biodegradable polymers (e.g., synthetic and natural) that have been used to develop micro and nano particles based formulations for antidiabetic drugs (Type 1 and Type 2) and how the various encapsulation strategies impact its therapeutic effect, including pharmacokinetics studies, drug release profiles and efficacy of the encapsulated drugs. This review also includes studies of different dosage forms such as oral, nasal, inhalation and sublingual for the treatment of diabetes that have been investigated using synthetic and natural biodegradable polymers in order to develop an alternative route to subcutaneous route for a better control of serum glucose levels.

Graphical abstract

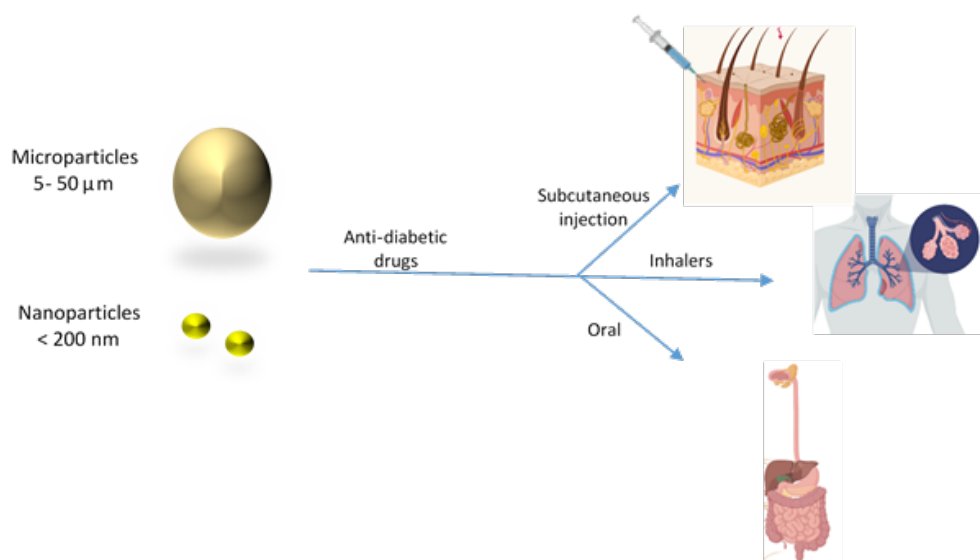


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Introduction

Diabetes mellitus is a metabolic disorder that is characterized by high glucose levels caused by defects in insulin secretions and actions. Diabetes mellitus is one of the long known chronic diseases. Nowadays, the number of people with diabetes is estimated to be over 400 million. Diabetes mellitus puts its patients at high risks of microvascular and macrovascular complications such as neuropathy, nephropathy, retinopathy, and cardiovascular comorbidities. In 2015, the number of deaths reported due to diabetes was 1.5 million deaths [1].

According to the American Diabetes Association, diabetes mellitus classified into type 1, type 2, gestational diabetes mellitus (GDM), and others. Type 1 diabetes mellitus (T1DM) affects between 5-10% of all diabetic patients, with 80-90% are children. T1DM is caused by the destruction of pancreatic B cells. On the other hand type two diabetes mellitus (T2DM) is the most common type of diabetes affecting more than 90% of all diabetic patients with obesity as a main contributing factor to the condition by increasing the insulin resistance [2,3]. For the treatment of T1DM, insulin via subcutaneous (SC) route is the conventional therapy to control blood glucose level, whereas oral antidiabetic drugs and insulin are the treatment options for T2DM [4,5]. Over the decades, several anti-diabetic drugs (Table 1) developed to help diabetic patients to control their condition.

Table 1: List of some medicines used in diabetes (Type 1 & Type 2), Maturity Onset Diabetes of the Young (MODY) and Gestational diabetes mellitus (GDM). [6, 7]

Type of antidiabetic drug	Generic name	Branded name	Medicinal forms	Diabetes	Pharmacokinetic properties	
Biguanides	Metformin hydrochloride	RIOMET [®] (500mg/5m)	Oral solution	Type 2 & GDM	6.5 hours	
		Glucophage [®] (500mg)	Tablets		The apparent terminal elimination half-life is approximately 6.5 hours.	
Sulfonylureas	Glibenclamide	Amglidia [®] (0.6 mg/ml)	Oral suspension	Type 2, neonatal& GDM (second & third trimester)	Elimination half-life is 5-10 hours	
		Gliclazide	Diamicron [®] (80 mg)	Tablets	Type 2	Elimination half-life between 10 - 12 hours
		Glimepiride	Amaryl [®] (1 mg)	Tablets	Type 2	Half-live of metabolites from 3 -6 hours
		Tolbutamide	Tolbutamide [®] (500mg)	Tablets	Type 2	Half-life 4.5 - 6.5 hours
Thiazolidinediones	Glipizide Pioglitazone	Minodiab [®] (5mg)	Tablets	Type 2 & MODY(1,3,&4)	Half-life of elimination from 2 - 4 hours	
		Actos [®]	Tablets	Type 2	Elimination half-life of unchanged pioglitazone 5 -6 hours and for active metabolites 16 - 23 hours	
Dipeptidylpeptidase-4 inhibitor	Vildagliptin Sitagliptin Saxagliptin	Galvus [®]	Tablets	Type 2	Elimination half-life approximately 3 hours	
		Januvia [®]	Tablets		The half-life following an oral 100-mg approximately 12.4 hours	
		Onglyza [®]	Tablets		The mean plasma terminal half-life values for Onglyza between 2.5- 3.1 hours	
Sodium glucose co-transporter 2 inhibitor	Linagliptin Canagliflozin Dapagliflozin Empagliflozin	Trajenta [®]	Tablets	Type 2	Half-life is 10.6 ± 2.13 hours for the 100 mg	
		Invokana [®]	Tablets		Half-life is 12.9 hours following oral dose of dapagliflozin 10 mg	
		Forxiga [®]	Tablets		Half-life of multiple doses of 5 mg linagliptin is 12 hours	
		Jardiance [®]	Tablets			
Glucagon-like peptide-1 receptor agonist	Dulaglutide	Trulicity [®]	Injection	Type 2	The mean apparent clearance is 0.111 L/h for the 0.75 mg dose, and 0.107 L/h for the 1.5 mg dose. Half-life of dulaglutide for both doses is 5 days	
		Exenatide	Bydureon [®]		Injection	Half-life 2.4 hours
		Liraglutide	Victoza [®]		Injection	Mean apparent clearance 1.2 L/h with an elimination half-life of approximately 13 hours
		Lixisenatide	Lyxumia [®]		Injection	Mean terminal half-life is approximately 3 hours
		Semaglutide	Ozempi [®]		Injection	Mean apparent clearance approximately 0.05 L/h and elimination half-life of approximately 1 week

The market sales for antidiabetic drugs was \$ 54.6 Billion in 2017 and it has been predicted to increase by 10.6% to reach \$ 110 Billion in 2024 [3]. The rise in diabetes prevalence necessitates an increase in the number of antidiabetic medications and formulations, not only to meet the market's needs but also to enhance the performance of the antidiabetic medications and bypass and challenges [3]. For instance, injections of peptides or proteins drugs are generally associated with short half-life; therefore, multiple daily injections are required. This can affect negatively on patients' compliance [4]. On the other hand, sustained-release formulations of peptides or proteins drugs are associated with fluctuated release profiles, such as high initial burst drug release. This can result in reaching the maximum drug serum concentration after administration and lead to side effects or result in loss of drug for sustained release formulations after the initial release of the drug, therefore, leading to loss of therapeutic effect [4, 8]. Oral administration is generally the most preferred route [9, 10, 11]. However, oral delivery of peptides and proteins drugs are associated with physiological barriers (e.g., low pH in the stomach, enzymatic degradations in the gastrointestinal (GI) tract and poor intestinal absorption) therefore low bioavailability [9, 10, 11]. In order to overcome such limitations associated with injection and oral route, pulmonary delivery of peptides and proteins has been explored [12, 13]. However, this route still requires injections to maintain the blood insulin concentrations [12]. The use of biodegradable nano and microparticles to deliver antidiabetic drugs have a significant impact on the therapeutic behaviour of these drugs. Micro and nanoparticles tend to exhibit different roles due to the different particle sizes between microparticles (e.g., >1 μm) and nanoparticles (e.g., 1-100 nm). However, the definition of micro and nanoparticles sizes can vary [5, 9]. Biodegradable microparticles can maintain the stability of protein or peptide drugs and provide controlled drug release. Besides, the microparticles as oral drug delivery systems demonstrate to deliver peptides and proteins drugs by protecting them from enzymatic degradation and improve intestinal absorption [5, 9]. Biodegradable nanoparticles facilitate the permeability across the intestinal cells and protect peptides and proteins drugs from enzymatic degradation [5, 9]. The present review surveys the role of micro- and nanoencapsulation of antidiabetic drugs using biodegradable polymers (e.g., natural and synthetic) and how the various encapsulation strategies impact its therapeutic effect (e.g., hypoglycemic effect), including pharmacokinetics studies, drug release profiles and efficacy of the encapsulated drugs.

Biodegradable polymers are classified into synthetic and natural polymers (Figure 1 and Table 2). Poly(lactide-co-glycolide) (PLGA), polyglycolic acid, polylactide (PLA), and poly(caprolactone) (PCL) are the Food and Drug Administration (FDA) approved synthetic polyesters. They are generally classified into three groups based on the polymer degradation kinetics: fast (PLGA, 1-6 months), medium (polyglycolic acid), and slow (PLA and PCL, > 12 months) [14, 15, 16].

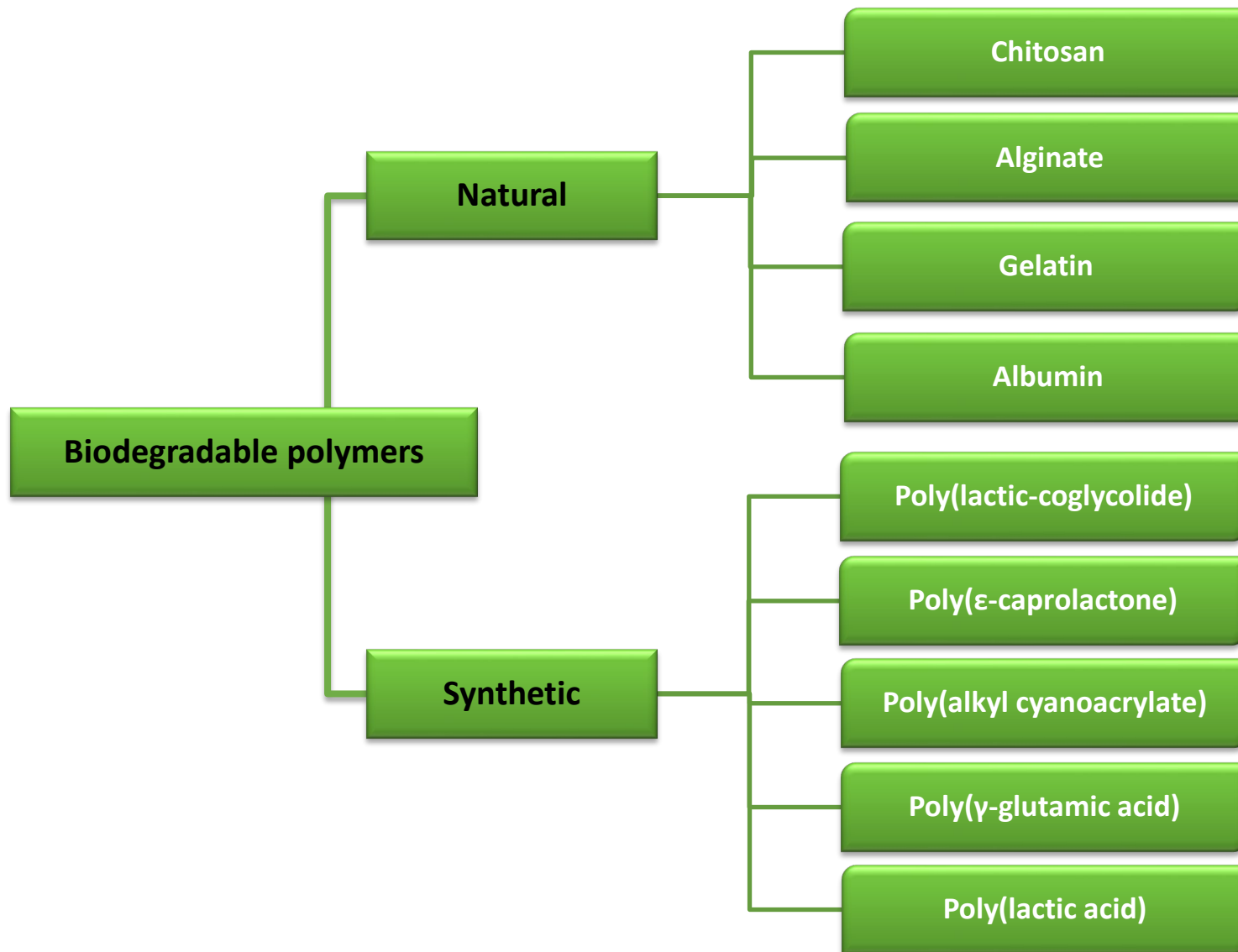
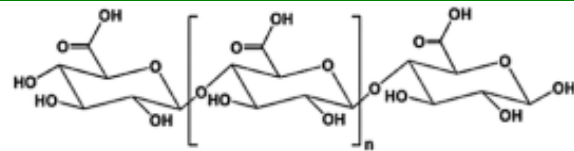
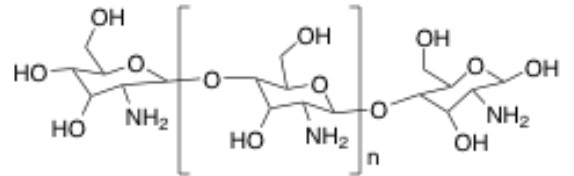
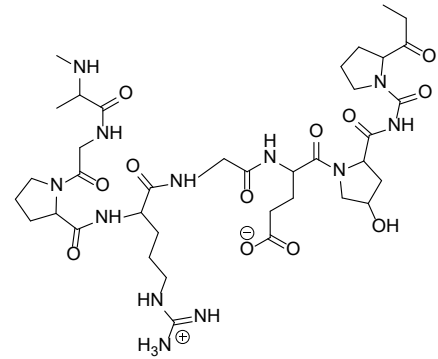
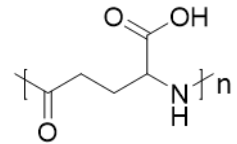
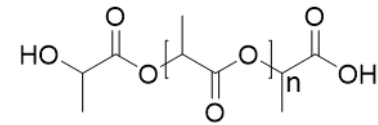


Figure 1: A schematic presentation of natural and synthetic biodegradable polymers.

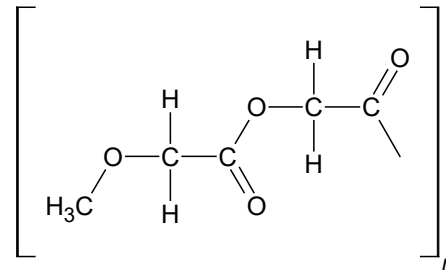
Table 2: Chemical structures of natural and synthetic biodegradable polymers.

Polymer	Structure /Empirical formula
Natural	
Alginate	
Albumin	$C_{123}H_{193}N_{35}O_{37}$
Chitosan	
Gelatin	
Synthetic	
Poly(γ -glutamic acid)	

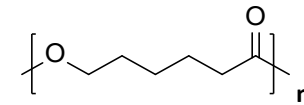
Poly(lactic acid)



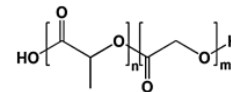
Poly(glycolic acid)



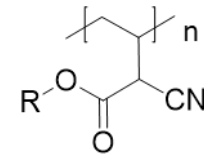
Poly(ϵ -caprolactone)



Poly(lactic-co-glycolic acid) (PLGA)



Poly(alkylcyanoacrylate)



Poly(lactic-co-glycolic acid)

Poly (d,l-lactic-co-glycolic acid)(PLGA) has been widely used as a therapeutic drug delivery system in protein or polypeptide formulations due to its good biocompatibility and biodegradability [5,16,17,18,19]. This polymer consists of various ratios of lactic acid and glycolic acid [8, 18, 20]. The degradation properties of PLGA can be controlled by changing the ratio between lactic acid and glycolic acid [20]. However, PLGA tends to generate acidic degradation products that lead to a decrease in the pH, causing the inflammatory reaction [16]. It is one of the most used synthetic polymers to produce nanoparticles for drug delivery purposes. PLGA allows the production of versatile nanoparticles since it may be combined with other polymers or coated with different ligands, attributing to important nanoparticle features that may enhance the uptake of loaded drugs.

Exenatide marketed as Byetta[®] by Astra Zeneca is a 39 amino acid synthetic polypeptide drug and the first glucagon-like peptide-1 receptor (GLP-1R) agonist approved by the FDA in 2005 and European Medicines Agency in 2007 for the treatment of type 2 diabetes [17,21, 22]. Byetta[®] is a short-acting GLP-1R agonist with twice-daily SC injection due to its short half-life of 2.4 hours [19, 22]. Therefore, in order to increase half-life the polymer based sustained release formulation of exenatide was developed using PLGA which is now marketed as Bydureon[®] by Astra Zeneca. Bydureon[®] is a long acting GLP-1R agonist that is administered subcutaneously once a week [17, 18, 19]. The Bydureon[®] PLGA microspheres (60 μm in diameters) were prepared by water-in-oil solvent evaporation method [12, 19]. Encapsulating exenatide into microspheres resulted in slow exenatide release through diffusion and drug metabolism leading to longer half-life and plasma concentration peak (2 weeks) after administration [22]. Cai *et al.* and Moonschi *et al.* reported that Byduren[®] demonstrated greater reduction in the levels of haemoglobin A1c (HbA1c) compared with twice daily injection of exenatide in the comparative trials. Also, Byduren[®] showed lower hypoglycaemia incidence and lower frequency of side effects (e.g., nausea and vomiting) with a low initial drug burst compared with the twice daily exenatide [12, 18]. Ji *et al.* reported a safety and efficacy study of exenatide once weekly vs exenatide twice daily in type 2 diabetes patients in randomised comparator-controlled study. The results showed that the level of HbA1c for patients on exenatide once weekly was significantly lower ($7.26\pm 0.07\%$) than the patients on twice daily exenatide ($7.57\pm 0.05\%$) at 26 weeks of the study (the average HbA1c was 8.7% before commencing the clinical study) ($P < 0.001$). It was also revealed that more patients on exenatide once weekly achieved the level of HbA1c $\leq 7.0\%$ ($p = 0.003$), $\leq 6.5\%$ ($p < 0.001$), or $\leq 6.0\%$ ($p = 0.003$) when compared to patients on exenatide twice daily [23].

Zhu *et al.* conducted a study using PLGA microparticles to sustain the release of exenatide. Exenatide-loaded microparticles were prepared by two different methods: ultra-fine particle processing system (UPPS) and spray drying. The particle size of microparticles prepared by UPPS was larger ($33.21 \pm 2.86 \mu\text{m}$) with a higher encapsulation efficiency (EE: $90.16 \pm 3.52\%$) than microparticles prepared by spray drying ($4.83 \pm 1.79 \mu\text{m}$, EE: $84.65 \pm 2.93\%$) [17]. Pharmacodynamics of a single injection of Exenatide-loaded PLGA microparticles prepared by UPPS and spray drying were studied in type 2 diabetes Sprague-Dawley (SD) rats by measuring the body weight gain and blood glucose and compared with the negative control group (sterile saline subcutaneously injected twice daily) and the positive control group (exenatide solution injected subcutaneously twice daily ($15 \mu\text{g}/\text{kg}$)) [17]. It can be noticed from the obtained results that UPPS and spray drying Exenatide-loaded PLGA microparticles with a single injection significantly inhibited the increase of the body weight for the first two weeks of the study, whereas the SD rats in the negative control (sterile saline) group showed a continuous bodyweight increase. Also, the hypoglycemic efficacy after a single injection of UPPS and spray drying exenatide-loaded PLGA microparticles was up to 18 days which can be considered markedly long [17].

In addition to the studies for SC route, PLGA based carriers have been studied for inhalation and used to sustain the pharmacological effect and reduce the daily doses for the development of inhaled drugs formulations for pulmonary delivery [24]. Ungaro *et al.* investigated the potential of pulmonary delivery of insulin loaded in PLGA and cyclodextrin (used as an osmotic agent) porous microparticles (VMD: $26.2 \pm 1.2 \mu\text{m}$) by assessing the *in vivo* deposition pattern and hypoglycemic activity of insulin loaded PLGA- cyclodextrin. The insulin loaded PLGA-cyclodextrin particles were prepared by the double emulsion-solvent evaporation technique and administered intratracheally to normoglycemic rats and induced diabetic rats. The *in vivo* deposition studies showed that insulin loaded PLGA particles reached the alveoli surface 30 min after administration and spread over the surface 90 min after administration. Upon testing the induced diabetic rats, insulin loaded PLGA (insulin dose: $0.5 \text{ IU}/\text{kg}$, $p < 0.05$ and $2 \text{ IU}/\text{kg}$, $p < 0.0001$) showed a significant reduction in blood glucose level compared to the control group treated with insulin solutions in the absence of PLGA (insulin dose: $4 \text{ IU}/\text{kg}$) [25]. Therefore, blood glucose levels can be reduced by the use of the PLGA based formulation with low doses. Similarly, Hamishehkar *et al.* evaluated the feasibility of pulmonary delivery for the controlled insulin release by preparing a dry powder inhaler formulation containing insulin loaded PLGA microcapsules blended with mannitol as a carrier and tested the aerosolization performance of the formulations [26]. Hamishehkar *et al.* also designed a PLGA microcapsule (VMD $4.04 \mu\text{m}$) dry powder system for sustained delivery of insulin via lungs. Insulin loaded PLGA microcapsules were prepared by oil in oil emulsification and solvent evaporation method and administered intratracheally to induced diabetic rats. The

pulmonary absorption and bioavailability of insulin was studied by monitoring serum insulin and glucose concentrations in induced diabetic rats. Pharmacokinetic analysis in diabetic rats showed that insulin loaded PLGA microcapsules demonstrated a sustainable reduction in glucose serum concentration for up to 48 h and the insulin serum residence time of insulin loaded PLGA microcapsules were significantly longer (34.1 ± 1.36 h) than NPH (long acting) insulin administered by SC route (6.32 ± 0.37 h) ($p < 0.001$). This demonstrated that insulin loaded PLGA microcapsules administered via intratracheal insufflation had longer sustained insulin profile in serum than NPH insulin via SC route therefore a prolonged insulin release from PLGA microcapsules. However, reaching the blood circulation from PLGA microcapsules was slower than the SC route. Additionally, the safety study assessed by lung histology for insulin loaded PLGA microcapsules showed acute inflammation and mild to moderate injuries (e.g., thickening of alveolar sacs walls) in rat lungs treated with insulin loaded PLGA microcapsules due to a drop in pH in the lung caused by the degradation of PLGA [13].

Some studies focused on other route, oral delivery of antidiabetic drugs such as insulin. Wu et al. designed a two-stage PLGA based oral delivery system for insulin using a capsule coated with pH-sensitive hydroxypropyl methylcellulose phthalate (HP55) to bypass the barriers in the gastrointestinal (GI) tract. Eudragit®RS was also used to facilitate the absorption in the intestine. This study was based on nanoparticle delivery system. Insulin loaded into PLGA-Eudragit®RS nanoparticles (particle size: 374-1426 nm) were prepared by the multiple emulsions solvent evaporation method and administered orally to induced diabetic rats. The hypoglycemic effects of the two-stage system were studied in the diabetic rats via oral administration (50 IU/kg) and compared with positive control of insulin solution by SC injection (5 IU/kg) and negative control of insulin in the absence of nanoparticles via oral feeding (50 IU/kg). The results showed that negative control of insulin in the absence of nanoparticles had no hypoglycemic effect, whereas the two-stage system and positive control of insulin solution by SC injection had a significant hypoglycemic effect in diabetic rats. There was a sharp decrease (90% of initial) observed in plasma glucose levels within 2 hours after SC administration of insulin solution. On the other hand, there was a slow reduction in blood glucose levels after oral administration of insulin loaded PLGA/ Eudragit®RS nanoparticles in the enteric-coated capsule and demonstrated a prolonged hypoglycemic effect [10]. Another study conducted by Malathi et al. developed insulin PLGA nanoparticles with the inclusion of D- α -tocopherol poly(ethylene glycol) 1000 succinate (TPGS, emulsifier) (particle size in diameter: 120-180 nm) for oral delivery of insulin. The insulin loaded PLGA-TPGS nanoparticles were prepared by water-oil-water emulsion solvent evaporation method and administered orally to induced diabetic rats (20 IU/kg). The *in vivo* studies showed that insulin loaded PLGA-TPGS nanoparticles suppressed the blood glucose level during the administration and

demonstrated a prolonged hypoglycemic effect for up to 24 hours. The serum insulin concentration level gradually increased after oral administration of insulin loaded PLGA-TPGS nanoparticles and achieved the maximum serum insulin level (6 μ U/dL) at 12 h, whereas control group treated with insulin in the absence of PLGA nanoparticles had the maximum serum insulin level (around 3 μ U/dL) at 3 h. This study demonstrated that loading insulin into PLGA based nanoparticles protected insulin from the enzymatic degradation in the GI tract therefore resulted in the effective and prolonged hypoglycemic effect. Also, the particle size of PLGA nanoparticles might have facilitated the absorption in intestine [27].

All these reported studies show that regardless of different delivery routes of administration (SC, pulmonary and oral), PLGA based micro and nanoparticles formulations for antidiabetic drugs have demonstrated to sustain hypoglycemic effect when compared to formulations without the use of biodegradable polymers. For example, encapsulating short acting exenatide into PLGA based microspheres led to the marketed formulation of long acting Bydureon[®]. This strategy has reduced the dose significantly from twice daily to once weekly. Therefore, biodegradable polymer-based formulations can offer the reduction of the daily doses for antidiabetic drugs. This is advantageous especially for patients on injection-based treatments as the frequency of injections can be minimised while the bioavailability and therapeutic efficacy of the drugs can be maintained or enhanced.

poly(ϵ -caprolactone)

Poly (caprolactone) (PCL) is a semi crystalline polymer with a low glass transition temperature (-54°C) [20,28]. This polymer has low toxicity making it suitable for colloidal drug delivery. PCL undergoes ester hydrolysis in physiological conditions and degrades. PCL has been used in manufacturing long-term implantable systems as the rate of its biological degradation is slow. PCL tend to generate less acidic degradants compared to PLGA. However, PCL is associated with intracellular resorption pathways and the degradation rate is slow (> 12 months) [16, 20, 28, 29]. This leads to the limited use of PCL for medicinal applications (e.g., general tissue regeneration) [16, 20, 28]. However, PCL can be used in combination with other polymers (e.g., polylactic acid (PLA) and PLGA) for designing drug delivery system and controlling drug release profile [20,28,29].

Barakat, Shazly & Almedany reported that oral bioavailability and therapeutic effect of gliclazide could be enhanced by incorporating the drug within a blend of PCL and Eudragit RS microparticles. Gliclazide formulated as an oral dosage form is a short-acting hypoglycemic drug and used for the treatment of type 2 diabetes. However, gliclazide is associated with limitations such as variable absorption rate in GI tract; therefore, the maximum dose could go up to 320 mg from 80 mg daily dose. This requires developing a formulation for the sustained drug release [29]. Barakat, Shazly & Almedany prepared the gliclazide loaded PCL/ Eudragit RS microparticles using a solvent evaporation method and administered the microparticles orally to the male white rabbits for *in vivo* evaluations (gliclazide dose: 40 mg kg⁻¹). The *in vivo* studies demonstrated that gliclazide loaded PCL/Eudragit RS microparticles had a slow reduction in plasma drug level with a peak concentration of 8 hours. In contrast, gliclazide solution in the absence of microparticles had a rapid reduction in plasma drug levels within 3 hours after oral administration [29]. Therefore, the use of PLC with Eudragit RS based microparticle formulations can be a feasible approach to maintain the low daily dose.

Sheikh Hasan et al. prepared microparticles containing insulin nanoparticles using PCL and PLGA for parenteral delivery of insulin. Their particle preparations involved two steps. First, insulin loaded PCL nanoparticles were prepared by water-in-oil-in-water solvent evaporation method and then microparticles containing insulin loaded PCL nanoparticles were prepared by water-in-oil-in-water solvent extraction method. The microparticles containing insulin nanoparticles were administered subcutaneously to fasted diabetic rats (20 IU/kg) for *in vivo* studies. The *in vitro* drug release study demonstrated a significant reduction in insulin initial burst release from PLGA microparticles containing PCL insulin nanoparticles (19% release after 15 min and 39% release at 24 h) compared to PLGA microparticles containing insulin without PCL nanoparticles (36% release in the first 15 min and 56% release at 24 h) and PCL insulin nanoparticles (41% release in the first 15 min and 50% release at 24 h). The *in vivo* studies on diabetic rats also showed that PLGA microparticles containing PCL insulin nanoparticles had a limited initial burst release within 30 minutes (insulin serum concentration: 433±71 µU/mL) and continuous insulin release with stable insulin serum levels for up to 72 hours, whereas PCL insulin nanoparticles in the absence of PLGA microparticles demonstrated a significant high insulin serum level within 30 minutes (insulin serum concentration: 2389±280 µU/mL)[4]. Both reported studies show that formulations of antidiabetic drugs associated with fluctuated release profiles can be overcome by the use of PCL polymer based formulations as they exhibit a slow reduction in plasma drug level with stable drug serum levels for a longer period.

Polyalkyl cyanoacrylates

Poly(alkyl cyanoacrylate) (PACA) is a biocompatible and degradable polymer and the properties of PACA are based on the type of PACA side chain and it can be produced from the rapid anionic polymerization of alkylcyanoacrylates upon contact with water. This polymer has good properties to be used in film preparation and as a glue in medical intervention for wound closure. PACA has been used to prepare nanoparticles as drug carrier systems [30]. Graf, Rades & Hook reported a study of oral delivery of insulin using PACA (i.e., poly(ethyl cyanoacrylate)) based nanoparticles prepared by using microemulsion as templates. The insulin loaded PACA nanoparticles dispersed in microemulsion templates (insulin loaded nanoparticle sizes: 200-400 nm) were administered intragastrically by gavage to fasted induced diabetic rats for *in vivo* studies (insulin dose: 100 IU/kg). The results showed that the insulin loaded PACA nanoparticles-based formulation demonstrated a consistent reduction in blood glucose level for up to 36 hours after intragastric administration (reduction to 68% of the initial blood glucose level achieved at 9 h). In contrast, insulin solution in the absence of nanoparticles and microemulsion templates showed no significant reduction observed in blood glucose level [31]. This study showed that although the insulin loaded PACA nanoparticles based formulation demonstrated a sustained glucose reduction for up to 36 hours, the glucose reduction started rather late (e.g. started 9 hours after intragastric administration) presenting a delay of insulin release [31].

Poly- γ -glutamic acid

Poly-gamma-glutamic acid (γ -PGA) is a high-molecular-weight polypeptide comprising γ -linked glutamic acid units and alpha-carboxylate side chains (Table 1) produced by certain strains of *Bacillus subtilis*. It has non-toxic, biodegradable, and non-immunogenic properties. Besides, PGA has anti-inflammatory and antiangiogenic properties.

Poly- γ -glutamic acid (γ -PGA) is a water soluble biodegradable polymer and has been used to prepare nanoparticles for oral insulin delivery [32]. Sonaje et al. studied oral delivery of insulin loaded γ -PGA based nanoparticles. The insulin loaded γ -PGA nanoparticles were prepared using an ionic-gelation method followed by freeze drying. The freeze dried insulin γ -PGA nanoparticles (nanoparticle size: 240-260 nm) were then filled into hard gelatine capsules and administered orally to induced diabetic rats for *in vivo* studies. The *in vivo* study was performed by comparing the blood glucose levels and plasma insulin levels between the formulations: insulin solution by SC injection (insulin dose: 5 IU/kg)

and oral administration of the freeze dried insulin loaded γ -PGA nanoparticles (insulin dose: 30 IU/kg) in induced diabetic rats. The results demonstrated that insulin solution (5 IU/kg) caused a significant reduction in glucose level at 2 h (75%) and reached maximum plasma insulin level (C_{max}) within 1 hour after administration (C_{max} : $119.6 \pm 7.4 \mu\text{IU mL}^{-1}$, AUC: $196.2 \pm 34.5 \mu\text{IU mL}^{-1}$). In contrast, the freeze dried insulin loaded γ -PGA oral formulations demonstrated a gradual and continuous reduction in glucose level for up to 10 hours (about 50%) and achieved maximum plasma insulin level at 5 h after administration (C_{max} : $49.9 \pm 2.4 \mu\text{IU mL}^{-1}$, AUC: $235.8 \pm 30.2 \mu\text{IU mL}^{-1}$)[33]. Another study reported by Su et al. also studied insulin oral delivery using γ -PGA to improve the oral bioavailability of insulin. Insulin loaded pH-responsive nanoparticles were prepared using chitosan in conjugation with γ -PGA and diethylene triamine pentaacetic acid (DTPA, a protease inhibitor) and administered orally to the induced diabetic rats for pharmacodynamic and pharmacokinetic profile studies. The results show that that insulin solution (insulin dose: 5 IU/kg) in the absence of γ -PGA based nanoparticles had a significant reduction in the blood glucose level within 2-3 hours and achieved the maximum plasma concentration at 1 h after SC administration (C_{max} : $101.5 \pm 6.2 \mu\text{IU/mL}$, AUC: $156.3 \pm 29.7 \mu\text{IU mL}^{-1}$). In contrast, oral administration of insulin γ -PGA based nanoparticles (30 IU/kg) showed a slow and prolonged reduction in blood glucose level for 10 hours and achieved a maximum insulin concentration at 4 h after oral administration (C_{max} : $39.2 \pm 2.8 \mu\text{IU/mL}$, AUC: $179.7 \pm 32.5 \mu\text{IU mL}^{-1}$) [34]. Both studies demonstrated a significant effect on blood glucose levels by delivering insulin orally to reach the intestines for absorption and achieved a prolonged reduction in glucose levels. Therefore, it can be indicated that using the γ -PGA nanoparticles based formulations is applicable approach for oral delivery of insulin and achieve a sustained hypoglycemic effect.

Poly lactic acid

Poly(D, L-lactic acid) (PLA) is widely used in drug delivery due to excellent biodegradable, biocompatible, and non-toxic properties. Similarly to PLGA, PLA has hydrophobic properties, hence requires surface modifications to be able to retain itself in the physiological environment. In addition, PLA nanoparticles are extensively studied for drug delivery with a controlled release due to their pros such as biodegradability, biocompatibility, and controlled particle size [35,36]. The PLA nanoparticles are usually negatively charged due to deprotonated carboxylic groups. Therefore, the repulsion between similar charges imparts stability to PLA containing systems in aqueous environments [35,36]. Nevertheless, PLA nanoparticles have some drawbacks as a drug delivery system due to their hydrophobic nature and low chemical stability. These limitations become significant when PLA nanoparticles are employed in oral administration because they are prone to be trapped by mucus via

steric or adhesive forces (mucoadhesion) [37]. Besides, PLA nanoparticles are removed rapidly via mucociliary clearance. Moreover, PLA particles have reduced stability in gastrointestinal fluids as the carboxylic acid groups become protonated at acidic pH causing a reduction in electrostatic repulsion between particles which consequently leads to their precipitation in gastric fluid [38, 39, 40, 41].

Zhang *et al.* used the PLA microsphere to deliver insulin, and polyvinyl sulfate potassium was added onto the surface as a film layer to protect the drug. The results showed a slow release of insulin from the loaded microspheres in acidic media and after 6 hours, 90% of insulin was released from the microspheres. A superior stability of the microspheres in acidic medium was also observed. This can be related to the high electrostatic attraction between polylactic acid and the film layers. On the other hand, the insulin release in phosphate buffer media (pH 8) was very low over 12 hours. The *in vitro* studies showed that the blood glucose level after intraperitoneal injection to diabetic rats was significantly different from that before the injection. However, there was no significant difference in the glucose level between 3 and 7 days after the injection. Consequently, the insulin-loaded microspheres successfully achieved a reduction in blood glucose levels. This formulation resulted in the protection of insulin within the film layer from being degraded by gastric enzymes. Additionally, the suitable size of microspheres caused good adherence to the gastric tract, and easy transportation to blood circulation. The *in vivo* results, along with *in vitro* outcomes, have shown a hypoglycemic effect with the insulin loaded microspheres [42].

The feasibility of using Pluronic PLA block copolymers as nanocarriers of insulin for oral delivery was investigated by Xiong *et al.* PLA-Pluronic vesicles were prepared using dialysis/nanoprecipitation methods for oral route of administration. The radii of these particles under scanning electron microscopy (SEM) was shown as 57nm. The *in vitro* release behavior and hypoglycemic effects of the orally-administered formulation were monitored up to 23 hours in fasted diabetic mice. The results indicated that the blood glucose concentration of oral insulin-loaded PLA-Pluronic vesicles reduced immediately and the highest blood glucose drop was achieved after about 5 hours. Furthermore, the blood glucose concentration remained at the same level for at least an additional 18.5 hours showing that these vesicles could be a potential candidate for oral insulin vehicles [43].

In another study, researchers attempted to formulate repaglinide containing biodegradable nanoparticles to decrease the drug-related side effect, which was cost-effective for patients. The polymeric nanoparticles were prepared using chitosan, PLA, and PCL by employing the solvent extraction method. The nanoparticles were of spherical shape and their size was found to be between 108-220 nm. The entrapment efficiency was high (81-92%), and the drug release pattern followed zero-order kinetics. These nanoparticles were then loaded in a transdermal patch to provide

prolonged drug delivery over a week in the diabetic patients. *In vivo* results showed a 76 fold increase in the therapeutic efficacy of repaglinide as compared to conventional oral administration. Moreover, the parameters such as area under curve (AUC), maximum concentration (C_{max}), time required to reach C_{max} (T_{max}), half life ($t_{1/2}$) and relative bioavailability estimated as 2218 $\mu\text{IU/ml/h}$, 41.88 $\mu\text{IU/ml}$, 36 h and 52.79 h, respectively [44].

A recently approved FDA drug known as “stevioside” was found to possess good antidiabetic activity, but its use is restricted in humans due to its low intestinal absorption. Barwal tried to improve the bioavailability of this new therapeutic agent by encapsulating the drug in pluronic-PLA block copolymeric nanoparticles. The spherical nanoparticles were prepared via nanoprecipitation method, which possessed the size range of 110-130 nm — their drug loading capacity was calculated to be $16.32 \pm 4\%$. The *in vitro* results indicated an initial burst release followed by a sustained release profile. Around 50% of the drug was released 25 ± 4 hours and completely released in 200 ± 10 hours. The *in vivo* studies of this drug delivery system are yet to be explored [45].

Another study reported insulin loaded PLA-di-lauroyl phosphatidylcholine nanoparticles. The nanoparticles were 107.5 nm in size (0.14 Polydispersity index, PDI) and had a Zeta Potential (ZP) of -6mV. The formulation managed to protect the drug from acidic pH with a loading capacity of 18%. The *in vitro* results indicated a 30 % release at pH 2.5 in the first 2 hours while 90% release at pH 6.8 after 8 hours. The *in vivo* studies were performed in streptozotocin (STZ)-induced male Sprague-Dawley rats, which demonstrated a 40% decrease in blood sugar levels in 4 h. Moreover, the pharmacological activity at an oral dose of 50 IU/kg was reported to be 11.2%, which was 10 times higher than free insulin [46].

In another study, glimepiride loaded PLA slow-release nanoparticles were prepared via o/w solvent evaporation technique and high-speed homogenizer. The prepared nano formulation possessed a size of 442.2 nm while the results of differential scanning calorimetry and fourier transform infrared spectroscopy indicated partial interaction between drug and polymer due to hydrogen bonding between N-H and carbonyl groups. The drug content and encapsulation efficiency was calculated to be 40.27 and 80.55%, respectively. These nanoparticles enhanced the therapeutic outcome of glimepiride by increasing drug release. Following first-order kinetics, the optimized batch showed a release of 73-78% up to 12 hours [47]. However, the record of animal studies and the route of administration was not discussed.

In summary, PLA has some advantages and disadvantages as a drug vehicle. Although PLA is safe to use and produces reduced size particles, the hydrophobic nature of the polymer makes it difficult to maintain its structure in gastric fluids therefore, oral delivery using PLA is still a challenge to overcome.

Chitosan

Chitosan is synthesized by partial deacetylation of chitin and is a copolymer of β -(1 \rightarrow 4)-2-acetamido-D-glucose and β -(1 \rightarrow 4)-2-amino-D-glucose units (Table 1), with the latter usually exceeding 80% [48]. It is a biodegradable polymer very suitable for pharmaceutical applications due to its low toxicity, low immunogenicity, and good biocompatibility. Chitosan and its derivatives, such as glycol chitosan, succinyl chitosan, arginine-chitosan, and aminated chitosan, etc. are efficient drug carriers for the oral route of administration [49].

Many studies have used chitosan to design particular systems to deliver antidiabetic drugs. Eilleia et al. conducted a study using microencapsulation technology to protect insulin from gastric and enzymatic degradation [50]. Porous microparticles have also been prepared using poly (d,l-lactide-co-glycolide) (PLGA) w/o/w emulsion solvent evaporation method. The insulin solution (5 mg/ml in 0.01 M HCl) was incubated with porous PLGA microparticles (10 mg) and the surfaces of PLGA microparticles were coated with chitosan by double freeze-drying. Insulin released from the microparticles was examined for its effect on lowering blood glucose levels. When compared with oral insulin suspension, SC insulin and uncoated insulin PLGA microparticles, chitosan-coated insulin PLGA microparticles coated with chitosan showed a significant reduction in blood glucose level ($15.8\% \pm 4\%$, $P < 0.001$). The blood glucose level rose to 70% after the first hour, then reduced to 50% and was maintained over 8 hours by using chitosan-coated microparticles. On the other hand, the oral suspension of insulin was unable to affect the glucose level due to the enzymatic degradation of insulin in the GI tract. Chitosan was believed to adhere to the upper intestinal part where insulin had minimum solubility resulting in low insulin absorption and sustained glucose reduction [50]. Zhang et al. concluded similar results of maintaining low blood glucose levels owing to the chitosan coating of insulin loaded PLGA nanoparticles [51].

Another recent study was conducted by Mumuni et al. to explore the effect of chitosan and mucin microparticles on insulin release. Insulin microparticles based on chitosan and snail mucin were prepared by the double emulsion method. The insulin release profile was studied in both acidic and basic medium of pH 1.2 and 6.8, respectively. The results showed that more than 80% of insulin was released over 12 hours in a prolonged mode. The batch with highest chitosan concentration showed a

prolonged release profile compared to other formulations, and this could be attributed to the high electrostatic interaction between insulin molecules and polymer's concertation. Mumuni et al. suggested that the combination of chitosan with snail mucin has a synergistic effect. Hence the addition of mucin improved the insulin loaded microparticles stability at a low pH. In addition, oral insulin loaded microparticles were tested on diabetic rats (Wister rats). Pharmacokinetics study was performed over 12 hours for oral insulin microparticles, solution and SC insulin solution. The results showed a reduction in blood glucose level after administration of insulin microparticles for the first 5 hours and a slow reduction over 12 hours. On the contrary, the administered SC insulin showed a rapid reduction within 1 hour whilst the insulin solution showed no significant difference ($p > 0.05$) [52].

The preparation of chitosan microparticles loaded with two antidiabetic drugs namely metformin and glibenclamide was investigated by Avram et al. They developed a binary formulation with an enhanced swelling degree and loading efficiency of the first-line treatment for patients with T2DM [53]. Metformin, for instance, has 50-60% bioavailability and 0.9 - 2.6 h half-life, which is considered low. Therefore, multiple daily doses are required to maintain the metformin therapeutic levels in the plasma. The use of glibenclamide in T2DM patients is also challenging as the drug has 45% bioavailability due to its poor dissolution properties. Glibenclamide doses are 2.5 - 5 mg/day as monotherapy or combined with metformin. Nevertheless, the combination of these medicines can lead to hypoglycemic effect, increase weight, and other gastric side effects. Avram et al. prepared three chitosan microparticles formulations to include single and combined therapies. Chitosan-metformin-glibenclamide microparticles were larger than chitosan-metformin and chitosan-glibenclamide microparticles. Also, these microparticles had the highest swelling degree in distilled water ranged from 226% to 310% when compared to chitosan single therapy (179%). Similarly, the highest loading efficiency was noticed in chitosan-metformin-glibenclamide microparticles with therapeutic dose of 15 mg for both drugs in 51% and 98% for metformin and chitosan, respectively. This can be a useful approach to prepare combined microparticles to enhance their properties [53, 54].

The therapeutic effect of insulin was also tested by incorporating the drug in chitosan nanoparticles. These nanoparticles were prepared in the presence of tripolyphosphate, a crosslinker, using the ionic gelation method (shown in Figure 2) and then the nanoparticles were loaded into the inner aqueous phase, comprised of 56% surfactant and 16% water in 28% oil, of w/o microemulsion to provide sustained release. The results indicated an increase *in vivo* stability and enhanced drug absorption through the GI tract. *In vitro* release study at pH 2.5 revealed that insulin release was significantly low

under higher chitosan ratios ($p < 0.05$). It was also reported that the emulsion-based nano encapsulated system enhanced the protective effect in acidic pH. The *in vivo* experiments in the Wistar Albino rat model demonstrates that these chitosan-coated insulin nanoparticles effectively reduced blood glucose levels over a period of 8 h after oral administration [54].

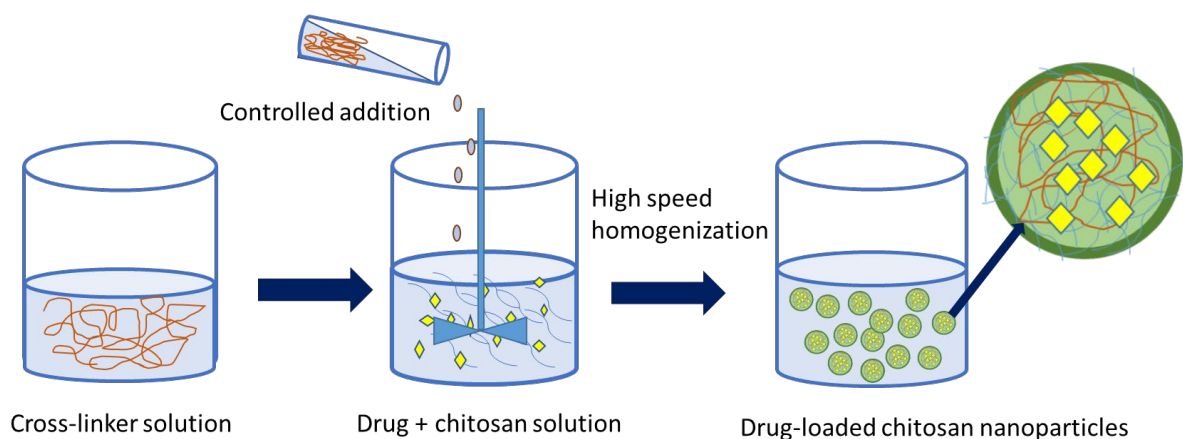


Figure 2: Ionic gelation method to prepare chitosan nanoparticles.

Similarly, Li-Chu and co-workers prepared chitosan nanoparticles for oral delivery of insulin. Trimethyl chitosan (TMC) was combined with fucoidan (FC), a polysaccharide isolated from brown algae, to prepare multifunctional nanoparticles which inhibited the α -glucosidase activity and improved transepithelial permeation of insulin via epithelial cell barrier of the intestine. TMC and FC self-assembled into spherical nanoparticles for insulin incorporation and prevented insulin degradation due to a pH-dependent release manner in GI tract fluids. The size of these nanoparticles was 276.7nm, Zeta Potential of 28mV, loading capacity, and encapsulation efficiency of 8.6% and 56.4%, respectively. The nanoparticles modified the barrier function of the Caco-2 intestinal epithelial cell monolayer and increased paracellular transport of insulin *in vitro*. These nanoparticles also demonstrated α -glucosidase inhibitory activity with an inhibition ratio of 33.2% at 2 mg/ml. The cytotoxicity results showed that caco-2 cells possessed the viability of >90% with <300 μ g/ml nanoparticles [49].

Another group of researchers proved extended control of glycemic levels *in* male albino rats using oral insulin chitosan nanoparticles. Insulin folate-chitosan nanoparticles of approximately 288 nm were prepared. The entrapment efficiency of these nanoparticles was also reported to be greater than 80%. The formulation successfully improved half-life of insulin and enhanced its stability upon exposure to gastric enzymes. The system was able to provide a sustained drug release of $38.92 \pm 4.52\%$ in PBS pH 7.4 over 8 hours, with only <10% release at pH 1.2. Moreover, the insulin intestinal permeability and

cellular uptake were three times higher than the solution form. No evidence of histopathological alterations was recorded, and the nanoparticles demonstrated glycemic control of 8 hours in rats [55].

Quercetin is another antidiabetic drug that was investigated. Quercetin is a natural bioflavonoid with a wide range of pharmacological properties, including antidiabetic activity, but its biomedical applications are limited due to its poor aqueous solubility, instability, low permeability, and extensive first-pass metabolic effect. Piyasi et al. studied novel pH-sensitive polymeric nanoparticles using succinyl chitosan and alginate. These nanoparticles possessed core-shell with corona morphology loaded with quercetin. The nanoparticles were of 91.58 ± 1.14 nm in size, with 0.43 Polydispersity index and -26.63mV zeta potential values. The drug encapsulation reported being over 90% with 59% loading capacity. The *in vitro* release studies showed that 16-27% of quercetin was release in gastric pH after 2 hours and 88-89% released in a simulated intestinal fluid due to significant swelling of nanoparticles at pH 6.8. *In vivo* results showed that quercetin loaded particles significantly downregulated the blood glucose levels as compared to free quercetin (control) and the blank nanoparticles, which did not show any hypoglycemic effects [56].

The studies mentioned here revealed chitosan and its derivatives have been proven successful to enhance the drug stability and bioavailability of antidiabetic drugs. As chitosan is associated with low immunogenic risks and good biodegradability, therefore it serves as a potential candidate for delivery of new antidiabetic drugs especially via oral route of administration.

Alginate

Alginate is a nontoxic, biodegradable polysaccharide obtained from marine brown algae. It is anionic copolymer comprised of 1-4 linked β -D-mannuronic acid and α -L-guluronic acid units of different composition and sequence depending upon the alginate source. Alginate shows a variable molecular weight, which is dependent on the enzymatic control during production and its extraction determines its degree of polymerization [57]. Alginates are generally used as an excipient in drug products due to its thickening, gel forming and stabilizing properties. However, due to its other desirable properties such as biocompatibility, biodegradability, pH sensitivity and mucoadhesiveness alginate also has been explored as an oral delivery matrix for proteins [58]. Alginate microspheres can be easily prepared but mechanical stability is an issue (Figure 3). Similarly, drug release is also observed to be too fast. To circumvent these issues, polymer-coated alginate microspheres and nanoparticles have proved promising results for modified drug delivery (Table 3) [59].

Kar et al. prepared alginate metformin loaded microspheres using ionic gelation technique. The microspheres were prepared from alginate blended with hydroxy propyl methylcellulose, methylcellulose, chitin and chitosan. The prepared microparticles had 85% entrapment efficiency and controlled metformin release for more than 8 hours. Microspheres of alginates blended with hydroxy propyl methylcellulose showed ideal release profile compared to other batches [60].

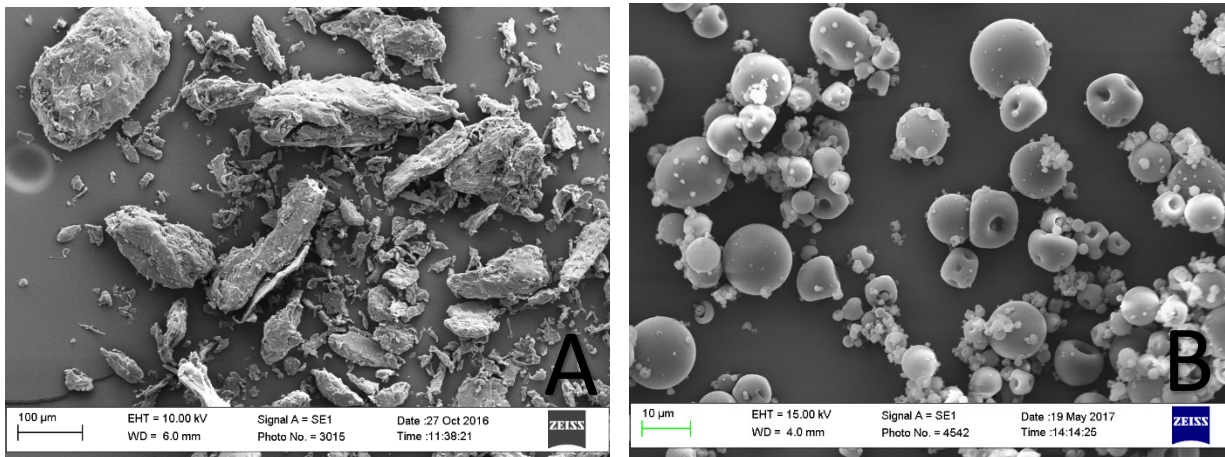


Figure 3: SEM images of raw powder of sodium alginate (A) , microparticles of spray dried sodium alginate (B).

Alginate-metformin microparticles were also investigated by Szekalska et al. who used spray drying to prepare the microparticles with the aim of prolonging the residence time of metformin utilizing the mucoadhesive properties of alginate. Alginate microspheres were successful in modifying and extending the release profile of metformin by Fickian diffusion. Moreover, the effects of alginate-metformin microspheres on the glucose uptake and the α -amylase enzyme inhibition were assessed by *in vitro* studies performed on *Saccharomyces cerevisiae* cells of bovine stomach mucosa [61]. It can be noticed in F6 which consists of cells incubated with microspheres in 2:1 ratio (drug: polymer) a high glucose uptake and the remaining glucose concentration in the medium was the lowest $3.91 \pm 0.69\%$ compared to the metformin alone and the control $6.51 \pm 1.01\%$, and $14.72 \pm 1.32\%$ (mean \pm SD), respectively. Also, F6 microspheres strongly reduced α amylase enzyme activity by $36.34 \pm 2.45\%$ compared to metformin effect $5.35 \pm 0.32\%$. Therefore, it was noticed that polymer amount had a strong inhibitory effect on the enzyme activity [61].

In 2018, Chakra et al. performed a study to sustain metformin release using alginate microspheres with gum to prolong the duration of action and minimise the gastric side effects of the drug. The microspheres were prepared by w/o/w emulsion solvent evaporation method and gum with sodium alginate were used as a matrix and ethyl cellulose as a coating polymer. The slow release of metformin,

dependant on the gum concentrations in the matrix, was achieved. Microspheres containing a high concentration of the gum showed a slow release which can be related to the high viscosity of the internal aqueous phase that increased with gum concentration and alginate. However, the increment of the gum concentration had a significant impact on drug release which was retarded from 7.53 to 10.82 hours with time required for 50% of drug release ($t_{50\%}$) and similar trend was noticed with 80% [62].

Also, Reis *et al.* used alginate to prepare insulin microparticles using internal gelation emulsification method. In this method, a solution of alginate with insulin protein was dispersed into a water immiscible solution. The gelation took place *in situ* by immediate calcium ion release from carbonate complex through a minor pH adjustment. *In vitro* release study was tested in pH 1.2 and pH 6.8 with a gastrointestinal simulated condition. The insulin release study showed a burst release of the drug (100%) at 5 min in acidic media and was not inhibited by alginate. Nevertheless, the protection of insulin secondary structure after 2 hours in isotonic PBS buffer (pH7.4) was detected which confirmed prolong drug efficacy [63].

Alginate crosslinked chitosan microparticles were also investigated by researchers. Szekalska *et al.* attempted to modify the drug release of metformin and studied the reduction in blood glucose levels in rats for 28 days. During the study the therapeutic efficacy of alginate-cross-linked microparticles was compared with non-cross-linked microparticles, commercially available metformin tablets in presence of placebo and carboxymethyl cellulose sodium salt (Control). The *in vivo* studies showed a gradual reduction in blood glucose level and a slower metformin absorption after orally administrated of non-cross-linked alginate microparticles and chitosan cross-linked alginate microparticles formulation to diabetic rats. This could be attributed to the prolonged drug release and the stable plasma drug concentration. Although, after 21 days the formulations achieved similar blood glucose level with metformin tablet, the effect of the formulations was gradual and stable. Moreover, the results showed a reduction of 15% and 33% in blood glucose level after using pure alginate for 3 and 18 days of treatment respectively. Therefore, alginate can be considered as a prospective antihyperglycemic agent. Nonetheless, the *in vitro* release studies showed a burst effect for all the formulations. This can be attributed to the hydrophilic nature of free metformin particles that present on the surfaces of the microparticles. Also, It was noticed that a sustained release profile was maintained for up to 6 hours with cross-linked microparticles compared to non-cross-linked alginate-metformin microparticles. This is owing to the swelling behaviour of the microparticles that were produced by the cross-linking of chitosan and alginate resulting in prolonged drug release from the swollen matrix. Besides, alginate

has the property to convert into alginic acid, in a gel form, at acidic pH, which can control the internal water content in the microparticles and affects the release profile [64].

Alginate nanoparticles were also investigated to deliver antidiabetic drugs. A study was conducted to design alginic acid nanoparticles along with nicotinamide as permeation enhancer for sublingual delivery of insulin, with the objectives of achieving good decrease in serum glucose level and increase in serum insulin level in diabetic rats. These nanoparticles were prepared using mild and aqueous process to avoid any detrimental effects on insulin stability. Alginic acid nanoparticles revealed strong bioadhesion compared to insulin solution with entrapment efficiency of 95%. Insulin release from alginic acid nanoparticles followed first-order kinetics. The insulin release profile of these nanoparticles was examined over a period of 12 hours which indicated very rapid initial burst (65%) in barely 2 h followed by slow release. The hypoglycemic effects and serum insulin levels were evaluated in STZ-induced diabetic wistar rats. The pharmacological availability and relative bioavailability of these sublingual insulin nanoparticles were compared with insulin SC injection. It was revealed that alginic acid nanoparticles at doses 5 IU/kg possessed high pharmacological availability of 100.2% to 125.1%. Similarly, the dose-corrected bioavailability with reference to SC injection (1 IU/kg) was also significantly higher (20%-25%) [65].

In another study, three types of insulin-loaded alginate-C18 nanoparticles were prepared by dropwise addition of insulin into alginate solutions under constant stirring and subsequently spray-dried. These nanoparticles were further dispersed into sodium alginate solutions to form beads which were then coated with tripolyphosphate cross-linked chitosan-oleic acid conjugate to produce multiple fold effect. These nanoparticles indicated low toxicity level, reduced size and zeta potential thus enhanced mucus penetration, intracellular trafficking and minimal insulin reabsorption tendency as a result of active COOH/COO⁻ sites of alginate were occupied by C18 conjugate.

The combination of insulin-loaded nanoparticles and beads in a single dosage form brought a remarkable blood glucose reduction of 25% in 0.5 hour followed by further decrease of approximately 46% within 24 hours, when compared to water, oral insulin, SC insulin and drug free nanoparticles-loaded beads. However, these nanoparticle-loaded beads initially showed a higher plasma glucose levels due to a small fraction of polysaccharide of beads being digested and absorbed to cause the surge. The use of beads, as carriers of insulin-loaded nanoparticles, prevented premature insulin release in the gastric cavity. Instead of using nanoparticles alone, the combined use of nanoparticles and beads as a single dosage form increased the blood glucose lowering extent of insulin synergistically and raised insulin level in blood. The nanoparticles in beads represented a vehicle that can be used in oral insulin delivery [66].

A study was conducted to prepare gliclazide loaded calcium-alginate beads using ionotropic gelation method. Alterations in polymer concentration, stirring speed, internal phase volume and type of surfactant used in external phase were made which affected the particle size, incorporation efficiency and flow properties of the beads. These alginate beads showed swelling and mucoadhesive properties, which helped in improving oral delivery of drug. *In vitro* testing revealed that “swelling” was main factor contributing to the control release of drug. *In vivo* studies were conducted in diabetic rabbits and indicated that hypoglycemic effect induced by these alginate beads was remarkably higher than conventional gliclazide tablet with a statistically significant difference of $p < 0.05$. The formulation successfully exhibited control release of drug as compared to its tablet form which showed a rapid release over 25 hours [67].

In another study, calcium-alginate nanoparticles were developed for sustained release of liraglutide. These nanoparticles were prepared via ionotropic controlled gelation method and coated with chitosan. The particles were characterised by dynamic light scattering technique, scanning and transmission electron microscopy. The formulation containing 0.5% alginate, 0.5% chitosan and 0.5% calcium chloride in volume ratio of 3:1:1 revealed a size of 100 nm under SEM. The loading efficiency and loading capacity of this formulation was 92.5% and 54.16%, respectively. The stability of these nanoparticles was reported to be 92.4% after freeze drying and 72.3% over subsequent storage at 4°C for 60 days. *In vitro* release results were carried out in simulated gastrointestinal conditions and drug release of 59.1% was observed after 6 hours. These results showed that chitosan coated alginate nanoparticle for oral delivery of liraglutide is a potential natural biodegradable polymer-based carrier system [68].

Overall, the highly sought-after properties of alginates make this polymer an ideal drug carrier and for oral route of administration. However, due to the stability issue of the alginate micro and nanoparticles, it is usually used in combination of other polymers such as chitosan etc, to impart mechanical strength to the system.

Table 3: Alginate and chitosan nanoparticles for antidiabetic drugs delivery. NPs: nanoparticle(s), L.E: loading efficiency , A.E: association efficiency, PVA: polyvinyl alcohol, E.E: encapsulating efficiency, L.E: loading efficiency, L.C: loading capacity, GK: Glucokinase

Polymeric nanoparticles	Drug incorporate	Route of Administration	OUTCOME		Reference
			Pharmacokinetic	Therapeutic	
Chitosan NPs (300-400 nm) L.E~55%	Insulin	Intranasal	↑nasal absorption	Significant rapid <i>in vitro</i> release ↑ <i>in vivo</i> release in albino mice as compared to insulin solution	[69]
Alginate/chitosan NPs (750 nm) A.E> 70%	Insulin	oral	↑oral absorption ↑oral bioactivity	↓basal glucose levels up to 40% for 18 hours	[70]
Chitosan NP	Insulin	oral	↑Interaction between rat intestinal epithelium & chitosan NPs after 3 hours	↓ Serum glucose levels in streptozotoc induced diabetic rats for 11 hours	[71]
Chitosan-mucin NPs combined with poloxamer & PVA E.E ~88-92%	Insulin	oral	Negligible release at pH 1.2 and >80% sustained release for 8 hours at pH 7.4	Fast onset of action in alloxan-induced diabetic rats. The NPs provided sustained release (12 hours) than subcutaneous insulin whose effect lasted for 4 hours	[52]
Fatty acid conjugated Chitosan Nanoparticles L.E and L.C >98%	Insulin	Liver-targeted SC delivery	↑Hepatocyte absorption ↑antidiabetic efficacy ↑↑ relative pharmacological availability than free insulin	↑↑ Relative pharmacological availability in liver than free insulin in diabetic mice	[72]

Polyurethane-alginate/chitosan core shell NPs E.E>90%	Insulin	oral	Diffusion-controlled and swelling-controlled release sustained over 20 hours	Slow onset of action but prolonged release and glucose control up to 6-10 hours in diabetic mice as compared to SC insulin (reduced blood glucose levels for 2 hours)	[73]
Alginate-chitosan NPs L.C~87%	Modified Insulin	oral	Insulin protection in simulated gastrointestinal fluid (SIF)	↑in cumulative insulin release (40%) in SIF than non-modified insulin with only 18% release	[74]
Chitosan-pectin biohybrid nanoparticles L.C ~ 92%	Metformin	Intra-peritoneal	↑ Upregulation of IRA, GLUT-2 and GK receptor gene expression ↓TNF-α, IL-6 in pancreas	↑ Glucose utilization in vital organs Controlled dyslipidaemia and renal impairment in diabetic rats	[75]
Alginate coated Zinc NPs	Metformin	oral	↑stability in acidic environment	Targeted release in intestine. ↑Effectiveness due to alginate coating.	[76]
Alginate-chitosan NPs E.E=52.48% L.C=47.01%	Insulin	oral	↓hypoglycemic effect lasted for 4 h	47% Blood glucose levels ↓ in STZ-induced albino rats at 5 h.	[77]

Poly acrylic acid

Developing controlled release systems is one of the main approaches for different research efforts. It was aimed to use biodegradable materials such as poly(D,L-lactic acid) (PLA) or poly(D,L-lactide-co-glycolide) (PLGA) to control the release of peptide and protein drugs [78].

Ibrahim *et al.* prepared polyacrylic acid and poly (lactide- co -glycolide) based microparticles loaded with 5% bovine insulin by multiple emulsion solvent evaporation methods (w/o/w). The microparticles were of sizes 40-53 μm with porous surfaces and the encapsulation efficiency between 75.18% - 79.63%. The release study results from polyacrylic acid and poly (lactide- co -glycolide) microparticles showed an early loss of 66% and 71% of insulin accordingly during the first 6 hours. This was attributed to protein accumulation and the hydrophobic interaction between the protein and polymers. However, the insulin amount was high and these results appeared in the stability studies after several weeks [42]. Also, polyacrylic acid was used in one of the old studies to prepare microbeads of insulin using emulsion solvent evaporation method. The results showed that more than half of the insulin was released within the first hour, and *in vivo* results showed insulin lasts for more than two weeks.

Gelatin

Gelatin is a natural polymer obtained from animal collagen and possess low antigenicity. It is isolated using acid or base hydrolysis of collagen by breaking the hydrogen bonds responsible for the stability of collagen [79]. Gelatin has been classified as type A or type B depending upon the type of hydrolysis method used in its preparation [79, 80]. Gelatin nanoparticles were first developed in 1978 by Mary *et al.* [81, 82].

Inoo, Bando and Tabata conducted a study to assess the insulin secretion by transport subcutaneously insulinoma cells and gelatin hydrogel microspheres in the back of rats to assess the insulin secretion from both of them. Model β cells were prepared by mixing gelatin hydrogel microspheres with insulinoma cells groups to sustain the cells vitality through studying the pathway of oxygen, nutrients and the effect of the mixed hydrogel microspheres on the function of cell aggregates. Chemical cross-linking of gelatin in w/o emulsion was used to prepare gelatin hydrogel microspheres. The insulinoma cells with or without gelatin hydrogel microspheres were loaded in a sac like tool and insulin secretion was evaluated before and after the subcutaneous transplantation on 3 and 7 days for all formulations. The results showed that insulin secretion was markedly high from the sac like tool containing insulin

insulinoma cells aggregates with gelatin microspheres compared with those without the microspheres. However, the secreted insulin amount was less after transplantation and it was similar between 3 and 7 days after transplantation by *in vivo* studies [83].

In another study Inoo, Bando and Tabat used the same obtained gelatin hydrogel microspheres previously for *in vivo* experiments on rat-derived insulinoma cells to compare between the effect of gelatin hydrogel microspheres and microspheres-free cell groups on the survival and glucose-induced insulin secretion. After 14 days of cultivation at the initial seeding density of 1.0×10^3 cells/well the results showed a good cell survival and reductase action for the cells with gelatin microspheres and a notably increment in insulin secretion with cell groups of large sized microspheres compared to the middle sized and the cell groups without microspheres. Hence, cell groups combined with large gelatin hydrogel microspheres can be considered to prepare the cells for treatment of type 1 diabetes [84].

Gelatin was used by Wang, Tabata and Morimoto to prepare animated microspheres for nasal drug delivery system of peptide drugs. The *in vitro* drug release study used to evaluate fluorescein-labeled insulin (FITC-insulin) and FITC-dextran as model drugs. The release of FITC-insulin from the animated gelatin microspheres was slower than from control gelatin microspheres, with 18.4% and 32.4% within 30 min, and 56.9% and 75.1% within 8 hours accordingly. However, the release of FITC-dextran from both gelatin formulations (animated and the active) was fast and no significant difference was observed between them. The *in vitro* study was used to test the hypoglycemic effect after intranasal administration of the formulations in healthy rats. The results showed a significant reduction in glucose level with nasal administration of animated gelatin microspheres in powder form in contrast to the intranasal suspension which had no significant effect on glucose level after at insulin dose of 5 IU/kg [85].

Ying-Zheng and his fellows prepared gelatin nanoparticles to investigate safety and bioavailability of pulmonary administered insulin. Insulin was attached to the surface of the gelatin nanoparticles which were further modified by poloxamer and D, L-glyceraldehyde solution. The ratio of gelatin to poloxamer played significant role in determination of physical characteristics such as size, PDI and ZP. The insulin loaded nanoparticles with 1:1 ratio had smallest size (250 nm), PDI (0.276) and highest ZP values of -21.1 ± 0.6 mV. The nanoparticles possessed a smooth surface with spherical shape. The formulation was administered to Sprague–Dawley (SD) rats via intratracheal route. A significant drop in initial blood glucose concentration from 100% to 37.5 ± 4.10 % was achieved within 4 h of administration of prepared gelatin nanoparticles in comparison with same dose (2 IU/kg) of insulin solution on its own where it dropped to only 65.32 ± 4.30 %. The bioavailability was also higher (76%

with gelatin nanoparticles Vs 42% for insulin solution. In addition, these nanoparticles assured the safety of lung by reducing insulin deposition in lungs [86].

Another study prepared chitosan- γ PGA hard gelatin nanoparticles using ionic gelation method for oral and SC delivery of insulin. The nanoparticles were as small as 241.5nm with 25.6 mV surface charge. An oral dose of 30 IU/kg was administered to STZ-induced male Wistar rats which showed 10% and 30% reduction in blood glucose levels in 5 hours with a continued hypoglycemic effect of 10 hours. The pharmacological activity of 20.1% was observed. The entrapment efficiency and drug loading was reported to be 70.6% and 18.3% respectively [87].

Similarly, another group of researchers also prepared chitosan and γ PGA gelatin capsule nanoparticles coated with Eudagrit via ionic gelation method and performed *in vivo* studies using STZ-induced rats. The formulation provided pharmacological activity of 21% and possessed a size of 250 nm and zeta potential of 25 mV. The *in vivo* results showed 40% decrease in blood glucose level in 4 hours and hypoglycemic effect was maintained up to 6 hours [88] .

Gelatin is an attractive polymer for preparing micor and nanoparticles with good natural abundance, high physical stability and low antigenic properties. However, the type of isolation technique may affect the purity and physicochemical properties of gelatin which should be considered in choosing this natural biodegradable polymer as a drug delivery vehicle.

Albumin

Albumin is a protein biopolymer and an emerging nanocarrier for protein-based drugs including oral delivery of insulin. It is most abundant plasma protein having molecular weight of 66.5kDa. It possesses high stability at pH 4 to 9 and thermal stability up to 60 °C. Besides, it has been preferentially used as oral drug delivery system for its biodegradable nature, low toxicity and non-immunogenicity making albumin an ideal material for drug delivery [89].

Mahobia and co-workers prepared egg albumin nanoparticles to deliver insulin orally. The microemulsion method was employed to prepare egg albumin nanoparticles in presence of glutaraldehyde as crosslinker. The nanoparticles were then placed in insulin PBS solution (pH 7.4) to load the drug. These were then subjected to swell up and absorb the drug until equilibrium was achieved. The size of these nanoparticles was reported to be 10-30 nm under Transmission electron microscope, while the *in vitro* cytotoxicity was investigated in fibroblasts and the albumin nanoparticles deemed non-toxic. It was observed that increase in albumin content up to a certain limit

increased insulin release proportionally. Formulations with 68% drug loading showed a maximum cumulative release up to 5mg while formulations with 33% drug loading exhibited 4 mg cumulative release [90].

Another group of researchers prepared chitosan-albumin coated particles for oral insulin delivery using a complex scheme based on emulsification gelation technique. Insulin was incorporated to be trapped between alginate-dextran sulphate pockets and a dual coating was applied by dropwise addition of chitosan and albumin, sequentially. The particles possessed average size of 300 nm, PDI \leq 0.30 and surface charge of -30.6 ± 0.8 mV. The encapsulation efficiency of nanoparticles before coating was $72.4 \pm 3.3\%$ while it was reduced to 21.9 ± 2.8 and $30.7 \pm 3.4\%$, after coating with chitosan and albumin, respectively. Similarly, the loading degree of nanoparticles also reduced from $10.1 \pm 2.8\%$ to $4.8 \pm 0.4\%$ and $6.2 \pm 1.4\%$ after chitosan and albumin coating respectively. The *in vitro* studies showed complete insulin release at pH 5.5 after 2 hours for both chitosan and alginate coated nanoparticles but a sustained release at pH 7.4 was observed only with albumin coated particles while in chitosan coated nanoparticles, the insulin release was halted due to insolubility of chitosan at that pH. *In vivo* permeability studies were carried out in a triple co-culture of Caco-2/HT29-MTX/Raji B model proved that dual coated nanoparticles had increase permeation across intestinal cell and longer retention in mucus layer than non-coated nanoparticles [91].

A similar type of nanoparticle formulation was developed by Woitiski and co-workers in 2010 where hypoglycemic effects of chitosan stabilized alginate-dextran nanoparticles coated with albumin were investigated. These were administered orally to wistar diabetic rats. The formulation carried negative charge therefore, the bioactivity of insulin was maintained bioactivity and pharmacological availability was improved by avoiding enzymatic degradation of insulin and via chemical and physical facilitation of permeation through the intestinal membrane. The nanoparticles were 396 nm (PDI 0.6) in size with a ZP of -38 mV. *In vivo* results indicated a drop of 40% in blood plasma levels with a sustained hypoglycemic effect maintained over 40hours. The oral bioavailability of 13% for a dose of 50 IU/kg was three times higher than free insulin. Confocal microscopy confirmed internalization of insulin in the small intestinal mucosa [92].

An efficient antidiabetic drug, exetanide administered via SC route is associated with noncompliance issues. The researchers attempted to coat the drug into a nano-in-micro delivery system in order to improve its gastrointestinal stability. The drug was loaded into a Bovine serum albumin (BSA)-dextran nanoparticles and tested for dynamic light scattering technique measurements. The size of the particulate system ranged from $190-360 \pm 4.7$ nm, PDI of 0.28-0.37 and ZP values of -40.3 ± 0.9 . The results were compared with available market SC formulation, Byetta®. The glycemic parameters of

this delivery system were investigated in diabetic ob/ob mice (severely obese due to leptin deficiency: gene mutation) and results suggested that the new formulation successfully lowered blood glucose levels raised insulin levels, decreased glycated hemoglobin and maintained the body weight of the mice [93].

Overall, albumin can be proposed as a potential future drug carrier as albumin based micro and nanoformulations show some promising results in terms of their half-life, excellent binding and stability. Nevertheless, using as a drug carrier on large scale, batch variation is expected due to its protein nature.

Conclusion and futuer perspectives:

Various studies have demonstrated that biodegradable polymer based formualtions controlled drug release, limited the initial burst drug release and enhanced hypoglycaemic/hypoglycemic effects when compared to conventional formulations that did not use micro or nanoparticles. In this review, micro-particles studies have generally focused on the controlled drug release in order to reduce the frequency of injection, limit the initial burst release and sustain hypoglycaemic/hypoglycemic effect, whereas nanoparticles studies have generally focused on oral delivery of antidiabetic peptides (e.g., insulin) to enhance the oral bioavialbility. Regardless of different delivery routes of administration (SC, pulmonary and oral), PLGA based micro and nanoparticles formulations have exhibited the reduction of the daily doses for antidiabetic drugs. This is advantageous especially for patients on injection based treatments as the frequency of injections can be minimised while the bioavialbility and theraperutic efficacy of the drugs can be maintained or enhanced. Studies reported on nanoparticles have demonstrated the protection of insulin from enzymatic degradations and enhanced intestinal absorption by encapsulating the drug into the biodegradable syntetic polymers (e.g. PLGA, and γ -PGA). Due to the hydrophobic nature of PLA, its structure integrity is difficult to maintain in gastric fluids, therefore, using PLA for oral delivery is still a challenge to overcome. Natural biodegradable polymers such as chitosan and gelatin have proven successful to control blood glucose levels with a continuous hypoglycemic effect. They also exhibit low immunogenic risks, yet these polymers had stability issues.

It is evident that polymeric nano and microparticles can play a significant role in enhancing drug pharmacokinetics and enable the antidiabetic drugs to bypass barriers in our body. These polymeric vehicles open the way for new routes of drug administration, offering new opportunities for patenting new drug delivery systems. Oral insulin has always been a dream and it is now a step closer to reality. In February 2020, Oramed Pharmaceuticals Inc announced the success of its phase 2b clinical trials.

An oral insulin developed by the company called ORMD-0801 succeeded to reduce HbA_{1c} by 1.26% after 8 weeks of treatment [94].

This paves the way to explore the oral delivery of other protein and peptide drugs. Polymeric vehicles can offer the opportunity to Glucagon-Like Peptide-1 and analog to be delivered orally without the need of modifying their chemical structures. Recently, Novo Nordisk and Oramed Pharmaceuticals developed oral GLP-1 analog (ORMD-0901) that use an excipient to protect the peptide together with protease inhibitor [95, 96].

The dose requirements for oral delivery studies are generally high (e.g., 30-100 IU/kg) in comparison to SC injections (e.g, 5 IU/kg). This could lead to a high cost of production.

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