Review

Nanotechnology based approaches for anti-diabetic drugs delivery

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\textbf{ABSTRACT}

Nanotechnology science has been diverged its application in several fields with the advantages to operate with nanometric range of objects. Emerging field of nanotechnology has been also being approached and applied in medical biology for improved efficacy and safety. Increased success in therapeutic field has focused several approaches in the treatment of the common metabolic disorder, diabetes. The development of nanocarriers for improved delivery of different oral hypoglycemic agents compared to conventional therapies includes nanoparticles (NPs), liposomes, dendrimer, niosomes and micelles, which produces great control over the increased blood glucose level and thus becoming an eye catching and most promising technology now-a-days. Besides, embellishment of nanocarriers with several ligands makes it more targeted delivery with the protection of entrapped hypoglycaemic agents against degradation, thereby optimizing prolonged blood glucose lowering effect. Thus, nanocarriers of hypoglycemic agents provide the aim towards improved diabetes management with minimized risk of acute and chronic complications. In this review, we provide an overview on distinctive features of each nano-based drug delivery system for diabetic treatment and current NPs applications in diabetes management.

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Diabetes mellitus (DM) is a chronic lifelong metabolic disorder that alters the life of billions of people throughout the world [1,2]. It can be classified into two major forms, namely Type 1 DM (T1DM) and Type 2 DM (T2DM) [1,3–6]. In DM, constant hyperglycemia may result in chronic micro- and macrovascular effects such as nephropathy, retinopathy, neuropathy, stroke and cardiovascular disease [1,3,5,7,8]. The number of diabetic patients are increasing tremendously worldwide, where a recent report has indicated the increase of 422 million patients in 2014 from 171 million in 2000, showing the sharp increase in the sufferers [9]. Adaptation of sedentary lifestyle and increasing in demographics with the age of >65 years suggesting this increased incidences of diabetes may be doubled, approximately 366 million in 2030 [10,11]. Therefore, management is needed to control diabetes conditions and consequence complications. Monitoring programmes make people aware for effective management diabetic through adaptation of proper low carbohydrate diet, regular physical exercise, and adherence to medication therapy, if needed [1,8,12–14]. The conventional medications used now-a-days to control hyperglycemic condition in DM are oral hypoglycemic agents (OHA) and parenteral preparations of insulin and glucagon-like Peptide-1 (GLP-1) receptor agonists [1,8,12,15–17].

Insulin, is a polypeptide hormone consists of 51 amino acids in two chains (A chain, 21 amino acids; B chain, 30 amino acid), joined together by two disulfide bonds (–s–s–) (Fig. 1A), that helps in regulating the uptake and storage of glucose in the liver and muscles [6,18]. It is produced by β-cells of pancreas and released via exocytosis process into the blood stream to help in utilization of peripheral glucose for generation of energy [6,18]. The coordinated responses which stimulate glucose oxidation and inhibit gluconeogenesis simultaneously led to the hypoglycaemic action of insulin. The plasma glucose concentration decrease when insulin directs the glucose transporters (GLUT 4) into the cell membranes and increases glucose transport into target cells (Fig. 1B) [10,19]. The aim of insulin therapy is to provide insulin replacement as close as possible in all patients. However, insulin resistance sometimes may happen during insulin therapy for the management of diabetic conditions [20,18]. Therefore, nanosize particles have comes out as for a more convenient, safe and non-invasive route for insulin delivery in order to overcome such limitations in diabetes management [2].
Conventional drug delivery systems often face limitations of lack of efficacy due to improper or ineffective dosage, diminished potency or altered effects due to drug metabolism and lack of target specificity [21,22]. Various approaches of nanocarriers adopted in drug delivery system are shown in Fig. 2. Application of nanocarriers may enhance activity against the combating diseases, increase detection sensitivity in medical imaging, decrease side effects by functionalizing their surface with synthetic polymers and appropriate ligands and by virtue of their small size [23–27]. This review has been highlighted in the importance of designing nanomedicines for anti-diabetic agent that has been approached to overcome the challenges of the conventional treatment process.

2. Nanocarrier based approaches in anti-diabetic drugs delivery

The word “nano” has been originated from Latin and the literal meaning of which is dwarf. Thus, nanotechnology science deals objects within the size range of $10^{-9}$ to $10^{-7}$m. Thus, strategies in successful delivery of therapeutic medications have incorporated the agents in the microenvironment of the nanocarrier, with the aim to suitably target for improved efficacy and safety. Synthesis of the nanocarriers are a challenging art of nano-research to get reproducible nano-products, at the same time, maintenance of the characteristics of the formulated nanocarriers to get reproducible results in the in vitro as well as in vivo experiments. Subsequent sections of the article will cover the various nanoparticle approaches for the improvement of diabetes therapy in hyperglycemic condition.

2.1. Liposomes based drug delivery system

Liposomes are small vesicles, consist of one or more phospholipid bilayers that are produced from natural non-toxic phospholipids and cholesterol [28–30]. These are the innovative technology and promising systems to serve as a transporter for the active molecules to the site of action within the biosystem [31–34]. The increase usage of liposome in
investigational system as well as commercially drug-delivery system is mostly because of their biodegradability, biocompatibility, and low toxicity to entrap both lipophilic and hydrophilic drugs, and also facilitate the site-specific/targeted delivery of drug [31–34]. Thus, a lot of studies have been conducted on liposomes to decrease drug toxicity and target specific cells for improved efficacy and safety [29]. Due course of delivery of lipid materials, the liposome fused with the cellular lipid membrane followed by release of liposomal content into the cytoplasm of the cell to produce its pharmacological action (Fig. 3). Table 1 of the article portrayed various liposomal approaches with the hypoglycaemic drugs.

In their research Zhang et al. investigated the capability of liposome modified with targeted ligand biotin (BLPs) to facilitate transportation of insulin through oral delivery, simultaneously investigated its cytotoxicity. By incorporation of biotin-1,2-distearoyl-sn-glycerol-3-phosphatidylethanolamine (DSPE) into the lipid bilayer of liposome, BLPs had been produced. The physiochemical properties of insulin-loaded liposomes have been affected by particle size and entrapment efficiency (EEf). The author has found out that lipid:cholesterol ratio of 3:1 appeared to have potential effect on holding more insulin in liposomes, with desirable membrane fluidity and decreased chances of insulin leakage from internal aqueous compartments. Apart from this, hypoglycemic effect was found to be affected by the biotin-DSPE proportion in liposomes, particle size of liposomes and also doses of the formulation. However, significant hypoglycemic effect was noticed with 153.7 nm liposomes which can further be described due to improved stability of liposomes for smaller diameter.

Fig. 3 – Mechanism of non-liposomal versus liposomal formulation containing anti-diabetic agents: Invagination of liposomal lipid layer on the lipid layer of the cell membrane and entry of drugs into the cells. liposomes concentrations. The results showed that BLP for insulin delivery are safe to be taken orally. Finally, BLPs showed 5.28 folds increase in pharmacological bioavailability compared to conventional liposomes, suggests that BLPs can be used as promising carriers for oral insulin delivery [35].
<table>
<thead>
<tr>
<th>Type of DM (1/2)</th>
<th>Polymer</th>
<th>Cell Line</th>
<th>Size Range</th>
<th>Drug(s) incorporated</th>
<th>Outcome</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1DM</td>
<td>Biotin-modified liposomes</td>
<td>Sprague – Dawley rats (220 ± 20 g)</td>
<td>Mean particle size under optimal conditions was about 160 nm</td>
<td>Insulin</td>
<td>1. Significant hypoglycemic effect was observed with 153.7 nm liposomes 2. More biotin-DSPE into lipid bilayer could reinforce the rigidity of liposomes due to high phase transition temperature of DPSE 3. It has proved that biotin receptor-mediated endocytosis facilitated BLP absorption through oral route 4. Relative bioavailability of BLPs was 5.28 folds compared to CLPs</td>
<td>[35]</td>
</tr>
<tr>
<td>T1DM</td>
<td>Chitosan-coated liposomes</td>
<td>Fasted male Kunmin mice with water for 8 h were used and divided into 10 groups</td>
<td>–</td>
<td>Insulin</td>
<td>1. A 0.2% concentration of chitosan was proved to be the best concentration in coating 2. Chitosan-coated liposomes could improve absorption of insulin from GT tract by reducing enzymatic degradation 3. The concentrations and molecular weights of chitosan has a great impact on the hypoglycemic efficacy of chitosan coated insulin liposome in normal mice after oral ingestion</td>
<td>[36]</td>
</tr>
<tr>
<td>T2DM</td>
<td>Glycerolphosphate–Chitosan Microcomplexation</td>
<td>Stomach and intestine tissues of Wistar rats</td>
<td>–</td>
<td>Metformin</td>
<td>1. GP/CH microcomplexes help to reduce water penetration rate into the biological system 2. Cross-linking of water-soluble polymers could restrict the system over hydration and polymer dissolution, improving mucoadhesion 3. GP/CH microcomplexes exhibited a good mucoadhesion in ileum and colon 4. A 2.5-times longer Tmax of metformin with a 40% improvement of the AUC/D value was observed with proposed microcomplexes 5. GP/CH microcomplexes are effective carriers of the highly water-soluble antihyperglycemic drug, allowing its controlled delivery and improved oral availability</td>
<td>[107]</td>
</tr>
</tbody>
</table>
| T1DM | Insulin- loaded folic acid functionalized polymer stabilized multilayered liposomes | 283 ± 9 nm | Insulin | 1. In FA-layersome, a sturdy structure formed due to strong electrostatic attraction produced between oppositely charged polyelectrolyte layers which further control phospholipid from external microenvironment.  
2. Higher cellular uptake of FA layersome further support targeting of FA receptor could be a superior targeting approach for orally administered bioactives  
3. Excellent hypoglycemic effect was reported in an in vivo for the developed stable FA-Ins-layersomes  
4. Long-lasting hypoglycaemic effect was observed with the developed and optimized formulation. It has been concluded that easy administration, patient-compliance with improved hypoglycaemic profile were achieved with the developed formulation |
| --- | --- | --- | --- | --- |
| T1DM | Sodium glycocholate liposomes | 150 nm | Insulin | 1. Particle size of 154 ± 18 nm with 30 ± 2% of entrapment efficiency were observed for the developed and optimized formulation  
2. Sodium glycolate liposome could be a potential approach for protein and peptide delivery through oral route, as bile salts protect liposomal rhINS from enzymatic degradation |
| T1/T2DM | 1. Liposomes containing SGC  
2. Liposomes containing sodium taurocholate  
3. Liposomes containing sodium deoxycholate | Rats | 150 nm or 400 nm | Insulin | 1. These developed and optimized liposomes after oral ingestion, exhibited mild and long-lasting hypoglycemia up to 20 h with increased insulin level.  
2. After oral administration of SGC-liposome, bioavailability of 11.0% and 8.5% were reported in diabetic and non-diabetic rats, respectively  
3. Maximum oral bioavailability as well as hypoglycemic effect were observed with SGC followed by sodium taurocholate, CH and sodium deoxycholate  
4. rhINS-loaded liposomes exhibited dose dependent and particle size dependent hypoglycemic effect | [39]  
(continued on next page)
<table>
<thead>
<tr>
<th>Type of DM (1/2)</th>
<th>Polymer</th>
<th>Cell Line</th>
<th>Size Range</th>
<th>Drug(s) incorporated</th>
<th>Outcome</th>
<th>Source</th>
</tr>
</thead>
</table>
| T1/T2DM        | 1. Glycocholate lipo-some (SGC-Lip)  
2. Conventional lipo- 
some (CH-Lip)       | Rats | Around 200 nm | Calcein, insulin | 1. Though, significant improvement in calcein release was observed in presence of bile salts in acidic pH but did not show any effect on particle size and its distribution pattern  
2. Glycocholate liposome protect insulin from enzymatic degradation in simulated GI fluid (SGF) as well as GI media from rats by encapsulating more insulin which further contributed to enhanced insulin’s oral bioavailability compared to conventional liposome | [38] |
| T1/T2DM        | 1. Dioleoyl-sn-glycero-3-phosphoethanolamine, dioleoyl-3-trimethylammonium-propane and cholesterol (DOPE/DOTAP/CH) with ratio of 2:1.5:2 plus 5 mol% of distearoylphosphatidyl ethanolamine polyethylene glycol (DSPE-PEG) | – | 265.4 ± 35.3 nm | Insulin | 1. PEG addition in Insulin-loaded cationic liposome resulted reduction in particle size as well as improvement in encapsulation efficiency  
2. DPSE-PEG addition in the formulation and changing the aqueous phase improved stability and encapsulation efficiency of the formulation and finally enhanced bioavailability of insulin was achieved | [108] |
| T1/T2DM        | 1. Compositions of lipids in liposomes were HPC/Chol (70/30 mol %) and HPC/Chol/PEG-DPPE (70/30/1 mol%) | BALB/c mice | Mean particle size (203.5 ± 20.5) nm | Insulin | 1. A 40% ethanol was found to ideal to achieve maximum encapsulation efficiency of insulin  
2. Pulmonary delivery of insulin liposome resulted targeted and homogenous distribution of insulin in the lung alveoli and enhanced lung retention-time, thus resulting in interestingly enhanced pharmacodynamic effect | [109] |
| T2DM           | 1. Anionic liposomes containing DSPE-PEG5G (10%), DPPC (27%), Cholesterol (36%) and DPPG (27%) | Rats | 131 nm | Glucagon-like peptide-1 (GLP-1) | 1. Maximum encapsulation of GLP-1 was achieved in anionic liposomes. The formulated highly dispersible liposomes were found to be within the diametric range of 130–210 nm  
2. Anionic liposomal delivery with the composition mentioned in column ‘Polymer’, exhibited the noticeable enhancement of therapeutic effects in experimental rats | [110] |
and facilitated uptake by receptor-mediated endocytosis through intestinal epithelia. Furthermore, the hypoglycemic effect of BLIPs was linearly corresponded when low doses are given and nonlinearity was expressed at high doses due to oversaturation of BLPs on enteroctyes biotin receptors. Finally, BLPs showed 5.28 folds increase in pharmacological bioavailability compared to conventional liposomes, suggests that BLPs can be used as promising carriers for oral insulin delivery [35].

Alternative approach of chitosan (CH)-coated oral insulin liposomal delivery has been developed and tested for its efficacy. Authors reported that increased zeta potential of the positively charged CH-coated liposome could lead to insulin leakage due to induced electrostatic interaction produced amongst surface CH of the liposome and the entrapped insulin inside the liposome. Accordingly, CH-coated insulin liposomes will have increased hypoglycemic efficacy when there is increased in molecular weight of CH on the surface and its concentration. However, it has been suggested that best CH concentration in the coating to protect insulin from enzymatic digestion was 0.2%. Further hypoglycemic effect of the liposomal delivery was investigated using Kunmin mice and it has been found that CH-coated insulin liposomes were as effective as parenteral insulin. It has been hypothesized that CH coated liposomes were protected from pepsin, trypsin and at the same time, increased mucosity, to increase the gastric residence time of the formulation. Additionally, CH molecule can transform to a more coiled configuration in pH7.4 solution and fixed on liposomes surface to act as a protective layer. Moreover, hypoglycemic efficacy of the CH1000 kDa coated liposomes showed advanced result compared to liposomes coated by other CH [36]. Another research group targeted folic acid (FA) receptor for improved antidiabetic potential of oral FA-layersome in insulin-loaded liposomes. The liposome was stabilized by substituting coating of anion polycrylic acid (PAA) and cation polyallyl amine hydrochloride (PAH) over the liposome. The robust FA-layersomes developed was found to be spherical in shape. Author suggested that intense electrostatic attraction formed within distinctly charged polyelectrolyte layers can form robust structure of FA-layersomes and could prevent degradation of lipid molecules from direct exposure to external environment. Additionally, addition of FA as a targeting ligand through synthesis of FA poly-allyl amine hydrochloride conjugation was used to provide benefits to FA transporters in gastrointestinal (GI)-tract. Besides that, Caco-2 cell and ex-vivo gastroenteric uptake investigations disclosed the greater uptake of FA functionalized layersomes as compared to conventional liposomes. Of concern, the advanced dosage form exhibits outstanding steadiness in biological fluids simulation and possessed an enhanced bioavailability. The pharmacodynamics studies of FA-layersomes have showed approximately 2-fold hypoglycemic effect with 20% increase in relative bioavailability compared to subcutaneously insulin administration. The effect of FA modification has been studied and the research found out that method of insulin encapsulation within layersomes could prevent GI degradation. The advanced dosage form showed 18 h sustained hypoglycemia effect, suggesting that FA layersomes are patient-beneficial and patient-compliant oral formulation to manage diabetes with improved therapeutic action [37].

The sustained and mild hypoglycemia effect of oral recombinant human insulin (rhINS) in liposomes is preferred when compared to subcutaneous injection of rhINS, as subcutaneous insulin often causes drastic decrease in blood glucose level, which leads to unwanted side effects such as dizziness. In order to accomplish oral delivery of insulin, it is important to protect insulin against proteolytic enzymes, e.g., pepsin or pancreatin that degrades and inactivates insulin in GI fluid. Liposomes containing bile salt (glycocholate) have successfully proved to prevent the encapsulated insulin from proteolytic degradation. Besides that, liposomes incorporated with sodium glycocholate (SGC-Lip) have improved liposomal membrane integrity, which results in slower release of encapsulated drugs in simulated GI fluids (SGF) than that of conventional liposomes (CH-Lip). According to a study conducted by Niu et al., the rapid release of calcine (a fluorescent dye) from SGC-Lip was attributed to low pH environment. However, result indicated that SGC-Lip has certain degree of insulin protection against enzymatic degradation when compared to CH-Lip, thereby contributed positively to oral bioavailability of insulin [38]. It was believed that smaller particle size of liposomes has higher rate of absorption across the cell membrane. However, in astudy, authors concluded that the hypoglycemic effect of insulin-loaded SGC-Lip was related to the particle size (150 nm or 400 nm) and was correlated to administrated dose proportionally [39]. Conversely, incorporation of sodium glycocholate in liposomal formulation is found to be advantageous in increasing efficacy of protease inhibition and permeation. Thus, a study regarding feasibility of glycocholate-containing liposomes in improving oral insulin delivery had been conducted by Mengmeng Liu et al. Liposomes with glycocholate sodium were formulated adopting inverted-phase evaporation which generated spherical and deformed liposomes with discernable lamella, owing to the glycocholate liposomes deformation. Liposomes of 154 ± 18 nm size were chosen optimized by the researchers based on good permeation across biomembranes and substantial EE of insulin. Additionally, 30% of glycocholate in liposomes could achieve optimal formulation where further increase of concentration has fluidizing effect on lipid bilayer that can cause leakage of the incorporated drug. Therefore, sodium glycocholate liposomes could be an excellent carrier for insulin which will protect the incorporated drug from enzymatic/others degradation [40].

Therefore, liposomal vesicles were found to be an effective tool for the delivery of peptides, insulin and GLP-1, as well as water-soluble OHA for the improved control over hyperglycemic stage during diabetes.

2.2. Niosomes based drug delivery system

Niosomes are synthetic microscopic vesicles, size of which lies in the nanometric scale, mostly formed by non-ionic surfactants incorporated with cholesterol as excipient [41–49]. Niosomes can be categorized into large unilamellar vesicles (LUV) (100–3000 nm), small unilamellar vesicles (SUV) (10–100 nm), and multilamellar vesicles (MLV). The categorisation is based on their sizes and bilayers [43]. Niosomes are said to
<table>
<thead>
<tr>
<th>Type of DM (1/2)</th>
<th>Polymer</th>
<th>Cell Line</th>
<th>Size Range</th>
<th>Drug(s) incorporated</th>
<th>Outcome</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1/T2DM</td>
<td>Span 60/cholesterol/N-TMC system</td>
<td>Caco-2 cell lines</td>
<td>100–180 nm</td>
<td>Insulin</td>
<td>A 4 folds increase insulin permeation was observed with niosomal NPs through Caco-2 cell monolayer was when compared with free insulin</td>
<td>[111]</td>
</tr>
<tr>
<td>T1/T2DM</td>
<td>Polyoxethylene alkyl ether, cholesterol</td>
<td>N/A (in vitro)</td>
<td>–</td>
<td>Insulin</td>
<td>The authors incorporated insulin in neosomes prepared using polyoxethylene alkyl ether as surfactants. Oral delivery of the developed insulin niosomes was found to be a promising tool with noble stability</td>
<td>[112]</td>
</tr>
<tr>
<td>T2DM</td>
<td>Span 40, Span 60, cholesterol</td>
<td>Female ovariectomized Wistar rats</td>
<td>242.5 nm, 259.7 nm</td>
<td>Insulin</td>
<td>Vaginal administration of insulin entrapped niosomes could deliver the active and effective therapeutic agent. This could further suggest a new carrier to deliver peptides through vaginal delivery</td>
<td>[50]</td>
</tr>
<tr>
<td>T2DM</td>
<td>Cholesterol, Span 40, Span 60, dicetyl phosphate</td>
<td>N/A (in vitro)</td>
<td>388 nm- 644 nm</td>
<td>Metformin</td>
<td>Study revealed that the niosomal nanocarrier could be an effective carrier with sufficient drug entrapment and prolonged release profile</td>
<td>[51]</td>
</tr>
<tr>
<td>T2DM</td>
<td>Span 40/cholesterol dicetyl phosphate and DOTAP</td>
<td>Adult male Wistar rats</td>
<td>223.5–384.6 nm</td>
<td>Metformin hydrochloride</td>
<td>Metformin hydrochloride-entrapped niosomal delivery is proved to be a promising sustained-release preparation with greater control on hyperglycemic condition</td>
<td>[52]</td>
</tr>
<tr>
<td>T2DM</td>
<td>Span 60, cholesterol</td>
<td>Male Wistar rats</td>
<td>144 nm</td>
<td>Repaglinide</td>
<td>Niosomes can be used efficiently for enhancing bioavailability of the entrapped drug, thereby, in the current study the delivery tool decreases repaglinide dosing frequency from BID to OD</td>
<td>[53]</td>
</tr>
<tr>
<td>T2DM</td>
<td>Cholesterol and span 20</td>
<td>Albino Wistar rats</td>
<td>145.3 nm</td>
<td>Pioglitazone</td>
<td>The prepared formulation has ability to retain the drug at the site of treatment for prolonged time and is capable of maintaining constant drug concentration for a longer duration due to sustain action were shown by in vitro and in vivo studies</td>
<td>[113]</td>
</tr>
<tr>
<td>T2DM</td>
<td>Span 60, cholesterol</td>
<td>Healthy normal Wistar rats</td>
<td>Gliclazide</td>
<td>Drug entrapment efficiency was largely depending on the cholesterol:surfactant ratio in the formulation. Additionally the developed formulation was proved to have good oral bioavailability of the entrapped drug in vivo</td>
<td>[114]</td>
<td></td>
</tr>
</tbody>
</table>
be potential carriers in drug delivery system for their property of acting as reservoirs for drugs to achieve maximum drug entrapment in sustained and prolonged drug release [43,47,48]. Besides, presence of hydrophilic, amphiphilic and lipophilic moieties in their structure, niosomes also can accommodate drugs with varying solubility [42,43]. Further, these agents offer excellent biocompatibility and low toxicity due to their nonionic nature [42,43,46]. A few studies were analysed to investigate the potential of niosomes in drug delivery system for diabetic therapy (Table 2).

Potential of niosomes has also been applied for vaginal delivery system of insulin. To obtain this, the niosomal nanocarriers were prepared via lipid phase evaporation technique through sonication, consisting two types of vesicles, Span 40 and Span 60, with the resultant particle sizes of 242.5 ± 20.5 nm and 259.7 ± 33.8 nm, respectively. The investigation of hypoglycemic effects and the pharmacokinetics of insulin were carried out after the vaginal administration of insulin vesicles in ovariectomized (to maintain the vaginal epithelium thickness) alloxan induced diabetic Wistar rats. The outcomes indicating a maximum reduction of blood glucose with insulin-Span 40 and insulin-Span 60 reached to 47.49% and 46.66%, respectively, and the levels were found to be lower even after 6 h of vaginal administration. Further, the bioavailabilities of the two formulations were found to be 9.11% and 8.43%, respectively, higher than that of subcutaneous administration. Prolonged insulin release profile from the niosomes after a day indicated that it can be a promising and effective therapeutic agent in vaginal administration that provides controlled and prolonged drug release in achieving significant hypoglycemic effect [50].

There are several studies has been performed with niosomal delivery system for improvement of delivery system of OHA. To avoid the limitations of metformin, niosomes were used to obtain sustained-release property, enhanced oral bioavailability as well as to minimize its untoward adverse effects and its dosing frequency. The preparation of niosomes was conducted with specified quantities of cholesterol, dicetyl phosphate and non-ionic surfactant. The EEf of niosomes was determined via centrifugation method and was found to be in the range of 83.66 ± 0.08 to 87.12 ± 0.05. The in vitro release, with best fit model of Korsmeyer-Peppas suggested the drug release mechanism of Fickian diffusion and showed prolonged release of drug for a period of about 8 h while the effect lasted only for 2–4 h with free drug solution. This sustained release was also shown the drug concentrations above therapeutic level and the limited presence of drug at non-targeted areas, and thus reducing unwanted side effects and enhanced patient compliance as the dosing frequency will be reduced. Finally, the niosomes were exhibited superior stability results by preventing degradation/release of the drug entrapped into the vesicles up to a period of 3 months at refrigeration temperature [51]. Similar, study was also conducted with metformin, where metformin hydrochloride (MH)-loaded niosomes were prepared adopting reversed-phase evaporation technique using Span 40 and cholesterol. The mean particle size of the MH-loaded niosomes was between 223.5 nm and 384.6 nm. From the pharmacokinetic study of MH-loaded niosomal preparation on Wistar rat groups, the intensity of hypoglycemic effect was increased and prolonged significantly as compared to free MH-solution. With niosomal preparation, the effect of drug was prolonged to 6–8 h where MH-loaded positively charged niosomes were able to sustain the drug release rate and could be contributed towards the hydrophobic barrier of lipid bilayer, which caused the drug to be released slowly. The mucoadhesive properties between negatively charged mucosal surface and positively charged niosomes also contribute to the MH absorption for longer periods. These liposomal deliveries for metformin have successfully contributed towards advantageous drug delivery system in achieving significant hypoglycemic effects, reduction in patient’s dosing frequency, as well as minimising the unwanted side effects of the drug [52]. Another OHA, repaglinide (RPG) was incorporated in niosomal delivery system, where RPG-loaded niosomes were prepared using span 60 and cholesterol with particle size in range of 144 nm–497 nm and EEf between 54–88%. The application of factorial design revealed that the concentration of span 60 and cholesterol significantly affect the dependent variables, particle size, and EEf. SEM investigations indicated the spherical shape of the niosomes and formation of vesicle whereas, FTIR spectroscopic analysis and DSC thermal analysis proved the drug and polymer compatibility. Results of in vivo pharmacodynamic study on male Wistar rats revealed significant decrease in blood glucose level involving RPG-loaded niosomes as compared to the conventional dosage form. Moreover, niosomal preparation also has the potential in order to maintain RPG therapeutic level for a longer period of time as reflected by constant decrease in blood glucose level for 8 h whereas, conventional dosage form resulted in decreased in glucose level initially followed by increased after 4 h. The sustained release effect can be possibly explained due to the fact that niosomes act as a substantial carrier through the epithelium into deeper mucosal layers where the encapsulated drug is slowly released [53]. Thus it can be inferred that potential nanocarrier like niosomes are promising delivery tool which can be used efficiently for enhancing bioavailability and reducing dosing frequency by achieving sustained control of hyperglycemic condition over a prolonged period of time.

2.3. Polymeric nanoparticle based anti-diabetic drug delivery system

National Nanotechnology Initiative has defined NPs as the structure with the sizes varies from one to one hundred nanometres in at least one dimension [54,55]. NP has widely been used in pharmaceutical, medical and biological field to deliver polypeptides, drugs, nucleic acids, proteins, genes, vaccines and so on [54,56–61]. Most importantly, they can reduce the risk of adverse events and enhance the drug utility [54,56–59]. Recently, researchers are more focusing on the modification of the NPs in order to advance it use in modern therapy [56–58]. For instance, the incorporation of surface ligand to the NPs can modify targeting potential towards the targeted site, may be with altered and favorable pharmacokinetic properties [56,57]. Indeed, it is not suspicious that the NP can be used as a perspective carrier for ant-diabetic drugs (Table 3).
Table 3 – Overview of nanoparticle-based drug delivery system for anti-diabetic therapy.

<table>
<thead>
<tr>
<th>Type of Carrier</th>
<th>Type of DM (1/2)</th>
<th>Polymer</th>
<th>Cell Line</th>
<th>Size Range</th>
<th>Drug(s) incorporated</th>
<th>Outcome</th>
<th>Source</th>
</tr>
</thead>
</table>
| Mucoadhesive NPs            | T1/T2DM          | Mucoadhesive NPs loaded with LMWP-linked insulin conjugates. | Caco-2/HT29-MTX Co-Cultured Cell Monolayers/Diabetic rats | 253.8 ± 6.4 nm | Insulin              | 1. An extended hypoglycaemia effect with a rapid onset of action was observed in experimentally induced diabetic rats with a bioavailability of being 17.98 ± 5.61%  
2. The developed NPs found to be advantageous in terms of its improved retention within the brass border of the intestinal wall, whereas decrease of degradation of conjugated insulin (by the proteolytic enzymes in the GI tract) released from the NPs during absorption from epithelia | [62]   |
| Dissociable “mucus-inert” agent coated trimethyl chitosan NPs | T1/T2DM          | The NPs composed of TMC-based insulin loaded polyelectrolyte complex core, and a dissociable coating of N-(2-hydroxypropyl) methacrylamide polymer (pHPMA) derivative | Diabetic rat and Mucus-secreting E12 cell line (which has been isolated from human colorectal adenocarcinoma HT29 cell line to mimic human epithelium) | 163.1 nm | Insulin | 1. pHPMA coated NPs exhibited a prominent hypoglycaemic response in diabetic rats following oral administration  
2. Incorporation of mucus inert agent in the NP formulation showed improved permeation of insulin by overcoming the multiple absorption barriers of mucosa tissue | [63]   |
<table>
<thead>
<tr>
<th>NPs</th>
<th>T1/T2DM</th>
<th>TPGS-emulsified PEG-capped PLGA NPs (ISTPPLG NPs)</th>
<th>Diabetic rats</th>
<th>180 ± 20 nm</th>
<th>Insulin</th>
</tr>
</thead>
</table>

1. ISTPPLG NPs were shown to enhance the loading efficiency of insulin into the NP with protection against enzyme-induced aggregation and degradation in SGF in vitro.

2. ISTPPLG6 NPs has shown the maximum encapsulation efficiency of 78.6% ± 1.2% and significantly modulate the increased serum glucose level in the experimentally induced diabetic rats for a period of 12 h.

3. Histopathological results showed that ISTPPLG NPs could help in restoration of the injury triggered by the action of streptozotocin in pancreas, liver and kidney of diabetic rats when compared to normal control, indicating its regenerative effects and biocompatibility.

(continued on next page)
<table>
<thead>
<tr>
<th>Type of Carrier</th>
<th>Type of DM (1/2)</th>
<th>Polymer</th>
<th>Cell Line</th>
<th>Size Range</th>
<th>Drug(s) incorporated</th>
<th>Outcome</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPs</td>
<td>T1/T2DM</td>
<td>Insulin loaded poly(ethylene glycol) capped poly(lactic-co-glycolic)acid NPs (ISPPLG NPs)</td>
<td>Diabetic rats</td>
<td>ISPPLG4 NPs- 140 nm</td>
<td>Insulin</td>
<td>1. Two different ISPPLG NPs exhibit a pronounced hypoglycemic effect with a reduced triacylglycerol, lipid peroxidation and cholesterol with a concomitant increase in HDL cholesterol level</td>
<td>[115]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ISPPLG2 NPs- 170 nm</td>
<td></td>
<td>2. Exhibits enhanced effect of glutathione peroxidase (GPx), Glutathione (GSH) and SUperoxide dismutase (SOD) and decrease in catalase (CAT) activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>90/10 (PLG2) and 78/22 (PLG4)</td>
<td></td>
<td>3. Enhanced expression of liver glycogen was observed in rats administered with ISPPLG NPs</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4. Histopathological examination revealed decreased intensity of degeneration along with decreased distention of sinusoids following treatment of ISPPLG NPs</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5. Capable of restoring the damages caused in the tissues of diabetic rats</td>
<td></td>
</tr>
<tr>
<td>SLN</td>
<td>T1/T2DM</td>
<td>Cetyl palmitate-based SLN</td>
<td>Diabetic rats</td>
<td>350 nm</td>
<td>Insulin</td>
<td>1. Following oral ingestion of insulin loaded SLN, the plasma glucose level of rats measured was lower compared to those with the administration of insulin solution and empty SLN</td>
<td>[69]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2. Effectiveness of the developed SLN was quite similar to that of the mucoclohesive NPs as discussed earlier in terms of its preventive action from degrading nature of the proteolytic enzymes in the GI fluid, and enhances the intestinal absorption of insulin</td>
<td></td>
</tr>
<tr>
<td>NPs</td>
<td>T1DM</td>
<td>Chitosan–sodium lauryl sulfate (SLS) NPs</td>
<td>Diabetic rat</td>
<td>253 nm</td>
<td>Insulin</td>
<td>1. This approach of oral insulin delivery was found to be successful where excess quantities of SLS and chitosan were interacted to get NPs within the size range of 250 nm. It serves as protective media from acidic environment of stomach to deliver insulin, whereas, in the SGF environment it found to be aggregated. 2. A modest activity of the orally absorbed insulin was observed as compared to the invasive SC delivery of insulin</td>
<td></td>
</tr>
</tbody>
</table>
| SLNs’ | T1/T2DM | Repaglinide-loaded SLNs prepared with various surfactants | – | 169 ± 11 nm | Repaglinide | 1. The SLNs’ physicochemical properties were affected by different types of surfactant. 2. The mixture of phosphatidylcholine/Pluronic F127 was incorporated in the current experiment for being the paramount surfactant/stabilizer (continued on next page)
Table 3 – (continued)

<table>
<thead>
<tr>
<th>Type of Carrier</th>
<th>Type of DM (1/2)</th>
<th>Polymer</th>
<th>Cell Line</th>
<th>Size Range</th>
<th>Drug(s) incorporated</th>
<th>Outcome</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPs</td>
<td>T2DM</td>
<td>Chitosan- or alginate-coated NPs in the form of nano-network gel</td>
<td>Diabetic Mice</td>
<td>NPs were 340 nm and 293 nm in size</td>
<td>Recombinant insulin</td>
<td>1. The network gel within nanometric range was formulated due to the generation of cohesive strength generated during withdrawal of external force, which permits convenient molding and injection. 2. A 3.6-fold improvement of the insulin release from the nanonetwork was observed as triggered by the increased glucose level in hyperglycemic stage. Thus, the nanonetwork can be considered as a smart device that release insulin in need in order to normalize the higher serum glucose, whereas, during normal glucose level it will stop release of further insulin. 3. This designed formulation could provide control for both self-regulated and long-term diabetes</td>
<td>[70]</td>
</tr>
</tbody>
</table>
Effective oral delivery of insulin by NP-based carrier systems is preferred by the society due to their convenience of administration and good patient compliance compared to the available parenteral preparations. Unluckily, the bioavailability of insulin through NP following oral administration is low as it is poorly absorbed in the GI tract due to the leakage of encapsulated insulin from NPs as well as their low permeability. Because of that, the insulin will then be degraded by the proteases present in the GI tract. To enhance the bioavailability of oral insulin NP delivery, insulin is conjugated with peptide that allows cell penetration and then encapsulated in mucoadhesive NPs. Through these approach the reservation of insulin into the NPs in intestinal mucus layer can be enhanced whereas the conjugated insulin freed from the NPs would not be deteriorated by the enzymatic degradations because of the short distance for the drug to reach the absorption site as well as the conjugates have high permeability through epithelia [62].

In a study by Sheng et al., insulin was conjugated with a cell-penetrating peptide-protamine, followed by encapsulation in the mucoadhesive poly(lactic-co-glycolic acid) (PLGA) NPs coated with N-trimethyl chitosan chloride. Based on the results obtained, a faster onset with long-lasting hypoglycaemia effect was shown in diabetic rats. A bioavailability of this designed oral delivery system of insulin was being 17.98 ± 5.61% compared to subcutaneous insulin injection. Interestingly, a significant improvement in this oral delivery system of insulin with improved bioavailability in experimental animals indicating better internalization of the mucoadhesive NPs by the cells as compared to the native insulin. Therefore, the conjugation of insulin with cell-penetrating peptides, and followed by encapsulation in mucoadhesive NPs could be an useful carrier for the oral delivery of insulin [62].

In another study, TMC NPs of insulin were then coated with N-(2-hydroxypropyl) methacrylamide copolymer (pHPMA) with the assumption to promote the permeation of NPs through mucus. The study showed the average size of the NPs was 163.1 nm after being coated with pHPMA (P-T-NPs). To determine the efficacy on mucus layer permeation of P-T-NPs, E12 cell line was used to imitate function of the epithelium. P-T-NPs were mostly found to be distributed beneath the mucus and lesser aggregations as compared to TMC-based NPs without pHPMA coating (T-NPs) and thus be able to conclude that P-T-NPs were more efficient to penetrate the mucus layer and gain direct access to epithelial cells. Consequently, P-T-NPs had higher apparent permeability coefficient (Papp) value than both, free insulin and T-NPs, which further support the statement permeability opinion of P-T-NPs. Subsequently, the hypoglycaemic effect of P-T-NPs on diabetic rats shown the most outstanding, about 36% decrease of maximal blood glucose level at 4 h without any risk of hypoglycaemia development [63].

Contrary, PLGA NPs are also known to capable of encapsulating and releasing drugs in a sustained-release rate as well as having the advantages of biodegradability, biocompatibility and being transmucosal carrier. However, the limitation of PLGA NPs was shown to be selective when interacting with surfaces of mucus layers. Furthermore, PEG molecules were found to be able to enhance the stability of NPs and their ability to transport across different mucosal surfaces. Most interestingly, the benefits of using D-α-tocopherol poly(ethylene glycol) 1000 succinate (TPGS) are their great efficiency of encapsulation and emulsification [64–66]. Consequently, the use of insulin-loaded TPGS-emulsified PEG-capped PLGA NPs (ISTPPLG6 NPs) as sustained-release oral delivery system of insulin was investigated with the aim to solve the limitations commonly encountered by orally administered insulin. In a reported study by Malathi et al., low-molecular-weight PLGA copolymers (70/30 [PLG6] and 80/20 [PLG4]) were synthesized and used in the production of ISTPPLG NPs of 180 ± 20 nm size. Results suggested that ISTPPLG NPs were able to enhance the loading efficiency of insulin and also prevent enzymatic degeneration and aggregation in stimulated gastrointestinal fluids in vitro. Surprisingly, the authors reported a 3-fold decrease of glucose level as compared to the free insulin-treated groups, and also exhibited the hypoglycaemic control over a period of 24 h. Additionally, the effect of ISTPPLG NPs reported a significant reduction in the ALT, urea, cholesterol and creatinine levels with the ability to induce restoration of damage in the pancreas, kidney and liver of diabetic rats caused by streptozotocin, indicating its regenerative effects and biocompatibility [67].

Another approach on ISPPLG NP formulation were expressed by using two different copolymers of PLGA, PLG2 (90% lactic acid and 10% glycolic acid) and PLG4 (78% lactic acid and 22% glycolic acid), forming ISPPLG2 and ISPPLG4 NPs comprising of size of about 140 nm and 170 nm, respectively. Surprisingly, these two subcutaneous ISPPLG NPs were able to exhibit hypoglycaemic effect with a reduced triacylglycerol, lipid peroxidation and cholesterol with a concomitant increase in HDL cholesterol level and enhanced liver expression of glycogen in diabetic rats. Antioxidant potential of the developed ISPPLG NPs, as evidenced by increase in GPx, GSH and SOD level and a decrease in CAT level, might be interconnected with the antioxidant property of vitamin E, a component of TPGS [68].

Solid lipid nanoparticles (SLNs) are submicron colloidal carriers within the size range between 50 and 1000 nm, consists of solid lipids dispersed either in plain water or in an aqueous solution of surfactant. It has been reported that SLN showed a great advantage of being drug delivery system such as improved bioavailability, prolong blood residence time and good tolerability. Consequently, insulin-loaded SLN are synthesized, which was then coated using cetyl palmitate. By comparing with insulin-loaded and unloaded SLN, the zeta potential and spherical particle size were found negatively charged and to be around 350 nm respectively. The submicron colloidal carriers were found negatively charged and incorporate insulin, to enableabsorption from M-cells on Peyer’s patches of GI tract. On evaluation, insulin association efficiency (AE) in SLN was reported over 43%, suggesting a lower AE of insulin-loaded SLN was expected for being a hydrophilic molecule. It is evident from the results that the solid matrix of SLN not only enhances the intestinal absorption of insulin but also partially provides protection for insulin from the proteolytic degeneration within the GI tract and controls the hyperglycaemic condition in diabetic animals [69].

Interestingly, one study regarding artificial “closed-loop” system was developed by using a mix of NPs, consisting of
Dendrimers are nano-sized, polymeric globular hyper-
delivery where the soft ferromagnetic property helps in
long-term and self-regulated diabetes control. Recently,
potential strategy of drug delivery for both management of
carrier for desire molecules [72,76,80–84]. The size and func-
tional end groups of dendrimers can be modified in order to
change their hydrophilicity, effective diameter and molecular
weight [72,73,85] (Table 4).

It has been well reported that PAMAM G4 mimics hypogly-
caea actions through reduction of higher plasma glucose
level and long-term markers of hyperglycemia in diabetic ani-
mal model [86]. Zheng et al. had conducted in vivo pulmonary
absorption studies of insulin and calcitonin to systemic circu-
lization using rats to determine the absorption enhancing
effects of different generations (G0 to G3) as well as 0.1–1.0%
(w/v) concentrations of PAMAMs [87]. The data revealed that
PAMAMs had significantly elevated the pulmonary absorption
of the entrapped components, thus increased the systemic
concentration of calcitonin and insulin. The experimental
results further indicate that the absorption enhancing effects
are generation dependent. PAMAM G3 has the greatest
absorption enhancement effect, followed by G2, subsequently
G1 and finally G0 with the least effect [87]. Meanwhile,
the absorption enhancing effects for the same generation of
PAMAMs was appeared to be concentration-dependent [87].
In bronchoalveolar lavage fluid, the protein release and activ-
ities of lactate dehydrogenase (LDH) were used to evaluate the
toxicity of PAMAMs with different concentrations and gener-
ations in lung tissues [87]. No significant increase in protein
production and LDH activities indicated the membrane of
lung tissues were not damaged by dendrimers [87].

In an alternative study, Labieniec et al. determined the
ability of PAMAM G4 in scavenging excessive glucose and tar-
geting modulation of declined metabolism of carbohydrate in
experimental animals [88]. However, PAMAM G4 was known
to be cytotoxic for almost a decade thus, the authors have
conducted a study to investigate the degree of cytotoxic effect
evaluated whether this risk would outweigh the benefits of
PAMAM G4 on ameliorating the deteriorative implications of
oxidative stress, carbonyl stress and hyperglycemia which
occurs in chronic untreated experimental diabetes. Authors
observed that diabetic animals administered with a vehicle
were suffered from enormously elevated plasma glucose level
whereas administration of PAMAM G4 manage to reduce the
plasma glucose level moderately, though it was still two times
higher than the healthy control. Their study also demon-
strated that the values of other major parameters which
includes glycated haemoglobin (HbA1c), AOPP, AGEs and
aminotransferases were normalized by PAMAM G4 to a nor-
mal physiological range. Hence, lowering blood glucose level
by PAMAM G4 dendrimer may play a crucial role to the
reduced of protein glycation and oxidation. In summary, pul-
monary delivery of PAMAM G4 can suppress long-term mark-
ers of poor metabolic control and normalise the plasma
glucose. However, there is still a drawback of increasing mor-
tality of STZ-induced diabetes rats due to PAMAM G4 cyto-
oxic effects [88].

Unique molecular architectures of dendrimers have made
them an essential component in gene delivery. Exendin-4, a
recently FDA approved glucagon-like protein-1 receptor ago-
nist, is administered intravenously to improve glucoregula-
tory effects via increasing secretion of insulin, while
regulates food intake, gastric emptying and glucagon secre-
tion, without producing any systemic toxicity. The effective-
ness of anti-diabetic treatment through exendin-4
expressing chimeric plasmid was investigated by Pyung-

encapsulated glucose-specific enzyme and recombinant insu-
lin in dextran NPs, coated with oppositely charged chitosan
(positively charged) and alginate (negatively charged) polysac-
charides. Through the mix of oppositely charged NPs, a gel-
like nano-network was able to form through electrostatic forces.
NPs with chitosan coating had average size of 340
nm whereas NPs with alginate coating had average size of
293 nm. After formation of insulin-loaded nano-network
(NNS), it was then injected into diabetic mice. A strong cohe-
sive property was formed within the nano-network and it was
able to allow convenient molding and injection of the drug.
Furthermore, the nano-network drug delivery system responded to change of glucose level significantly in the body,
whereas there was an increase of 3.6-fold in release rate of
insulin from the NPs when the level of blood glucose was on
hyperglycemic state. While the rate of release of insulin at
hyperglycemic state was gradually increased to a maxi-
mum level and declined after a certain period of time. Thus,
the authors stated a that the release of insulin through
nano-network drug delivery system was facilitated through the
level of blood glucose, which high blood glucose level
stimulates the release of insulin from the nano-network gel
while low blood glucose level inhibits the release of insulin from the gel, mimicking the hemostatic mechanism of insulin
release from the β-cells of islets of Langerhans [70].

Thus, NPs could provide a comprehensive platform to improve the delivery of both, peptide based and organic hypo-
glycaemic agentsin the control of high plasma glucose level for a prolonged time during diabetes, minimising the fre-
cquency of ingestion. Although various factors are involved in the improvement process, thus close monitoring of such
parameters including particle size, polymeric coat, surface
charge, characteristics of the polymer, etc. could deliver a
potential strategy of drug delivery for both management of
long-term and self-regulated diabetes control. Recently,
magnetic core has been introduced into the nanoparticulate
delivery where the soft ferromagnetic property helps in delivery of the nanocarriers particularly to the site of action to avoid the systemic adverse events of the drugs [71].
Thus, it is obvious that polymeric nanoparticulate delivery system can be incorporated in the delivery system of for
successful systemic delivery of anti-hyperglycemic agents,
although such researches need extension towards clinical
settings.

2.4. Poly(amidoamine) dendrimer based drug delivery
system for anti-diabetic drugs delivery

Dendrimers are nano-sized, polymeric globular hyper-
 branched macromolecules with tree-like morphology in 3D
nanostructure which comprises of a central core and
branched monomers with different reactive end groups on
the surface [72–76]. The family of dendrimers consists of poly(amidoamine) (PAMAM), poly(propyleneimine) (PPI), liquid
crystalline (LC), core shell (tecto), peptide, glycol and hybrid
dendrimers [77–79]. Their unique chemical and physical
properties are the main contributing factors to the widely usage in
pharmaceutical and biochemical applications as an effective
carrier for desire molecules [72,76,80–84]. The size and func-
tional end groups of dendrimers can be modified in order to
<table>
<thead>
<tr>
<th>Type of Carrier</th>
<th>Type of DM (1/2)</th>
<th>Polymer</th>
<th>Size range</th>
<th>Drug(s) incorporated</th>
<th>Outcome</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAMAM dendrimer (G2, G3, G4)</td>
<td>Streptozocin induced diabetes</td>
<td>–</td>
<td>–</td>
<td>Human pancreatic insulin</td>
<td>Protein ratio and generation of PAMAM dendrimer have strong effect on insulin fibrillation</td>
<td>[80]</td>
</tr>
<tr>
<td>Cationic PAMAM-NH₂ dendrimers, neutral PAMAM-OH dendrimers and anionic PAMAM-COOH (G3, G4)</td>
<td>–</td>
<td>Amidoamine</td>
<td>–</td>
<td>Bovine pancreatic insulin</td>
<td>1. Dissolve prion protein and amyloid fibrils aggregates 2. Only cationic dendrimers decreased the insulin aggregation 3. Small concentrations of dendrimer prevent or decrease the formation of misfolded structures of protein</td>
<td>[91]</td>
</tr>
<tr>
<td>Arginine-grafted poly (cystaminebisacrylamidediaminohexane) (ABP)-conjugated poly (amido amine) (PAMAM) dendrimer (PAM-ABP)</td>
<td>T2DM</td>
<td>Arginine-grafted bioreducible poly (disulfide amine) (ABP) cationic polymer and PAM-ABP dendritic polymer</td>
<td>Polyplex has the size below 80 nm</td>
<td>Bovine insulin</td>
<td>1. Improved glucoregulatory effects, as well as increased insulin secretion</td>
<td>[89]</td>
</tr>
<tr>
<td>PAMAM dendrimer, (G4)</td>
<td>Streptozocin induced diabetes</td>
<td>Ethlenediamine core</td>
<td>–</td>
<td>–</td>
<td>Both intraperitoneal and subcutaneous routes were equally effective in suppressing the long-term markers of severe hyperglycemia. But subcutaneous routes have less adverse effects in the treated animals</td>
<td>[86]</td>
</tr>
<tr>
<td>PAMAM Dendrimer (G0, G1, G2, G3)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Insulin and calcitonin</td>
<td>1. In an in vivo study in rats, pulmonary absorption of calcitonin and insulin were significantly increased where the absorption-enhancing effects are generation dependent (G3 &gt; G2 &gt; G1 &gt; G0) 2. The developed formulation were safe in experimental animals</td>
<td>[87]</td>
</tr>
<tr>
<td>PAMAM dendrimer (G4)</td>
<td>Streptozocin induced diabetes</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Adequate number of surface amino groups should be present in PAMAM dendrimer to decrease cytotoxicity exerted by polycationic group as well as to reduce non-enzymatic modification of biomacroolecules</td>
<td>[88]</td>
</tr>
</tbody>
</table>
Hwan Kim et al. using two-step transcription amplification plasmid system with arginine-grafted poly (cystaminebisacrylamidediaminohexane) (ABP)-conjugated PAMAM dendrimer (PAM-ABP) to improve incretin-based gene therapy. PAM-ABP/chimeric DNA polyplex increased ectopic cells expression of exendin-4, thereby, up-regulation of exendin-4 induce increased cAMP levels in the β-cells of the pancreas resulting in increased active protein kinase K which finally elevate insulin production. Hence, dendrimeric delivery of exendin-4 system provides a feasible anti-diabetic agent for better incretin gene therapy in the future [89].

Apart from the delivery approaches of hypoglycaemics, several approaches have been made to check the effect of dendrimers on the structural integrity of peptides. In this context it is important to mention that insulin can be structured as hexamer, dimer or monomer, whereas the monomer is the active form to produce its biological activity. This insulin undergoes aggregation, misfolding and fibrillation after repeated administration, may be due to the changes in environmental condition, agitation of the formulation, concentration of protein, temperature and ionic strength, thus the stability of insulin is affected [90]. The thermostability of human insulin is investigated using DSC and the results revealed that 60 °C and 82 °C appeared to be the two phase-transitions for insulin. However, after dendrimers were added at 0.6 μmol/L, the first peaks disappeared and there was an elevation of the second peaks. Hence, Nowacka et al. hypothesized that addition of dendrimers will form hexamers in the solution due to ionic interaction between the active surface group of dendrimers and ions of the peptide chain. Further, the effect of PAMAMs on insulin fibrillation is highly dependent on the generation of dendrimers and dendrimer:protein ratio [80]. In addition, the authors also revealed that the zeta potential of insulin with negative value changed to positive with the co-administration of PAMAM dendrimers due to such ionic interaction [90]. To further study regarding effect of dendrimers on the aggregation of insulin, dithiotreitol (DTT) had been used to induce insulin denaturation by reducing disulfide bridges and causing destabilization of protein structure. It was revealed that small concentration of higher generations PAMAM dendrimers could inhibit the aggregation process, prevent or decrease the formation of misfolded structure of protein as well as disrupt the existing fibrils [91]. In a similar target to deliver insulin using the PAMAM dendrimer tool where the authors observed that the secondary structure of the insulin molecule was not altered when incorporated into the delivery tool. Further studies demonstrated that there was no alteration of circular dichroism spectrum space, although the delivery tool could not found to be effective in the inhibition of aggregation induced by DTT [90].

Labieniec et al. conducted an experiment to evaluate the efficiency of PAMAMs G2 and G4 in non-enzymatic modifications of primary amino groups in polyamine compounds and BSA. Authors clearly demonstrated that PAMAMs at the used concentrations neither interacted with BSA nor affected the protein conformation. In addition, PAMAM dendrimers do not form firm complexes with the proteins while non-glycated poly(L-lysine) significantly form, as evidenced by the decreased fluorescence of BSA [92].
Table 5 – Overview of micelle-based drug delivery system for anti-diabetic therapy.

<table>
<thead>
<tr>
<th>Type of Carrier</th>
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<th>Drug(s) incorporated</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Micelles</td>
<td>T1/T2DM</td>
<td>Pluronic® F68, Pluronic® F108, Pluronic® F127, Soluplus®</td>
<td>A549, Calu-3, THP-1, Raw 246.7, and U937</td>
<td>&lt;300 nm</td>
<td>Lyophilized human insulin with potency ≥27.5 U/mg</td>
<td>Good deposition and compatibility into the lungs has been observed with powder delivery system containing aerodynamic diameter of &lt;6 μm and they fail to present significant in vitro toxicity for respiratory cell lines</td>
<td>[105]</td>
</tr>
<tr>
<td>Micelles</td>
<td>T2DM</td>
<td>PEG-PE micelle</td>
<td>BALB/c male mice</td>
<td>9 nm radius sphere</td>
<td>Lyophilized porcine insulin</td>
<td>The formed micelles are found to be advantageous in prevention of aggregation of insulin. This action is achieved by preventing DTT from doing this and also by obstructing the hydrophobic interaction between A and B chain of insulin</td>
<td>[103]</td>
</tr>
<tr>
<td>NPs and Micelles</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Insulin</td>
<td>Insulin was released rapidly from self-assembled polymeric micelle</td>
<td>[104]</td>
</tr>
<tr>
<td>Self-assembled polymeric micelles</td>
<td>–</td>
<td>PEG-b-PDPA block copolymers</td>
<td>–</td>
<td>–</td>
<td>Co-loaded with insulin and glucose oxidase</td>
<td>The complex micelles displayed glucose responsiveness under of hyperglycemic condition, indicating that in response to physiological glucose level, the complex micelle had a potential role for self-regulated insulin delivery</td>
<td>[102]</td>
</tr>
<tr>
<td>Micelles</td>
<td>T1/T2DM</td>
<td>PEG-b-P(Asp-co-AspFB) and a P (Asp-co-AAG)</td>
<td>–</td>
<td>135.1 nm</td>
<td>Insulin</td>
<td>The complex micelles displayed glucose responsiveness under of hyperglycemic condition, indicating that in response to physiological glucose level, the complex micelle had a potential role for self-regulated insulin delivery</td>
<td>[101]</td>
</tr>
</tbody>
</table>
Polymeric micelles are formed by self-assembled amphiphilic copolymers via aggregation into a core–shell micellar structure when its concentration reached the critical micellar concentration \([93–99]\). The outer hydrophilic layer forms the shell to provide protection and functional groups for further micelle modification. Meanwhile, the hydrophobic moiety forms the core of micelles where hydrophobic drugs can be loaded \([95–98,100]\). Nowadays, most of the drug delivery fields have been widely applying self-assembled polymeric micelles from amphiphilic polymers due to their enhanced pharmacokinetics, bio-distributions as well as preventing protein degradation by enzymes \([95–99,101,102]\) (Table 5).

As we have discussed earlier, instability of pharmaceutical formulations is closely associated with the aggregation of insulin. Various modern techniques, viz. circular dichroism, turbidity assay, bis-ANS binding assay, MALDI-TOF MS, thioflavin-T (ThT) binding assay and agarose gel electrophoresis have been adopted by Fang et al. in their study, where the authors determine the reconversion efficacy to the native configuration on DTT-denatured insulin by the PEG-PE micelle. The results revealed that PEG-PE micelle have a negative charged-layered hydrophilic nano-cage-like structure which is able to prevent aggregation by capturing insulin A and B chains induced by DDT and interfering hydrophobic interaction. The reduced insulin A and B chain in the nanocage are also able to recognize each other and 30% of native insulin is formed as measured by hypoglycemic activity analysis in mice. Finally the authors concluded that PEG-PE micelle is a potential artificial chaperone for in vivo and in vitro protein refolding \([103]\). Further, a stable micelle system can also be formed by addition of cross-linkable hydrophilic groups with the hydrophobic polymer. An example of micelle complex is the formation of an amphiphilic block copolymer by conjugation of multifunctional PEG with biodegradable hydrophobic polymers. The branches of PEG create a structure which is cross linkable in the micelle system. Other than that, the formation of phenylboronic acid-containing block copolymer and a glycopolymer complex resulted in formation of glucose-responsive micelles. The micelle complex with PEG shell makes it stable against aggregation, respond faster to the change of glucose at the physiological pH and more sensitive to glucose level \([104]\).

On the other hand, micelles with stimuli responsive functional units on the surface can work as cargo which only responds to specific stimulus signal. Through the smart-cargo-release behavior approach, the efficiency of therapy could be increased whereas the side effects can be reduced. Li et al., had designed a self-assembled PEG-block-poly(2-diisopropylaminoethyl methacrylate) (PEG-b-PDPA) block copolymer to form glucose-responsive micelles. With the concept of glucose oxidase catalyzed glucose degradation, the insulin and glucose oxidase were co-loaded with the nanomicelles. From the developed formulation, insulin was released rapidly due to the glucose variation in the microenvironment. The tertiary amine groups in PDEA blocks were protonated and caused the expansion of hydrophobic PDPA core which will eventually speed up the release of cargo from the carrier explained the mechanism of rapid cargo release induced by pH. Overall, these micelles may be considered to be applied for the delivery of insulin \([102]\).

It has been reported by Andrade et al. that amphiphilic polymers such as Pluronic®F68, Pluronic®F108, Pluronic®F127 and Soluplus® were extensively applied on the production of lyophilized formulations to deliver insulin via inhalation. The researcher has been widely studied phenylboronic acid (PBA)-based polymer in the management of diabetes patients due to its potential applications-responsive insulin delivery. Thus to utilise the effectiveness of PBA, a formulation was experimented, although affecting the in vitro release of insulin form micelles; thus, PBA was found not to confer any glucose-sensitive properties to formulations. Conversely, results have shown that powders for inhalation are more advantages delivery system compared to liquid formulations due to their higher long-term stability, improved patient compliance since these are only breath-actuated as well as absence of gas propellants. However, powders contain certain properties including density, size and shape of particles that would influence the particles’ aerolization and deposition characteristics. However, authors determined that the compatibility of powders with aerodynamic diameter <6 μm have good deposition in the lungs and the in vitro toxicity for respiratory cell lines was not significant. Overall, the formulations based on polymeric micelles have shown potential properties to develop insulin delivery by inhalation in the future \([105]\). Keeping in view, a novel drug delivery system using PBA-based glucose-responsive has been improved by decreasing the apparent pK\(_a\) for application in the physiological pH, which lead to the improvement of the rate of responsiveness for self-regulated drug release, enhancing its responsiveness towards uncontrolled blood glucose level. Thus, another glucose-responsive complex micelles were synthesized by self-assembly of a PBA-containing block copolymer PEG-b-(aspartic acid-co-aspartamidophenylboronic acid) (PEG-b-P(Asp-co-AspPBA)) and a PAsp-based glycopolymer (aspartic acid-co-aspartglucosamine) P(Asp-co-AGA) by Yang et al. \([101]\). Interestingly, the results in the PBA based complex micelles displayed response to the glucose level under physiological pH 7.4 with 2 gL\(^{-1}\) glucose which is the condition of hyperglycemia. This indicated that the external glucose concentration was the main contributing factor that decide the release of insulin from the complex micelles. Further, presence of PEG shell contributed towards advantage of stability against aggregation. Furthermore, the self-assembly of the two polymers with a hydrophilic P(Asp-co-AGA)/P(Asp-co-AspPBA) core enabled faster response to control higher circulating glucose at neutral pH. However, better glucose sensitivity was obtained by decreasing the apparent pK\(_a\) of the PBA/AGA complex. Most importantly, the poly(aspartic acid)-based polymers together with the glycosyl moieties that utilized in the system has ensured that the PBA-based complex
micelles to have a good biodegradability and biocompatibility. Shortly, this type of complex micelle is hope to be a potential candidate for self-regulated insulin delivery in the management of diabetes patients [101].

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Apart from these, there have many more studies been conducted to improve oral delivery of insulin, one of them involves insulin-loaded nanocomposite. The test carried out by using SLS and polyelectrolyte complex to form a micelle of insulin-chitosan complex. SLS micelles formed had an average NP size of 253 nm, and were then introduced into diabetic rats. Based on the results obtained, the author concluded that insulin-chitosan complex micelle was able to protect the insulin from being eliminated and also improve the activity of absorbed insulin through oral delivery when compared that of the subcutaneous delivery [106].

Finally, micelle based nanocarriers are also found to be a developing field of peptide delivery for the protection of molecular structure of the peptides, which could be project towards control of high glucose level in diabetic patients. These micelle could also found to be an important delivery to control the release of incorporated drug inside the microenvironment based on increased glucose level after food ingestion of food, as well as due to change in plasma physiological pH, thus it will mimic the role of β-cells of Langerhans of pancreas in the control of hyperglycemia.

3. Conclusions

Over the past decades, the emergence and potential of nanotechnology in drug delivery systems have been developed and explored by many researchers. The rapid development of nanotechnology inevitably provides alternative approaches to overcome limitations in existing conventional delivery of antidiabetic drugs, improving the care of patients with diabetes. Different nanocarriers based delivery systems has been discussed here, including liposomes, niosomes, NPs, dendrimers and micelles. Those nanocarriers have clearly shown higher efficiency in drug delivery with increased bioavailability, dose proportionality, decreased toxicity and reduced dosing frequency. Additionally, for protein based oral delivery nano-system has also been approached for successful transfer of drugs to the biological circulation escaping the degradability potential of the proteases in the GI environment. Further, the control release triggered by the increased glucose level in the plasma or consequent change in pH could simulate the action of pancreas in the control of hyperglycemic condition. As the management of diabetes is acknowledged with the continuous monitoring of blood glucose levels and insulin or other OHA administration, safety of nanotechnology for long term use in enhancing drug delivery is placed under investigation. Following this, a guidance was issued by FDA to strengthen the safety of development of clinical use nanotechnology-based products. All in all, in order to convert nanotechnologies from basic research into clinical products, it is extremely important to gather up the efforts amongst scientists in different disciplines. Experts from various disciplines required to cooperate closely in order to revolutionize innovation from novel laboratory into commercially viable medical products. Better cross training is believed to have higher possibility in yielding splendid proposals with a higher possibility of success. Consequently, the future of nanotechnology in drug delivery systems remains wide open.

Disclosures

There is no conflict of interest and disclosures associated with the manuscript.

References


