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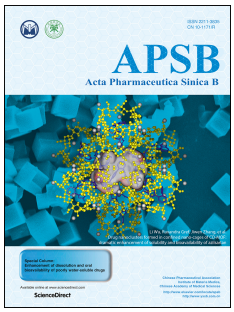

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REVIEW

Fighting type 2 diabetes: Formulation strategies for peptide-based therapeutics

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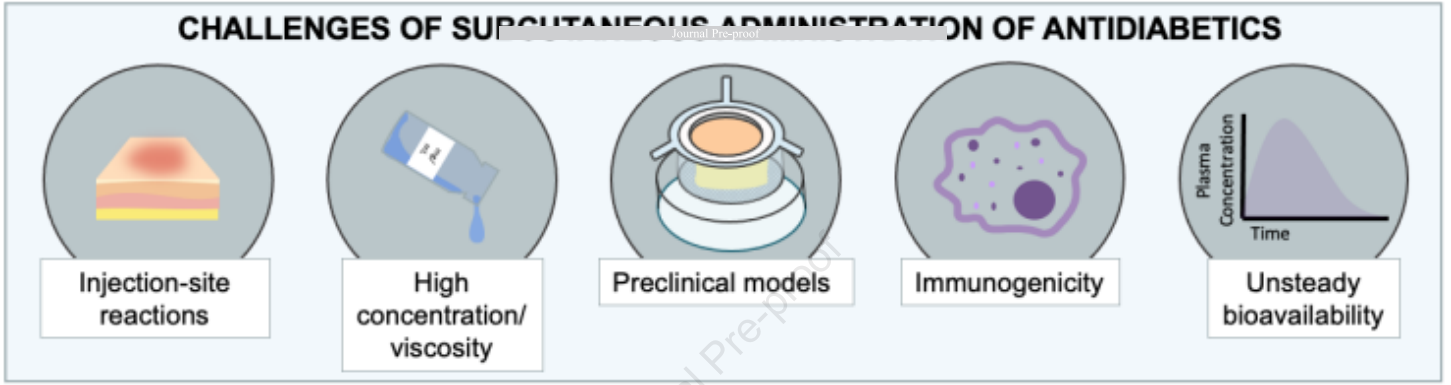
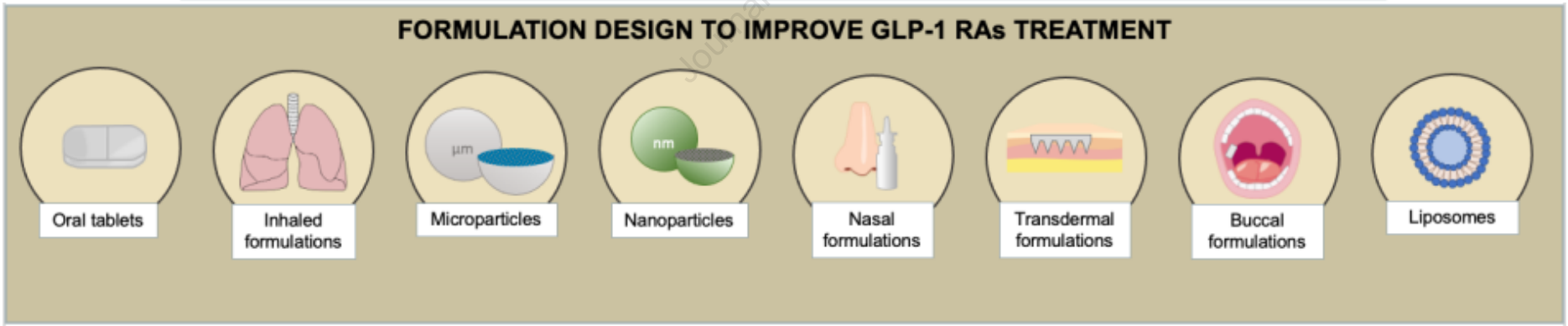
Running title: Formulation strategies for peptide-based antidiabetics





Abstract Diabetes mellitus is a major health problem with increasing prevalence at a global level. The discovery of insulin in the early 1900s represented a major breakthrough in diabetes management, with further milestones being subsequently achieved with the identification of glucagon-like peptide-1 (GLP-1) and the introduction of GLP-1 receptor agonists (GLP-1 RAs) in clinical practice. Moreover, the subcutaneous delivery of biotherapeutics is a well-established route of administration generally preferred over the intravenous route due to better patient compliance and prolonged drug absorption. However, current subcutaneous formulations of GLP-1 RAs present pharmacokinetic problems that lead to adverse reactions and treatment discontinuation. In this review, we discuss the current challenges of subcutaneous administration of peptide-based therapeutics and provide an overview of the formulations available for the different routes of administration with improved bioavailability and reduced frequency of administration.

KEY WORDS Type 2 diabetes mellitus; Glucagon-like peptide-1 receptor agonists; Exenatide; Subcutaneous administration; Amylin mimetics; Drug delivery systems; Biotherapeutics; Peptide delivery; Controlled-release formulations; Microparticles; Nanoparticles

1. Introduction

Subcutaneous delivery of biotherapeutics has attracted increasing attention across many disease areas and has shown to be effective, well-tolerated and generally preferred by patients and healthcare workers over the intravenous route[1]. The subcutaneous route has been indicated in providing an alternative for intravenous infusions, which involve invasive and time-consuming procedures that represent an economic burden for healthcare systems. Indeed, since the first subcutaneous formulations were approved by regulatory bodies, significant progress has been made towards developing therapies for several disease areas, including rheumatoid arthritis, multiple sclerosis and diabetes mellitus (DM)[2].

Particularly, a considerable number of these subcutaneous medications can be administered at home by patients or caregivers. Given the positive impact that home administration had on patient adherence to treatment and on reducing costs and resources, it became evident the benefits of switching from intravenous infusions to subcutaneous injections. However, significant developmental issues and knowledge

gaps remain that hamper the progression of subcutaneous biotherapeutic formulations[3]. In 2020, the Subcutaneous Drug Delivery and Development Consortium identified several major challenges that need to be addressed before the complete implementation of the subcutaneous route to deliver biotherapeutics. Among others, the need for technological advances to successfully deliver high-dose/volume formulations, the incomplete bioavailability of subcutaneous formulations and the concerns about the higher immunogenicity of the subcutaneous route compared to the intravenous route can be cited[4].

Patients subjected to chronic treatment regimens that require multiple administrations, such as diabetic patients, particularly benefit from the subcutaneous drug administration strategy. DM is a metabolic disorder characterized by pancreatic β-cell dysfunction and insulin resistance that has become a major global health problem[5]. In 2019, the International Diabetes Federation (IDF) estimated that nearly 500 million people have DM, and the number of cases is expected to increase rapidly in the upcoming years. DM not only causes premature mortality (DM was the direct cause of approximately 4 million deaths in 2019) and reduces patient's quality of life, but also represents a high economic burden for any health system; in 2019, DM investment reached USD 760 billion (capital expenditure increased by 4.5% since 2017), and the IDF expects it to continue growing [6]. Although major milestones have occurred in the development of alternative drug delivery systems, DM management can be improved further, especially for patients who have failed common DM therapies and need complex drug combinations.

Since the discovery of insulin in the early 1920s, the first peptide to be isolated and administered therapeutically, other protein-based drugs have been developed[7,8] (Fig. 1). Particularly, insulin paved the way for the employ of the subcutaneous route as an option for the administration of proteins. Although other routes (*e.g.*, pulmonary and oral) for the administration of peptides have been explored, for the time being, the subcutaneous route remains the most suitable route [8].

In 2005, exenatide (Byetta®), a glucagon-like peptide-1 receptor agonist (GLP-1 RA), was approved by the US Food and Drug Administration (FDA) for type 2 diabetes mellitus (T2DM) treatment[9]. GLP-1 RAs are administered in combination with other antidiabetic drugs [10]. Besides a proven efficacy in glycaemic control, additional benefits of GLP-1 RAs therapy include reduced cardiovascular risk, lower risk of hypoglycaemia and good tolerance by the patient[11]. However, due to the short half-life

of exenatide (2.4 h in humans)[12] multiple injections are required, with gastrointestinal adverse events and injection-site reactions being the most common adverse effects derived from this repetitive administration[13].

In the same year, another antidiabetic drug, pramlintide (Symlin®), a synthetic amylin analogue, was approved by the FDA as an adjunctive treatment for patients with T1DM and T2DM in which glycaemic control was not achieved with insulin therapy[14,15]. Amylin mimetics are a promising class of antidiabetic drugs that have also been shown to be useful for weight loss, especially in combination with other agents, such as leptin[16]. However, they are underused in clinical practice due to their poor pharmacokinetic properties[17]. Amylin plays an important role in the control of food intake, and the alteration of this control could be responsible for the increase in obesity rates, so the development of other amylin mimetics with improved potency and pharmacokinetics may be a promising approach for future treatment options[16].

Although much has been accomplished in the field of DM management, with five GLP-1 RAs and one amylin mimetic already commercialized for subcutaneous administration (Fig. 1)[13,14], it is still necessary to improve and develop novel formulations with longer circulation times to decrease the frequency of administration[18–20]. This review aims to provide an overview of the current situation and recent advances in the production of safe and effective systems for the subcutaneous administration of T2DM drugs (non-insulin), in particular, GLP-1 RAs. Additionally, ongoing research for new drug delivery systems and alternative routes for GLP-1 RAs administration will be discussed.

2. Subcutaneous administration of biotherapeutics: General considerations

As alluded, the development of novel peptide and protein formulations for subcutaneous delivery as an alternative to the conventional intravenous infusions is of primary importance to improve therapy effectiveness. The increment in the half-life of the drugs would reduce the frequency of injections, which together with the possibility of home administration would positively impact patient compliance with medication and, ultimately, could reduce healthcare costs.

Despite extensive clinical use of the subcutaneous route, the specific mechanism underlying the subcutaneous absorption of peptides and proteins remains to be fully understood. Many academic papers have described the anatomy and components of the subcutaneous tissue and how its particular characteristics can affect the absorption of

drugs administered by this route[21–24]. Briefly, the subcutaneous injection administers the formulation into the hypodermis—between the muscle and the dermis—which is composed of loose connective tissue (areolar tissue) and adipose tissue. This site of injection is also irrigated by blood and lymphatic capillaries, which play a fundamental role in the absorption of large peptides (>16 kDa) [1,25]. The extracellular matrix (ECM) is produced by fibroblasts and is mainly composed of collagen and elastin fibres and glycosaminoglycans[23]. Collagen and elastin are two structural proteins that determine the mechanical properties of the subcutaneous tissue, while glycosaminoglycans are responsible for the viscoelasticity of the ECM[26], controlling its hydraulic conductivity[21]. The difficulty for water to diffuse through the subcutaneous tissue prevents the rapid spread of the formulation from the administration site[21].

The limited proteolytic activity of the subcutaneous tissue makes it ideal for the administration of biotherapeutics. However, the unsteady bioavailability of proteins after subcutaneous administration is a major concern; whereas intravenous injections introduce the drug directly into the systemic circulation, a drug administered subcutaneously has to be absorbed from the subcutaneous tissue to the systemic circulation by a combination of vascular and lymphatic vessels[4]. In this sense, several factors can affect bioavailability. On the one hand, the person-to-person variability regarding subcutaneous tissue thickness, tissue pH, temperature, or hydrostatic and osmotic pressure within the tissue. On the other hand, the characteristics of the formulation (*e.g.*, volume and viscosity), the charge and hydrophobicity of the drug, and ECM binding interactions or excipient interactions could also affect the bioavailability of the protein or peptide[1,4,21,23]. Other factors, such as the patient's physical activity and the application of heat or massage at the injection site can also affect absorption[3,4]. Thus, several challenges should be addressed before obtaining an ideal subcutaneous formulation (Fig. 2).

The immunogenicity incidence of this route of administration is another concern that should be studied in depth. A more detailed understanding of the behaviour of the immune system in the subcutaneous tissue is needed, especially in the case of interactions between the immune system and the drug. In addition, more immunogenicity data for drugs used both subcutaneously and intravenously are required to address specific issues, such as the greater immunogenicity of subcutaneous than intravenous administration, which remain controversial[4].

Furthermore, there is a lack of appropriate *in vitro* and *in vivo* models to help better understand and predict the absorption and bioavailability of subcutaneously administered biotherapeutics. Although various *in vitro* and *ex vivo* models have been proposed, these exclude variables that should be considered, such as immune cell interactions[1,21,22]. *In vivo* animal models based on rodents, pigs or monkeys are usually employed during the preclinical development of peptide and protein therapies. However, no animal model could perfectly reproduce human characteristics and the collected data must be handled carefully[1]. The absence of data prior to clinical trials adds uncertainty and if during pharmacokinetic studies the bioavailability results do not turn out as expected, the project is terminated, resulting in a waste of resources[4].

The administration of formulations with high protein loads is another major challenge in the field of subcutaneous administration. Some biotherapeutics, such as monoclonal antibodies, require high doses, leading to a choice between developing high-volume or high-viscosity formulations [21]. The maximum volume for subcutaneous injections is approximately 1 to 2.5 mL. Larger volumes are associated with injection pain, injection site adverse effects (inflammation, bleb formation, or induration), and injection site leakage[4,21,25]. Furthermore, high protein concentrations are commonly associated with high viscosities, difficulties with protein solubility, protein aggregation, and decreased protein stability[27]. Injection back pressure is also increased in large subcutaneous injection volumes and high viscosity formulations, increasing injection forces, chances of drug leakage, pain, and tissue deformation[21,28].

Different strategies have been applied to achieve higher volumes at the site of injection. Permeation enhancers, like hyaluronidase[3], are already being used for commercial products and allow injection of volumes from 5 to 100 mL. Other strategy is the employ of technologies to reduce the viscosity of high concentration formulations. For example, EXCELSE™ technology allows injections with a volume of 1 mL with concentrations up to 250 mg/mL without increasing the viscosity[29]. It uses a mixture of amino acids that covers regions of the drug without modifying it to avoid aggregation and stability problems. Arestat® is a technology that also allows switching between intravenous to subcutaneous administration of biotherapeutics among other possibilities, however, there is little information available on how it works[30]. Finally, new trends are emerging such as the formulation of proteins as physical protein complexes, microparticles, spherical microbeads, or paste formulations for their successful sustained release after subcutaneous administration[4].

3. Glucagon-like peptide-1 receptor agonists and amylin mimetics

3.1. Glucagon-like peptide-1 receptor agonists pharmacology and current state

Glucagon-like peptide-1 (GLP-1) is a 30-amino acid peptide that acts as an incretin hormone. It is produced by the epithelial endocrine L-cells in response to food intake and is responsible for various effects throughout the body, as GLP-1 receptors are distributed in the pancreas, gastrointestinal tract, brain, heart, and the kidneys[31]. The main function of GLP-1 is to strictly stimulate insulin secretion when sugar levels rise (incretin effect) and to inhibit glucagon secretion helping to lower glucose levels. Moreover, GLP-1 enhances β-cell functions and inhibits β-cell apoptosis, delays gastric emptying thereby reducing postprandial glucose levels and regulates appetite and energy intake by activating GLP-1 receptors in the Central Nervous System[13,31,32]. Despite its potent anti-diabetic effect, GLP-1 is rapidly metabolized by the enzyme dipeptidyl peptidase IV (DPP-4) (its half-life is <1 min) [9] and, therefore, its clinical application is limited.

To overcome the bioavailability problems of GLP-1, some peptide analogues with a longer half-life have been formulated. Exenatide, with homology to GLP-1 of approximately 50% (the same for lixisenatide), was the first to be commercialised. It was developed from exendin-4, a molecule isolated from Gila monster venom. Other GLP-1 RAs, such as liraglutide, dulaglutide, or semaglutide, were directly developed from GLP-1 and their homology is >90% [11]. GLP-1 half-life extension strategies included amino acid sequence modification (exenatide and lixisenatide), fatty acid attachment (liraglutide and semaglutide), sustained-release microparticles (exenatide), fusion with human serum albumin (albiglutide), or fusion with the fragment crystallizable (Fc) region of a monoclonal antibody (dulaglutide) [13].

Five GLP-1 RAs are currently commercialized: exenatide (Byetta®, Bydureon®), liraglutide (Victoza®), lixisenatide (Adlyxin® in US and Lyxumia® in EU), dulaglutide (Trulicity®) and semaglutide (Ozempic®). GlaxoSmithKline (GSK) marketed albiglutide (Tanzeum®) from 2014 to 2017 but stopped marketing the drug due to its limited prescription[33]. The mechanism of action of GLP-1 RAs is similar to that of GLP-1. As they are glucose-dependent, when blood glucose levels increase, GLP-1 RAs lower blood glucose levels by binding and activating GLP-1 receptors that induce insulin secretion and inhibit glucagon secretion[9,11,13,32]. GLP-1 RAs differ from each other in molecular size, molecular structure, and pharmacokinetics [34]. Thus, their half-

lives depend on the molecule and the delivery system. On this basis, the administration can be twice a day, once a day or once a week[11].

Common adverse reactions of this class of drugs include gastrointestinal events (*e.g.*, nausea, diarrhoea, vomit, decreased appetite, dyspepsia and constipation), injection-site reactions, hypoglycaemia (when combined with insulin or an insulin secretagogue), macrovascular outcomes, acute pancreatitis, acute kidney injury, thyroid C-cell tumours, immunogenicity and hypersensitivity[35–41].

Real-world data from T2D patients who started GLP-1 RAs therapy suggest high discontinuation rates. In an 18 months study, 42.5% of patients discontinued once weekly exenatide (Bydureon®), of which 16% were due to adverse reactions[42]. The average time to discontinuation of Bydureon® treatment was 6 months for adverse reactions reasons and 12 months for other reasons. Other studies agree on the low continuity of treatment with GLP-1 RAs (liraglutide, exenatide once weekly and exenatide twice daily) after 12 months or more[43,44]. More data are needed to conclude the reasons for the high discontinuation rates or changes in treatment.

3.2. Relation between GLP-1 RAs administration volume and injection-site reactions

Table 1 lists the data on the dose/volume relationship for each commercialized GLP-1 RA. As shown, volumes range between 0.02 mL for Byetta® and 0.75 mL for Ozempic®, and doses range between 5 µg for Byetta® and 50 mg for Tanzeum®. Although all administration volumes are within the maximum range for subcutaneously administered volumes, injection-site reactions continue to be a common side effect of GLP-1 RAs. In particular, Bydureon® has shown severe injection-site reactions with or without subcutaneous nodules, such as abscesses, granulomas, cellulitis, and necrosis. Subcutaneous nodules form more frequently with Bydureon® therapy than with other GLP-1 RAs (77% of subjects experienced at least one subcutaneous nodule during treatment in clinical trials) due to the use of a microsphere-based formulation[36]. These nodules were temporary and generally, no medical intervention was needed[32]. Additionally, Bydureon®-treated patients have a higher incidence of injection-site reactions (17.1%) than Byetta®-treated patients (12.7%)[45]. In both Bydureon® and Byetta®, injection-site reactions are associated with high titers of anti-exenatide antibodies[36,37], and Bydureon® shows a higher percentage of antibody-positive patients than Byetta®[46]. In the placebo-controlled trials of Tanzeum®, 18% of treated patients experienced injection-site reactions (including hematoma, erythema, rash, hypersensitivity, haemorrhage, or pruritus) that led to discontinuation of treatment

within 2% of participants[39]. As with exenatide, injection-site reactions caused by albiglutide appear to be mediated by the immune system[47].

However, the percentage of injection-site reactions for the remaining analogues was lower: 0.2% for Ozempic®, 0.5% for Trulicity®, 2% for Victoza®, and 4% for Adlyxin®[35,38,40,41]. According to Supporting Information Table S1, the administration volume of Ozempic®, Trulicity®, Victoza®, and Adlyxin® is less than 1 mL, and their concentrations are low (≤ 6 mg/mL). Both low volume and concentration may be responsible for the greater acceptance of these formulations compared to the other analogues. Byetta®, Bydureon®, and Tanzeum® show a higher proportion of injection-site reactions, which may be related to the higher immunogenicity of these formulations. In addition, Bydureon® includes 50:50 poly(D,L-lactide-*co*-glycolide) (PLG) polymer microspheres in its formulation[36], which may account for the higher proportion of injection-site reactions and nodules formation[32,45]. Tanzeum® was used in a small volume (0.5 mL) but in high concentrations (60 and 100 mg/mL). High protein concentrations are associated with protein aggregation and protein particle formation[21], which can increase immunogenicity[48] and thus injection-site reactions.

3.3. Immunogenicity issues

In general, protein and peptide pharmaceuticals are considered potentially immunogenic. Subcutaneous immunogenicity is suggested to be higher than IV, mainly because of the higher exposure to the lymphatic system and to antigen-presenting cells[1,4]. Immunogenic effects may alter pharmacokinetics, produce safety risks (injection-site reactions, hypersensitivity, or anaphylactic reactions), and attenuate therapeutic effects[1,49].

The immunological effects of GLP-1 RAs were evaluated in several clinical trials. Studies with Byetta® (add-on to either metformin or a sulfonylurea, or both) showed that 38% of treated patients had anti-exenatide antibodies, of which 6% had high antibody titers. Glycaemic control failed in 3% of them[37]. On the other hand, the immunogenicity data were higher for Bydureon®. In various clinical trials, 43%–49% of patients had antibody formation. Moreover, 6% of patients showed an attenuated glycaemic response[36]. Low anti-exenatide antibody titers do not appear to have a significant effect on the glycaemic response; however, in some patients, higher titers are associated with a significant reduction in efficacy[46].

In the case of Adlyxin®, 70% of the patients tested positive for antibodies and 2.4% of them showed an attenuated glycaemic response. Allergic reactions and injection-site

reactions were more likely to occur in antibody-positive patients[35]. For Victoza®, immunogenicity ranged from 0.9% to 8.6%, depending on the trial. However, antibody formation was not associated with reduced efficacy of Victoza® or with adverse effects related to immunogenicity [41]. Immunogenicity for the remaining GLP-1 RAs was lower: 5.5% for Tanzeum®, 1.6% for Trulicity® and 1.0% for Ozempic®[38–40]. For these, immunogenicity was not related to the neutralization of glycaemic control.

The clinical significance of the immunogenicity of GLP-1 RAs is not yet clear[49]. As the data suggest, human GLP-1 derived GLP-1 RAs appear to be associated with lower antibody titers than exendin-4 derivates (Byetta®, Bydureon®, and Adlyxin®)[49]. However, these data should be interpreted carefully because antibody detection depends on several factors (assay sensitivity and methodology, sample taking time and processing, accompanying medications and underlying conditions). For these reasons, data obtained from different trials or with different GLP-1 RAs cannot be directly compared with each other[35–41].

3.4. In vitro and ex vivo models for subcutaneous injection

The subcutaneous drug delivery market size is expected to grow by 9.7% between 2020 and 2027[50]. This fact, coupled with the growing trend to minimize the use of experimental animals, makes the development of a reliable *in vitro* or *ex vivo* model an urgent task. These models should make it possible to predict the pharmacokinetic parameters of the drug, the interactions of the formulation components with the subcutaneous environment, the immune response and the toxicity. In addition, there is a strong need for models capable of conducting long-term, yet stable, studies to evaluate controlled release drug delivery systems.

Recently, some companies have developed *in vitro* and *ex vivo* skin models for different purposes. Particularly, Genoskin has launched Hyposkin®, the first *ex vivo* skin model to test subcutaneous injections[51]. This model contains a real human skin biopsy that can last at least 7 days. Basically, it is a culture insert that contains donated human skin from abdominal surgery and a patented matrix and culture medium (Fig. 3).

Another company, Straticell, has developed several skin models, including skin cells in monolayer cultures, *in vitro* reconstituted human epidermis, and *ex vivo* skin explants[52]. However, none of the models is specifically designed for subcutaneous injection. Another *in vitro* model, called Scissor (Subcutaneous Injection Site Simulator), uses a dialysis-based injection chamber with acellular ECM components immersed in a container of a bicarbonate-based physiological buffer to emulate the

movement of the injected biotherapeutic from the subcutaneous tissue to the systemic circulation[22]. It allows real-time monitoring of the drug and the medium (ECM components, pH, ionic composition, interstitial pressure and temperature). Scissor is now marketed by Pion Inc.[53] and some studies have been performed to predict the bioavailability of eight monoclonal antibodies injected subcutaneously with a strong correlation with data obtained *in vivo* in humans [54].

Each of the models described above has its limitations. While the latter model focuses on pharmacokinetic prediction, other researchers have turned their attention to *in vitro* models for immunogenicity prediction[55]. However, there is not yet available a model of subcutaneous administration that allows continuous drug and medium monitoring, emulation of subcutaneous tissue properties and interactions, prediction of immunogenicity and pharmacokinetics and feasibility for long-term studies.

3.5. Amylin mimetics

Amylin is a 37 amino-acid peptide produced by the β-cells that acts as a glucoregulatory hormone and an energy metabolism regulator. It is co-secreted with insulin in response to blood sugar levels[56]. However, its physicochemical properties make it easily precipitable, so its clinical application is troublesome. Pramlintide, an analogue of human amylin, was approved by the FDA in 2005. It replaces amino-acids in positions 25, 28, and 29 of human amylin with proline, improving the problem of precipitation[17]. Also, it is used in combination with insulin in both T1DM and T2DM[16]. Even though it was approved in 2005, its clinical use is quite limited due to its inconvenient administration; pramlintide requires 3 to 4 subcutaneous injections daily and does not appear to adequately mimic the natural release profile of amylin. The wide variations in plasma levels throughout the day lead to dose-related gastrointestinal adverse reactions[16,17,56]. Moreover, Astra Zeneca has just launched it in the US as Symlin®[14], but other amylin analogues with improved pharmacokinetics are under development, especially for obesity treatment[16].

4. Formulation strategies to improve the pharmacokinetics and bioavailability of GLP-1 RAs

Along with attempts to improve the subcutaneous administration of GLP-1 RAs, many efforts are being made in the development of new formulations with enhanced pharmacokinetic properties and, above all, with characteristics that could improve

patient acceptability. To this aim, very different approaches are being taken, both by academy researchers and pharmaceutical companies. This section reviews the different drug delivery systems proposed for GLP-1 RAs: oral tablets, buccal formulations, liposomes, nasal formulations, transdermal formulations, pulmonary formulations, nanoparticles, and microparticles (Fig. 4).

4.1. Oral tablets

Until 2019, GLP-1 RAs were only available as an injectable treatment, so despite their efficacy, they were underused. In 2019, Rybelsus®, the first oral GLP-1 RA, was approved by the FDA and marketed by Novo Nordisk. Rybelsus® is an oral semaglutide formulation (tablets of 3, 7, or 14 mg) that is administered once a day [57]. It uses Eligen® technology (Emisphere Technologies, Inc.) to enhance the semaglutide absorption in the stomach. Eligen® technology uses a small fatty acid derivative, sodium *N*-(8-[2-hydroxybenzoyl] amino) caprylate (SNAC), to prevent degradation of semaglutide and improve its oral bioavailability (Fig. 5)[58]. SNAC allows semaglutide to utilise passive transcellular transport of the gastric epithelium and its effect depends on time and concentration[59]. The bioavailability of semaglutide is estimated to be approximately 0.4% to 1% after administration of Rybelsus®[57].

Another company, Oramed Pharmaceuticals Inc., has been developing ORMD-0901, an oral exenatide formulation based on the company's POD™ technology [60]. Although the company has presented some promising results, there is little information on the product and it is still in the company's pipeline.

4.2. Buccal formulations

The oral mucosa allows the drug to directly access the systemic circulation, thereby avoiding first-pass metabolism. Moreover, the buccal route of administration is much more convenient than the subcutaneous route and causes fewer problems than the nasal, transdermal, vaginal or pulmonary routes[61]. The main drawback of the buccal route is the low bioavailability of larger molecules that require the use of several strategies to be absorbed, such as the use of absorption enhancers or enzyme inhibitors to improve their pharmacokinetics[62].

Despite the efforts that have been made to develop an effective formulation for buccal administration of peptides, this remains a challenge. Some attempts have been made for the buccal delivery of insulin (an oromucosal spray and a dissolvable film

with embedded gold nanoparticles) but they failed to reach the market due to its low efficacy and variable pharmacokinetics[63].

For instance, ArisCrown technology (Arisgen SA) has been used for the buccal administration of exenatide (ARG011). This technology uses biodegradable cyclic compounds (crowns) to selectively and reversibly mask (with non-covalent interactions) peptide functional groups. The modified peptide is then included in a lipid formulation optimised to maintain the properties of the peptide[64,65]. Preclinical studies have been conducted with buccal exenatide in mice and monkeys. In mice, buccal administration of exenatide controls blood glucose levels in a manner equivalent to an intraperitoneal injection of the unformulated peptide[66]. In monkeys, the buccal formulation of exenatide included in a buccal patch controls blood glucose levels in a manner equivalent to the subcutaneous formulation[67]. Despite the promising results, the development of this formulation was cancelled because Arisgen SA is currently closed.

4.3. Liposomes

GLP-1 was formulated in liposomes to improve its pharmacokinetics and pharmacological effect [68]. Anionic, cationic, and non-ionic liposomes were prepared and 130–210 nm liposomes with moderate size homogeneity and high dispersibility were obtained. Anionic liposomes showed the highest encapsulation efficiency (80.2%) and the best pharmacokinetic parameters, as well as an evident improvement in pharmacological effects (Fig. 6).

Although anionic liposomes have shown great promise, intravenous administration requirements are a significant drawback for their future development and commercialisation.

Exendin-4-loaded liposomes coated with chondroitin sulfate-g-glycocholic acid (EL-CSG) were also developed for oral delivery[69] and the role of bile acid transporters in the absorption of exendin-4 was evaluated. The average size of liposomes was 230 nm and their loading efficiency was 77%. Experiments in rats showed a relative bioavailability of 19.5% (*versus* subcutaneous administration). The antidiabetic effects (HbA1c, body weight, blood lipid concentration) were evaluated for four weeks and were found to be equivalent to those obtained with the subcutaneous administration of free exendin-4.

4.4. Nasal formulations

The nasal route of administration, in addition to being non-invasive, allows avoiding the first-pass metabolism. Nasal delivery of small lipophilic molecules is feasible as they

can reach therapeutic levels in the bloodstream. However, peptides and proteins must be co-administered with a nasal absorption promoter due to their usual low nasal absorption[70].

4.4.1. Nasal Microparticles

A nasal formulation consisting of a capsule filled with approximately 60 µm particles of recombinant human GLP-1 amide bound to calcium carbonated and coated with corn starch was developed and tested in a double-blind placebo-controlled study[71]. Each capsule contained 1.2 mg of recombinant human GLP-1 and a device for intranasal delivery of drugs was used for the GLP-1 microparticles delivery.

GLP-1 treated patients did not develop any serious adverse reaction after nasal administration. The C_{max} was 47.2 pmol/L and the T_{max} was 8.1 min. GLP-1 induced insulin secretion, inhibited glucagon secretion and improved glycaemic control markers in the medium term. However, the short study period (2 weeks) makes long-term trials necessary to evaluate the safety, efficacy and tolerability of this treatment. Comparison with other GLP-1 RAs formulations also may be desirable. Moreover, the addition of a nasal absorption promoter should be considered to improve the bioavailability of the formulation and to reduce associated costs.

4.4.2. Nasal thermosensitive hydrogels

Other approach to formulate exenatide for nasal administration was based on the creation of exenatide-loaded chitosan-based thermosensitive hydrogels[72]. Chitosan was shown to activate protein kinase C transduction pathways by opening tight junctions in epithelial cells and increasing permeability. The chitosan/glycerophosphate thermogelling systems were lyophilised for storage and then redissolved in the presence of a metal salt ($CaCl_2$ or $MgCl_2$). The hydrogel formulation redissolved with $MgCl_2$, compared to that redissolved with $CaCl_2$, preserved the stability of the exenatide, increased transport through Calu-3 cell monolayers, and increased the bioavailability of exenatide in rats after nasal administration (Fig. 7).

This formulation significantly decreased food intake and body weight in high-fat-fed rats compared to an exenatide solution and appears to be suitable for nasal administration of exenatide for fat reduction, but further studies are needed to evaluate its efficacy in DM treatment, its safety and its tolerability.

4.4.3. Nasal solution with a cell-penetrating peptide

Recently, it has been suggested that Alzheimer's disease could be considered type 3 diabetes [73], and it has been proposed that insulin and GLP-1 RAs could improve learning and memory by increasing glucose uptake in the neuronal cells of the hippocampus. For this reason, it could be interesting to develop nose-to-brain drug delivery systems for exendin-4 and GLP-1. Solutions with different concentrations of exendin-4 or GLP-1 and penetratin (L- or D-penetratin) were formulated and administered intranasally to anaesthetised ddY mice [74]. Intranasal coadministration with L-penetratin increased the systemic absorption of both peptides, and in the case of exendin-4, its concentration in the olfactory bulb increased significantly as shown in Fig. 8. The concentration in the whole brain was much lower than in the olfactory bulb. However, the distribution in the brain showed a significant increase in exendin-4 levels in the hippocampus. Exendin-4 plays a role in the hippocampus by mediating memory and learning. According to this study, L-penetratin could act as an absorption enhancer, but further research is required to improve this approach.

4.4.4. Nasal spray

Other approaches have been undertaken to develop a nasal formulation of exenatide. For example, MDRNA Inc. (previously Nastech Pharmaceutical Company Inc.) and Amylin Pharmaceuticals developed an exenatide nasal spray that reached phase II trials[75]. The exenatide spray formulation comprised a viscosity enhancer, methyl-3-cyclodextrin, a surfactant, tartrate buffer for pH control, and a chelating agent for cations[76]. However, its development was interrupted, and it was never approved.

In 2014, Aegis Therapeutics LLC (now Neurelis Inc.), offered a license to develop, market and sell a patented exenatide nasal spray that used its Intravail® technology[77]. Intravail® is a group of absorption enhancers belonging to a class of compounds called alkylsaccharides (sugars and one or more alkyl chains covalently bound together)[78]. Although the company has commercialised other nasal formulations, the development of the exenatide product has been interrupted.

4.5. Transdermal formulations

The transdermal administration of exenatide could have some advantages compared to current treatments. Transdermal delivery of drugs is non-invasive and painless, and patients can self-administer the drug. In addition, this route is usually well tolerated.

However, achieving skin penetration of proteins and biotherapeutics is a major challenge. The problems faced by the drug when penetrating through the skin can be solved by permeabilizing the outermost layer of the skin, the *Stratum corneum.* This can be achieved by creating microchannels using microneedles or by temperature modulation using radiofrequency. These strategies allow painless drug absorption[79].

4.5.1. Microneedle patches

Exenatide dissolving microneedles patches were created using low-molecular-weight sodium hyaluronate and using the micromould casting method [80]. Dissolving microneedles showed a high loading capacity, and likewise, they did not leave sharp biohazard residues. Materials selection and geometric shape are particularly relevant in dissolving microneedles due to their weak mechanical properties. In this case, sodium hyaluronate was selected for its biocompatibility and high hydrophilicity, and pyramidal microneedles were chosen for its high mechanical strength. The patches showed similar pharmacokinetics and hypoglycaemic effect to subcutaneous delivery. Exenatide was released and absorbed almost completely from the patch within 2 min.

Despite the potential of this system, it still has some drawbacks. For instance, the high hydrophilicity of sodium hyaluronate may be a problem in high humidity environments because the stability of exenatide and the mechanical strength of the formulation could be compromised. Storage should be strict to ensure long-term stability, patches should be stored in waterproof packaging with a dry nitrogen atmosphere and the addition of sucrose or trehalose should be considered. In any case, administering the patches twice a day could be inconvenient for the patients, but their safety profile should be further evaluated.

Another formulation consisting of dissolving microneedle arrays was developed employing carboxymethyl cellulose and using the centrifugal lithography method[81]. Three fundamental factors in the manufacture of microneedles were optimised (temperature during manufacture, pH and concentration of the polymer) to ensure the activity of the drug once encapsulated in the microneedles and stored. The formulation showed no reduction in activity after eight weeks of low temperature storage.

In other study, indissoluble alginate microneedle array patches loaded with dual mineralized particles containing exendin-4 and glucose oxidase were prepared (Fig. 9)[82].

These patches act as a closed-loop system, releasing exendin-4 while immobilizing glucose oxidase in a glucose-responsive manner. Two different mineralised particles

were used; copper phosphate encapsulates glucose oxidase and calcium phosphate encapsulates exendin-4. In the hyperglycaemic state, glucose oxidase reacts with glucose and lowers the pH. Calcium phosphate responds to the decrease in pH by releasing exendin-4, while copper sulphate prevents the release of glucose oxidase. The mineralised particles improved the mechanical resistance of the microneedles by crosslinking with the alginate, promoting skin penetration. These microneedle patches achieve exenatide release on-demand, without patients having to monitor their blood glucose.

Despite the positive results obtained in preclinical studies, the patches must be redesigned for clinical translation, and dose adjustment, as well as the length and morphology of the microneedles and the frequency of application, must be adapted for human administration.

4.5.2. Transdermal drug delivery through temperature modulation

TransPharma Medical Ltd. developed a product called ViaDor®, which has now reached phase 1. It uses a radio frequency-based physical heating technique to enhance drug diffusion through the skin[83]. However, limited information is available and its development has been interrupted.

4.6. Pulmonary delivery

Several formulations have been developed to deliver inhaled GLP-1 and GLP-1 RAs[84]. For instance, Afrezza® is an inhaled insulin approved by the FDA in 2014 using Technosphere® technology[85]. Technosphere® is a powder formulation composed of fumaryl diketopiperazine (FDKP) microparticles where the peptide is encapsulated. The size of the microparticles ranges from 2 to 5 μm. The Technosphere® microparticles dissolve due to the physiological pH of the alveoli, releasing the peptide for absorption[86].

The pharmacokinetics and pharmacodynamics of Technosphere® GLP-1 (7–36) amide (MKC253) were studied in healthy and diabetic humans. When administered, MKC253 is rapidly absorbed (peak concentrations within 5 min) and rapidly degraded (baseline values within 30 min). In addition, it stimulates insulin and C-peptide secretion but hardly reduces glucagon secretion[87]. As this study was a proof-of-concept, further studies are required for the optimisation of the formulation and its inhalation device. Furthermore, the negative experience with Afrezza® in the market (inadequate insurance coverage, new adverse effects and safety concerns and the emergence of new

therapeutic alternatives) highlights the need for more in-depth studies before commercialising new pulmonary formulations [88].

4.7. Nanoparticles

GLP-1 RAs nanoparticles (NPs) have been extensively investigated in recent years, especially for oral administration. Table S1 shows a summary of studies since 2017 that have designed and tested GLP-1 RAs NPs. Currently, these studies are just proof of concepts and none are intended to be marketed in a short period of time. However, the development of this technology is expected to develop further in the coming years.

4.8. Microparticles

Biodegradable microparticles based on PLGA polymers have proven to be useful drug delivery systems in biomedical applications such as adjuvant/vehicle for vaccines, tissue engineering, sustained-release systems or cancer treatment[113–117]. In 2012, the FDA approved Bydureon® for the treatment of DM[36]. Bydureon® uses Medisorb® technology, which is based on PLGA microparticles with a size of 60 µm that provide an extended-release of exenatide when administered subcutaneously[32]. Drug release occurs in three steps: an initial burst release, a lag phase, and a drug diffusion phase. The burst release phase, shown in Fig. 10, is mainly caused by surface and easily accessible drug molecules, and can transitorily increase drug concentrations in the blood, which can cause adverse effects.

Since the approval of Bydureon®, research on GLP-1 RAs microparticulate systems has focused on improving the pharmacokinetic and pharmacodynamic properties of Bydureon®. To this end, various approaches have been developed for the release of GLP-1 RAs.

4.8.1. PLGA microparticles

Most of the published works on GLP-1 RAs microparticles have focused on the use of PLGA as an encapsulating polymer and exenatide as a model drug. After Bydureon® release, several efforts have focused on improving the original formulation. For instance, several batches of microspheres with polymers of different molecular weight and composition have been prepared by different methods and employing different solvents [118]. According to this study, microspheres prepared with 65 KDa 50:50 PLGA using a water-in oil-in oil (W/O/O) emulsion-solvent extraction method showed the highest encapsulation efficiency (98.0±9.4%) and loading efficiency (4.53±0.44%). Besides, by using heptane as the only hardening solvent, the microspheres had a round

shape, smooth surface and good dispersion. All microspheres prepared by the W/O/O emulsion-solvent extraction method provided low burst release *in vitro* (Fig. 11), attributable to the non-aqueous processing medium (silicon oil).

However, it was observed that the use of organic solvents such as silicon oil, dichloromethane, heptane, or ethanol in the process was not appropriate for obtaining microparticles. Also, mechanical dispersion methods were not effective in controlling the size distribution of the microparticles, which led to problems of inconsistency in the results. In another study, exenatide-loaded PLGA microspheres of uniform size were fabricated using the Shirasu Porous Glass premix membrane emulsification technique combined with water-in oil-in water (W/O/W) emulsion solvent extraction method. Microspheres of approximately 20 µm were obtained with high encapsulation efficiency[119]. Moreover, exenatide-loaded PLGA microparticles prepared by spray drying were compared to those prepared by the ultrafine particle processing system (UPPS) based on the disk rotation principle[120] (Fig. 12). This technique provided a simple and scalable alternative to reproducibly manufacture microparticles under mild preparation conditions.

The UPPS microparticles were larger in size and showed higher encapsulation efficiency than the microparticles obtained by spray-drying. In *in vitro* studies, UPPS microparticles released exenatide in a stable and sustained manner, whereas in the *in vivo* studies in rats, the antidiabetic effect was observed for one month.

Using the same technique, dimpled exenatide-loaded PLGA microparticles were prepared to investigate the mechanism of formation and release characteristics [121]. The microparticles showed high encapsulation efficiency (91.50±2.65%) and a sustained drug release for two months with a reduced initial burst. Additionally, effective drug concentrations were maintained for three weeks after a single injection.

In other work, hollow microparticles loaded with GLP-1 were compared to solid microparticles prepared by a modified oil-in-water (O/W) emulsion solvent evaporation technique[122]. The purpose of the study was to reduce the accumulation of acidic products of PLGA degradation and to reduce polymer-peptide interactions. It was concluded that in the hollow microparticles 93% of the extracted peptide was active, while in the solid microparticles only 58% of the peptide was active. Thus, the *in vitro* release of the hollow microparticles on Day 14 was 88±8% compared to 33±6% for solid microparticles. In the same year, a novel GLP-1 analogue with a longer half-life was synthesised and encapsulated in PLGA microspheres by the double emulsion-

solvent evaporation method[123]. Zinc was incorporated to slow initial burst release and achieve uniform drug distribution.

Also for the purpose of reducing the burst release, PLGA microspheres containing exenatide-loaded lecithin NPs were prepared by a modified solid-in oil-in-water (S/O/W) emulsion technique[124]. The microparticles released the drug for more than 60 days and showed reduced burst release. An encapsulation efficacy of 66.33%±3.75 was obtained, and the size of the microparticles was 5.29±0.98 μm with a Span value of 1.32±0.01.

In a more recent study, exenatide-loaded inside-porous PLGA microspheres with outside layers were fabricated using a W/O/W emulsion method with a microfluidic technique [125]. To form the porous microspheres, NH_4HCO_3 was chosen as the porogen. Briefly, to form the microparticles, an emulsion was first prepared with W_1 (exenatide and NH_4HCO_3 solution) and O (PLGA in dichloromethane), then this emulsion was mixed with W_2 (PVA and NaCl solution) through the microfluidic cross chip. Solid microspheres were obtained after stirring to volatilize the dichloromethane. The porous microspheres had low burst release and absence of a lag phase with high encapsulation efficiency, improving the release profile of existing exenatide-PLGA microparticles.

More studies have been published to evaluate various parameters of PLGA microparticles that were affecting the encapsulation of GLP-1 RAs[126–128]. Conversely, only a few authors have proposed different polymers to PLGA to encapsulate GLP-1 RAs to overcome its severe limitations in the pharmacokinetic profile of the drug, such as minimizing initial burst release or extending the time between administrations. In addition, there has not been much innovation nor have many alternatives been proposed in the methodology used to encapsulate these drugs, which are usually techniques based on the emulsion-solvent evaporation method. This method has several drawbacks, such as the presence of organic solvent residues in the final formulation, a wide particle size distribution or being hardly scalable for the industry.

4.8.2. Hydrogel microparticles

Elaboration of hydrogel drug delivery systems for the subcutaneous administration of exenatide has also been proposed. For instance, a drug delivery system based on [Gln^{28}]exenatide (a more stable analogue of exenatide) covalently bound to hydrogel microspheres has been developed by means of a self-cleaving β-eliminative linker[129–131]. For preparing the microparticles, a large pore tetra-PEG hydrogel polymer was used.

This system can be administered once a month and has already been tested in cats for feline DM with positive results.

In another study, a gel matrix microsphere gel deposition system was developed by encapsulating exenatide-loaded hydrogels in PLGA microspheres encapsulated into blank hydrogels[132,133]. A 46 days release *in vitro* without burst release was achieved also obtaining stable blood glucose for 20 days *in vivo*.

5. Challenges and future perspectives

To date, it is estimated that 9.3% of the world population (aged 20–79 years) suffers from DM and it is expected that his percentage will continue to increase until reaching a global prevalence of more than 10% in the next 10 years, which makes DM one of the most prevalent chronic diseases in the world[6]. Improving efficacy, safety and patient compliance continue to be the primary goals of DM management. Advances are likely to come from the development of novel subcutaneous treatments capable of effectively controlling glycaemic levels, being also more convenient for patients to ensure their adherence to therapy. Commercialised formulations of GLP-1 RAs and amylin mimetics still present pharmacokinetic problems that limit the effectiveness of the therapy and cause frequent adverse effects[16,134]. In addition, the high cost of these treatments means that their prescription is limited to specific cases. For example, the NICE guideline for T2DM management in adults only considers a GLP-1 RA as part of triple therapy (with metformin and a sulfonylurea) when the conventional therapy with three oral antidiabetics is ineffective, not tolerated or contraindicated[135]. In addition, GLP-1 RAs are only recommended in cases where the patient has a body mass index (BMI) of 35 kg/m^2 or higher, when the patient presents psychological or medical problems associated with obesity, or in those cases in which the patient has a BMI less than 35 kg/m^2 and insulin therapy is not appropriate. They are also recommended in cases where weight loss would benefit other obesity-related comorbidities.

The obtention of longer acting GLP-1 RAs and amylin mimetics that allow a prolonged and constant release of the drug is an area of ongoing research. However, there is some controversy regarding the use of short-acting *versus* long-acting GLP-1 RAs. One of the mechanisms by which GLP-1 RAs control postprandial blood glucose is by slowing gastric emptying[136]. In long-acting GLP-1 RAs, this mechanism appears to be reduced compared to exenatide twice daily (Byetta®), which is associated with lower postprandial glucose levels[137]. Therefore, there may be a place in the market for a

GLP-1 RA with improved pharmacokinetics and tolerability, compared to exenatide twice daily, for prandial administration.

Alternatives to injectable formulations such as transdermal, nasal and oral routes have also been proposed. These alternative routes could be especially convenient for patients with motor or visual impairments, very common in DM. In particular, there is a growing interest in making oral delivery of GLP-1 RAs and insulin a reality. The recent marketing of Rybelsus® is a proof of this[57]. However, oral peptide therapeutics still have to face several challenges, such as poor oral absorption, pH and enzymatic instability along with food effect and pharmacokinetic variability. Significant investments should be made in formulation design and development to provide sufficient oral bioavailability before oral GLP-1 RAs and insulin assume an important role in the DM management[10].

Regarding transdermal drug delivery, development work is focused mainly on minimally invasive microneedle patches. Conventional approaches to fabricate microneedle patches include micromoulding, lithography and coating techniques[79]. However, these techniques are usually time-consuming and difficult to scale-up. Innovative technologies such as 3D printing could offer an alternative for the manufacturing of these devices. Stereolithography (SLA) and semi-solid extrusion (SSE) 3D printing have already proven capable of creating microneedle patches with high degrees of complexity and reproducibility in a fast and cost-effective manner[138,139]. The use of biocompatible resins and materials helps to circumvent toxicity problems; however, regulatory and technical challenges still remain before the adoption of 3D printing in the clinical practice[140,141].

GLP-1 RAs and other protein and peptide drugs also represent interesting targets for intranasal administration. However, these molecules are charged, hydrophobic and usually present high molecular weights, and thus are poorly permeable through lipid barriers such as the nasal. Some pharmaceutical strategies have been applied to overcome the low permeability as well as the physicochemical instability in the nasal mucosa to increase drug bioavailability, such as the use of nano and microparticulate systems, enzyme inhibitors and permeation enhancers. Of particular interest is the inclusion of GLP-1 RAs in chitosan-based thermosensitive hydrogels. Chitosan is well recognised as a polymeric absorption enhancer capable of opening tight junctions in epithelial cells and thereby increasing permeability[142]. Despite the potential showed by

these formulations, further research and investment is needed before optimised intranasal therapies can reach the market.

Microparticulate systems have also shown great promise for GLP-1 RAs delivery. Since PLGA is well accepted by regulatory bodies and has been shown to be effective in delivering biotherapeutics, many of the microparticle formulations approved by the FDA to date are PLGA based[143]. One example is found in Bydureon®, the polymer microsphere formulation of exenatide, a once-a-week product[36]. Once injected subcutaneously, the microspheres form a matrix drug reservoir *in situ* and exenatide is released in a discontinuously, with an initial low burst release in the first 48h and two peaks of drug release in the second and seventh week. This discontinuous rate of drug release is due to the properties of PLGA, the main drawback of these formulations being the impossibility of releasing the drug at a steady rate[13]. Novel drug encapsulation methods must be developed that employ biocompatible polymers and avoid the use of organic solvents or high temperatures. These methods must not only be industrially scalable and allow a loading efficiency close to 100% to avoid the loss of drugs as expensive as biotherapeutics, but also capable of producing formulations that allow a constant drug release without major fluctuations in blood drug levels.

Aside from the development of improved formulations, the establishment of *in vitro* and *in vivo* models for preclinical testing of such formulations is almost equally important. The lack of *in vitro* dissolution tests for biotherapeutics and the high variability between subcutaneous tissues from different animal models are major obstacles during the development of new biotherapeutic formulations[144]. Although novel methods, such as the *in vitro* methodology called Scissor for Subcutaneous Injection Site Simulator[22], represent important advances to achieve better reproducibility and more suitable experimental conditions to test the formulations, none of the proposed models completely mimic the human subcutaneous environment. Future preclinical models should allow long-term studies of the pharmacokinetics, bioavailability and immunogenicity of sustained-release formulations.

The joint use of improved subcutaneous formulations with longer circulation times that allow more separate administrations is forecast to be a game-changer on DM management as well as on patient's quality of life.

6. Conclusions

GLP-1 receptor agonists and amylin mimetics have the potential to become a key treatment for diabetes mellitus. This review article summarises the current challenges of subcutaneous administration of these drugs as well as the emerging strategies for the development of GLP-1 receptor agonist formulations with improved bioavailability. Also, a broad overview of currently marketed dosage forms is provided, from oral nanoparticles to subcutaneous injections for the controlled release of biotherapeutics. Although all these new strategies can greatly benefit diabetes mellitus therapy, peptide-based drug delivery systems still have many challenges to face, mainly related to pharmacokinetic and safety issues.

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Author contributions

Carlos Bendicho-Lavilla wrote the manuscript. Iria Seoane-Viaño, Francisco J. Otero-Espinar and Asteria Luzardo-Álvarez conducted review and editing of the manuscript. Francisco J. Otero-Espinar and Asteria Luzardo-Álvarez are the corresponding authors. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

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This review summarizes the current challenges of subcutaneous administration of peptide-based antidiabetics and provides an overview of the formulations available for the different routes of administration.

Figure 1 Milestones in GLP-1 and amylin therapy development since their discovery.

Figure 2 Challenges of subcutaneous administration of biotherapeutics.

Figure 3 HypoSkin® scheme.

Figure 4 Formulation strategies for the administration of GLP1-RAs.

Figure 5 Effect of SNAC effect on the oral absorption of semaglutide. SNAC enhances the transcellular absorption of semaglutide by increasing the pH.

Figure 6 (A) Activity of GLP-1 formulations in an intraperitoneal glucose tolerance test in rats. (B) Serum GLP-1 concentrations in rats after intravenous administration of GLP-1 formulations. Figure reproduced and modified with permission from Ref. 68.

Figure 7 Exenatide blood levels *vs.* time profiles after subcutaneous (SC) or intranasal (IN) administration of exenatide (EXT) in solution or included in $CaCl_2$ or $MgCl_2$ hydrogels. Values are expressed as the mean±SD (n=5). Figure reproduced and modified with permission from Ref. 72.

Figure 8 Systemic absorption and brain transport of GLP-1 and its analogue exendin-4, after single intranasal administration of GLP-1 and exendin-4 with or without L- or D-penetratin (2.0 mM) to male ddY mice. Each data point represents the mean±SEM of n=3–4. *P<0.05, **P<0.01 indicate significant difference with the control group receiving GLP-1 or exendin-4 solution. Figure reproduced and modified with permission from Ref. 74.

Figure 9 (A) Photograph of the patch with a microneedles array. Scale bar, 0.5 cm. (B) SEM image of the microneedles array. Scale bar, 500 μm. (C) Single microneedle. Scale bar, 100 μm. Figure reproduced and modified with permission from Ref. 82.

Figure 10 (A) Plasma concentrations of exenatide after a single extended-release exenatide injection (0 to 12 weeks) (B) Plasma concentrations of exenatide after repeated weekly exenatide injection (0 to 27 weeks). Figure reproduced and modified with permission from Ref. 13.

Figure 11 In vitro release profiles of exenatide loaded PLGA microspheres prepared with PLGA of different molecular weights and different copolymer compositions in

HEPES buffer pH 7.4. Figure reproduced and modified with permission from Ref. 118.

Figure 12 Schematic illustration of spray dryer (A) and ultrafine particle processing system (UPPS) (B) for the manufacture microparticles. Figure reproduced and modified with permission from Ref. 120.

Table 1 Dose/volume relationship in GLP-1 RAs.

Administration	GLP-1 RA	Tradename	Dose	Volume	Concentration
Twice daily	Exenatide	Byetta® [37]	5 µg	0.02 mL	250 µg/mL
			10 µg	0.04 mL	
Once daily	Liraglutide	Victoza® [41]	0.6 mg	0.1 mL	6 mg/mL
			1.2 mg	0.2 mL	
			1.8 mg	0.3 mL	
	Lixisenatide	Adlyxin® (US), Lyxumia® (EU) [35]	10 µg	0.2 mL	50 µg/mL
			20 µg	0.2 mL	100 µg/mL
Once weekly	Exenatide (Controlled release)	Bydureon® [36]	2 mg	0.65 mL	3.08 mg/mL
	Albiglutide	Tanzeum® [39]	30 mg	0.5 mL	60 mg/mL
			50 mg	0.5 mL	100 mg/mL
	Dulaglutide	Trulicity® [40]	0.75 mg	0.5 mL	1.5 mg/mL
			1,5 mg	0.5 mL	3 mg/mL
	Semaglutide	Ozempic® [38]	0.25 mg	0.1875 mL	1.34 mg/mL
			0.5 mg	0.375 mL	
			1 mg	0.75 mL