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Highlights

- Cheaper production costs and extended life of TPPs in ODDFs leads to ease of transport and storage
- TPPs in ODDFs are age-appropriate dosage forms increasing compliance in paediatrics and elderly
- Co-administration of TPPs with excipients such as SNAC enhances absorption across mucosa
- Buccal and sublingual delivery of TPP-loaded ODDFs increases bioavailability of products

Advances in Formulation and Manufacturing Strategies for the Delivery of Therapeutic Proteins and Peptides in Orally Disintegrating Dosage Forms

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Abstract

Therapeutic proteins and peptides (TPPs) are increasingly favoured above small drug molecules due to their high specificity to the site of action and reduced adverse effects resulting in increased use of these agents for medical treatments and therapies. Consequently, there is a need to formulate TPPs in dosage forms that are accessible and suitable for a wide range of patient groups as the use of TPPs becomes increasingly prevalent in healthcare settings worldwide. Orally disintegrating dosage forms (ODDF) are formulations that can ensure easy-to-administer medication to a wider patient population including paediatrics, geriatrics and people in low-resource countries. There are many challenges involved in developing suitable pharmaceutical strategies to protect TPPs during formulation and manufacturing, as well as storage, and maintenance of a cold-chain during transportation. This review will discuss advances being made in the research and development of pharmaceutical and manufacturing strategies used to incorporate various TPPs into ODDF systems.

Keywords

Oral proteins and peptides, enhanced mucosal delivery, mucosal vaccines, stability, personalised medicines, orally disintegrating dosage form

1. Introduction

Healthcare and medical research is now expanding to accommodate unconventional therapeutic agents such as oral biologics. Consequently, there is an increased need for the development of novel approaches required to deliver these therapies. However, to achieve the best outcomes, in terms of health benefits and treatments, simplicity of formulation design is crucial to ensure optimal treatments that are accessible and agreeable to the patient, as well as be cost-effective to manufacture, store and transport. The oral route of delivery is the most common and desirable route of administration for therapeutic drugs or active pharmaceutical ingredients (API) due to the many advantages offered, such as: ease of administration, painless and non-invasive route of delivery, increased patient compliance and acceptability leading to greater therapeutic benefits, and possibilities of self-administration without the need for experienced healthcare professionals to intervene, thereby reducing the burden of costs incurred by public-health services. Nevertheless, oral delivery of therapeutic proteins and peptides (TPPs) does not come without its issues and challenges. The nature of TPPs, their mechanism of absorption, and susceptibility and tolerance to the harsh environment of the gastro-intestinal tract (GIT), are fundamental factors that will determine efficacy and therapeutic benefits, without patients experiencing unwanted side-effects. For solid oral dosage forms, the size of the dosage form, palatability and taste, pathological and physical condition of the patient (e.g. dysphagia, patients with disabilities, paediatrics, geriatrics) are just some of the factors that can hinder successful treatment therapies. It follows that the search for innovative novel oral dosage forms is a necessity that allows more effective treatment options and choices, for those patient groups who would otherwise be overlooked.

According to a recent published report, the global peptide synthesis market is projected to reach 474.3 million USD by 2025, and the global oral proteins and peptides market is currently estimated at around 643 million USD and likely to grow to 8233 million USD by 2028 (Dan et al., 2020).

There are many advantages of TPPs, including high-specificity to the site of action causing reduced incidents of interference with normal physiological homeostasis leading to fewer adverse effects (Haggag YA, 2018, Zizzari et al., 2021). On the other hand, the biggest challenge facing oral delivery of TPPs is their susceptibility to enzymatic degradation in the gastric environment leading to low bioavailability and pharmacokinetic profiles, due to poor intestinal absorption (Zhu et al., 2021). Consequently, the majority of TPPs are formulated for parenteral administration. In recent times, research into evaluating the oral routes for delivery of TPPs has been making significant progress to address and overcome these inherent drawbacks.

One such area of growing interest to deliver TPPs are orally disintegrating dosage forms (ODDFs) (Almukainzi et al., 2019), examples of which include orally disintegrating tablets (ODT) (Nagar P, 2011), orally disintegrating films (ODF) (Nagaraju et al., 2013) and orally disintegrating mini-tablets (ODMT) (Khan et al., 2021, Comoglu and Dilek Ozyilmaz, 2019). The advantages of ODDFs are rapid disintegration within the oral cavity, without water, in the presence of very little saliva leading to potential pre-gastric absorption (if the therapeutic candidate exhibits suitable properties) through the oral mucosa (buccal or sublingual absorption), therefore minimising entry into the gastric cavity; desirable criteria for delivery and absorption of TPPs, that characteristically possess hydrophilic properties and are therefore soluble in biological media. Furthermore, strategies such as encapsulation of TPPs within polymeric nano/microparticles (Vardaxi et al., 2022) or lipid-based carriers (Haddadzadegan et al., 2022) can provide protection and shielding from low pH and digestive enzymes of the stomach and intestines (Brayden et al., 2020, Alqahtani et al., 2021).

An excellent example of the application of TPPs in ODDFs to enhance and improve the quality of life of patients, is the development of desmopressin ODT. Desmopressin is a synthetic peptide which is prescribed for the symptomatic relief from nocturnal polyuria caused by chronic conditions such as diabetes insipidus. The ODT formulation can be taken by patients at night, in bed, without water to provide symptomatic relief from multiple nocturnal urinary voidance episodes thus allowing improved sleep. Desmopressin is one of the very few TPPs to have been formulated into an ODT for use in clinics. There are several Phase 3 clinical trials that have been conducted to evaluate the safety and efficacy of the ODT formulation of desmopressin, as well as assessment of optimal therapeutic benefits of this novel formulation (Yamaguchi et al., 2020, Weiss et al., 2020). The delivery of bioactive peptide compounds in ODDFs has also been applied to the cosmetic industry where a collagen peptide loaded into a buccal patch was clinically tested on females for three months and skin samples were subsequently analysed for hydration and elasticity (Kim et al., 2021). Intestinal oral absorption of collagen is limited as it is easily degraded in gastric acid, thus a buccal delivery system was developed as an alternative method of administration for enhanced systemic absorption. The study demonstrated successful buccal absorption of the collagen peptide with results showing improvement in skin quality.

There is widespread acceptance in healthcare research of the need for a personalised approach to treatments and therapies (Vicente et al., 2020, Vogenberg et al., 2010) where the ‘one-size-fits-all’ concept is being replaced with the knowledge and understanding that tailoring therapies to suit individual patients or patient groups is a much more effective way of ensuring beneficial health outcomes. The purpose of this review is to provide a comprehensive understanding of the pharmaceutical strategies that are used to develop ODDF systems for the delivery of TPPs. An overview of the physical and biochemical barriers to the absorption of TPPs, as well as strategies

employed to increase absorption is discussed in this review, to highlight the factors that must be considered when developing formulation strategies for enhanced protection and absorption of TPPs. Additionally, the advantages of using well-established and simple manufacturing technologies to fabricate TPPs in ODDF systems are discussed, showing the promise of delivering complex APIs in a simple formulation and its implications for easier transport and storage to treat vulnerable patients worldwide. Furthermore, novel manufacturing technologies are also demonstrating promise in this area indicating a potential shift in manufacturing approaches applied to complex molecules. Considerations of future perspectives for delivery of TPPs in an ODDF will also be discussed.

Challenges in the formulation of oral therapeutic proteins and peptides

Mechanisms of absorption of TPPs in the oral cavity and gastrointestinal tract

The extent of absorption of an intact TPP, across mucosal surfaces into the systemic circulation, is determined by encounters with physiological barriers designed to either transport small peptides that have been first digested and degraded in the intestinal lumen, and then subjected to further possible degradation by lysosomal enzymes in the intracellular cytoplasm of enterocytes. The oral cavity, on the other hand, presents buccal and sublingual routes of delivery which allow absorption into the systemic circulation without the delay in absorption through the GIT, therefore bypassing the hostile environment of the stomach and small intestine as well as avoidance of hepatic first pass metabolism (Morales et al., 2021, Trincado et al., 2021). Consequently, ODDFs developed for sublingual and buccal delivery are also being investigated as possible sites for rapid delivery and absorption of TPPs that can be used in emergency or ‘on-demand’ medical events (Bae et al., 2018, Zhu et al., 2018). An additional consideration, in terms of administration of TPPs, is mucosal vaccines. The formulation of ODDFs as vaccine delivery systems is well underway and several examples will be presented below. The mucosal sites are very attractive for the induction of mucosal immunity and studies have demonstrated that delivery of vaccine antigens to the mucosa can elicit immune responses to distant sites far from the site of administration, including systemic immunity (Holmgren and Czerkinsky, 2005, Miquel-Clopés et al., 2019).

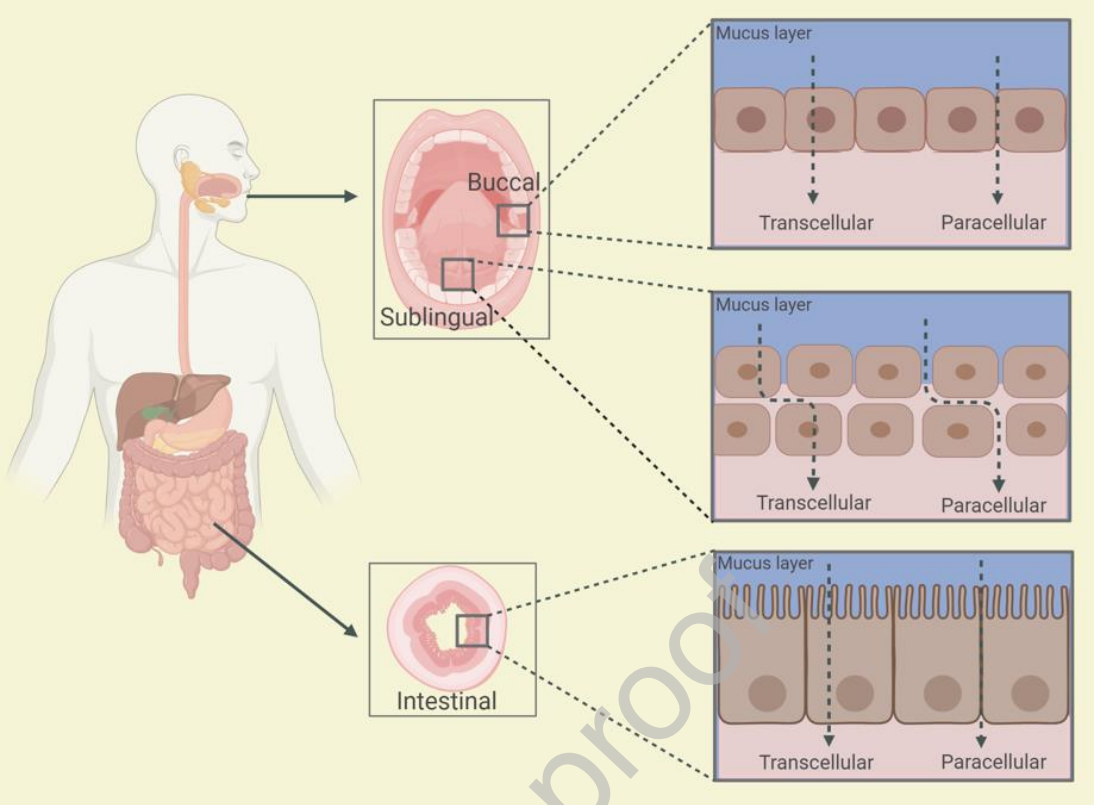

Fig 1: The mechanisms of transport for TPPs across buccal, sublingual, and intestinal epithelium. Paracellular transport is the passive diffusion through the tight junctions between adjacent cells and is the preferred route for hydrophilic TPPs and is dependent on molecular size and charge. Formulation strategies are usually required to facilitate transport between the intercellular spaces, particularly for large molecular weight TPPs. The permeability potential of sublingual epithelial cells is greater due to the loose packing allowing faster rates of absorption. Passive transcellular diffusion, where TPPs are transported through the cells, along a concentration gradient, is the favoured route for neutrally charged or lipophilic TPPs as molecules must pass through the lipid bilayer of the epithelial cells. Drawn on Biorender.com.

The possible pathways for the absorption of TPPs across mucosal surfaces such as the intestinal lumen, buccal and sublingual regions predominantly involve the paracellular route of transport (Figure. 1). This route comprises the passive diffusion of molecules/substances through the aqueous intercellular spaces, also known as the 'tight junctions' between epithelial cells that line the mucosal surfaces (Ménard et al., 2010). There is a large body of evidence to show that tight junctions are involved in the transport of macromolecules and is the rate-determining step for their permeation (Laksitorini et al., 2014, Ménard et al., 2010, Monaco et al., 2021). This is the most likely route of transport for TPPs because they exhibit poor membrane partitioning (Ménard et al., 2010, Edelblum and Turner, 2015). There are very few clinically approved oral TPPs in use due to the many challenges of achieving appropriate therapeutic plasma-concentrations. Table 1 lists some examples of TPPs that are currently licensed for oral administration, and Table 2 details a selection of TPPs that are under clinical trials.

Passive transcellular diffusion is the transport of molecules across the mucosal membrane via passive diffusion through epithelial cells, along a concentration gradient (Fig. 1). This mechanism of

absorption for TPPs is inherently reliant on the physicochemical properties of the molecule. The physicochemical characteristics of TPPs will determine the extent of diffusion through the enterocytes. In general, diffusion depends on molecular weight and size, charge, hydrogen bond potential and structural conformation (Burton et al., 1991). Most TPPs have low lipophilicity and high molecular weight and so their absorption is limited by this route. Transcellular transport is further divided into different mechanisms, such as carrier mediated transport (active transport and facilitated diffusion) and endocytosis (pinocytosis, phagocytosis, receptor mediated endocytosis and transcytosis) (Xu et al., 2019, Artursson et al., 2001). Cyclosporin A is one of the few oral peptides that has a high bioavailability (>30%) owing to its conformational flexibility where it is resistant to hydrolysis in biological fluids (Wang and Craik, 2016) and can passively diffuse across lipophilic cell membranes (Dougherty et al., 2019), by adopting a 'closed' structure which shields the amino acid backbone from being cleaved by gastric and intestinal enzymes, and also increases the lipophilicity of the molecule.

In order to increase the permeability of drugs such as TPPs, reversible methods for reducing the barrier potential of the buccal mucosa have been employed (Pather et al., 2008). For example, the buccal absorption of a potent peptide, *Stichodactyla helianthus* neurotoxin (ShK), being investigated for the treatment of autoimmune diseases such as multiple sclerosis and rheumatoid arthritis, was evaluated *in vitro*, as well as *ex vivo*. Although the permeation of the peptide was found to be poor when evaluated on untreated porcine buccal mucosal tissue, the addition of surfactants and bile salts to the formulation was found to be a successful enhancement strategy. The mechanism of absorption was not fully understood, however it was postulated that the addition of chemical enhancement agents caused a reversible disruption of the paracellular route of diffusion thereby increasing permeation of the peptide through the intercellular spaces between the epithelial cells of the buccal mucosa. Another approach used to enhance delivery of TPPs to the systemic circulation is by loading TPPs into nanoparticle delivery systems. Kotak et al (2020) encapsulated salmon calcitonin (SCP) into Hydroxyapatite nanoparticles (HAP-NPs) for bone targeted delivery using the sublingual route of administration. HAP was selected because it is a natural bone component and therefore, the HAP-NPs accumulate around bone. The C_{max} obtained for SCP-HAP-NPs translated to ~15% bioavailability which was much higher than the commercially available intranasal formulation that only exhibited 3% bioavailability.

Table 1: Examples of clinically approved oral TPPs

TPP	Structure	Therapeutic indication	Dosage form	References
Insulin	51-amino acid linear	Type I and 2 diabetes	Buccal oral	(Heinemann and

		Approved for use by FDA in 2009	spray (Oral-lyn®)	Jacques, 2009) (Iyire et al., 2016)
Standardised allergens	Wide variety of secondary structure compositions with tertiary folds	Allergy immunotherapy Approved for use by FDA in 2014	Sublingual tablet	(Dall'antonia et al., 2014, Lund et al., 2019)
Cyclosporin A	Cyclic undecapeptide with non-canonical N-methylated amino acids	Graft-versus host disease in transplant patients Oral dosage form approved for use by FDA in 1983	Capsule	(Dougherty et al., 2019, Kolata, 1983, Wang and Craik, 2016)
Oral Desmopressin acetate	Nonapeptide with a six amino acid ring structure. Synthetic analogue of vasopressin	Central diabetes insipidus Primary nocturnal enuresis Oral dosage form approved by FDA in 2008	Tablet Sublingual tablet	(Drucker, 2020, Lundin and Artursson, 1990, Pantzar et al., 1995)
Semaglutide	31-amino acid linear GLP-1 receptor agonist analog	Type 2 diabetes Oral dosage form approved for use by FDA in Sept 2019	Tablet	(Drucker, 2020, Brayden et al., 2020, Chen et al., 2022, Twarog et al., 2021)
Octreotide	Cyclic octapeptide, somatostatin-analog	Long-term maintenance of Acromegaly Oral dosage form approved for use by FDA in 1988	Tablet	(Drucker, 2020, Brayden et al., 2020, Tuvia et al., 2012)

Table 2: Examples of TPPs in clinical trials (clinicaltrials.gov).

TPP	Sponsoring company	Disease or purpose of the study	Mode of Administration	Clinical trial status	Trial Number
Insulin	Oshadi Drug Administration	Diabetes	Oral	Phase I	NCT01120912
	Technical university of Munich	Type 1 diabetes	Oral capsule	Phase II	NCT03364868
	Oshadi Drug Administration	Type 1 diabetes	Oral	Phase II	NCT01973920
	Technical university of Munich	Type 1 diabetes	Oral capsule	Phase II	NCT02620072
	Oramed Ltd	Type 2 diabetes	Oral	Phase III	NCT04754334
Leuprolide	Enteris Biopharma Inc	Endometriosis	Oral tablet	Phase II	NCT02807363
Parathyroid	Rani Therapeutics	Hypoparathyroidism	Oral RaniPill®	Phase I	NCT05164614

hormone	Entera Bio Ltd	Hypoparathyroidism	capsule Oral	Phase II	NCT03516773
Salmon calcitonin	Nordic Bioscience A/S	Osteoarthritis	Oral tablet	Phase III	NCT00704847
GABA (B) R1 (extracellular) blocking peptide (GB001)	Gossamer Bio Inc Zhejiang Echon Biopharm Limited	Chronic rhinosinusitis Observational study	Film-coated oral tablet Buccal spray	Phase II Phase I	NCT03956862 NCT05125211
Oral peptide BBT-401-1S	Bridge Biopharmaceutics	Ulcerative colitis	Oral capsule	Phase I	NCT03482648
Grass pollen peptide (gpASIT+TM)	BioTech Tools S.A.	Seasonal allergic Rhinoconjunctivitis	Entero-coated capsules	Phase I	NCT00813046
Optiquel® (B27PD)	National Eye Institute (NEI)	Uveitis	Oral capsule	Phase I/II	NCT01195948
MV130 (whole-bacteria vaccine)	Immunotek S.L.	Induction of trained immunity	Sublingual suspension	Phase I/II	NCT05208060

Barriers to oral delivery of TPPs in the oromucosal cavity

Sites of absorption within the oral cavity are particularly advantageous for the delivery of TPPs in comparison to the GIT due to lower enzymatic activity, more favourable pH conditions and drainage of blood vessels directly into the jugular vein leading to the superior vena cava, therefore by-passing hepatic first-pass metabolism. Sublingual and buccal mucosa provide suitable absorption sites for macromolecules since they comprise 60% of the surface area within the oral cavity (Patel et al., 2011). The sublingual mucosa is thinner which makes it suitable for rapid absorption whereas the thicker mucosal layer of the buccal region can be used if slow onset and longer duration of action is required (Morales et al., 2021). Therefore, buccal and sublingual sites are particularly useful, as an alternative to the oral route, for the absorption of fragile TPPs that would otherwise have extremely low bioavailability when absorbed through the intestinal epithelium (Pather et al., 2008, Hua, 2019). Nevertheless, similar to other mucosal surfaces, the oral cavity comprises absorption barriers that must be overcome. The two main routes of transport for molecules across the oral mucosa is paracellular and transcellular transport (Pather et al., 2008) where the paracellular route is favourable for small hydrophilic moieties such as TPPs and diffusion is dependent upon a concentration gradient. The transcellular route is suitable for lipophilic compounds where transport is mediated by passive diffusion. Below is a brief overview of the anatomical structure of the buccal and sublingual mucosa including differences and similarities which are exploited to obtain different rates of absorption from rapid absorption in the sublingual mucosa to a more controlled release or topical application using the buccal region of the oral cavity. By understanding the anatomy of the

oral mucosa, TPP candidates can be identified and developed for incorporation into ODDFs for effective delivery systems that are patient-friendly and non-invasive.

Advantages and limitations of buccal and sublingual delivery

There are several types of mucosal tissue found within the oral cavity, 40% of which consist of keratinised mucosal regions that make up the gum (gingiva), palatal mucosa, inner side of the lips and parts of the tongue. Keratinized mucosa contains lipids such as ceramides and acylceramides organised in a compact, ordered (lamellar) structure in the intercellular spaces (Guo and Pratap Singh, 2019). It is unlikely that significant amounts of TPPs, or macromolecules in general, would penetrate in between the compact epithelial cells of keratinized tissue. In contrast, 60% of the mucosal surface in the oral cavity, namely the buccal and sublingual region, consist of non-keratinised tissue (Guo and Pratap Singh, 2019). The permeability potential of the sublingual and buccal mucosa allows for the absorption of a wider range of molecules because they consist of non-keratinised tissue existing in a more amorphous and fluid state offering less hindrance to absorption of substrates (see Fig 1). Due to the loose packing of non-keratinised tissue in the buccal and sublingual region, it has been suggested that the preferable route to absorption for molecules is the paracellular route by passive diffusion. Although the transcellular route of transport co-exists with other mechanisms of absorption, the paracellular route provides the least resistance to molecules crossing the buccal and sublingual mucosa (Guo and Pratap Singh, 2019, Hua, 2019).

The main difference between buccal and sublingual tissue is the degree of stratified epithelial layers: the buccal epithelia is composed of 40-50 layers of non-keratonised cells amounting to a thickness of 500-800 µm, and the sublingual mucosa consists of 8-12 non-keratonised layers of epithelial cells resulting in a thickness of about 100-200 µm (Morales et al., 2017). Other significant differences that can impact formulation design and drug delivery is that the buccal mucosa provides an area of 50 cm^2 for absorption, in comparison to 27 cm^2 for the sublingual mucosa (Morales et al., 2017). Furthermore, saliva does not accumulate on the buccal surfaces whereas saliva tends to pool and accumulate in the sublingual region. Both epithelia are well-vascularised, in comparison to the GIT, with blood vessels draining directly into the jugular vein, therefore avoiding hepatic metabolism. Consequently, pharmaceutical agents targeted for delivery in the buccal and sublingual mucosa will be rapidly absorbed into the systemic circulation (Morales et al., 2021). Figure 2 summarises the barriers to absorption of TPPs in the oral cavity.

There is a layer of gelatinous mucus covering the buccal and sublingual epithelium that varies from 40 to 300µm (Teubl et al., 2013, Hua, 2019) and plays an important role in mucoadhesion of formulations such as films and tablets. Mucins, composed of disulfide-linked subunits, adhere to the

surface of the oral epithelium and represent another penetration barrier (Cone, 2009). Saliva is secreted from salivary glands and primarily consist of water (95-99%), enzymes, inorganic salts, lipids, and glycoproteins (mucins), with a pH ranging between 6.2-7.4 (Hua, 2019) and a normal (unstimulated) flow of approximately 100-500 mL/min. An increase in salivary flow due to stimulatory factors can impede the attachment of drug delivery systems to the oral mucosa, as can swallowing, talking and chewing (Fábián et al., 2012).

The buccal and sublingual mucosa has a low surface area available for molecular absorption (relative to the GIT), and the constant flow of saliva can reduce the transport of macromolecules across the mucosa through clearance of such molecules into the throat. In addition, molecules with a strong taste are not desirable for buccal and sublingual delivery and therefore taste issues must be considered prior to application (Almukainzi et al., 2019). Although some of these limitations may not be controlled, the benefits associated with buccal and sublingual mucosal administration outweigh the limitations, making it a promising route for the administration of macromolecular therapeutics, such as TPPs.

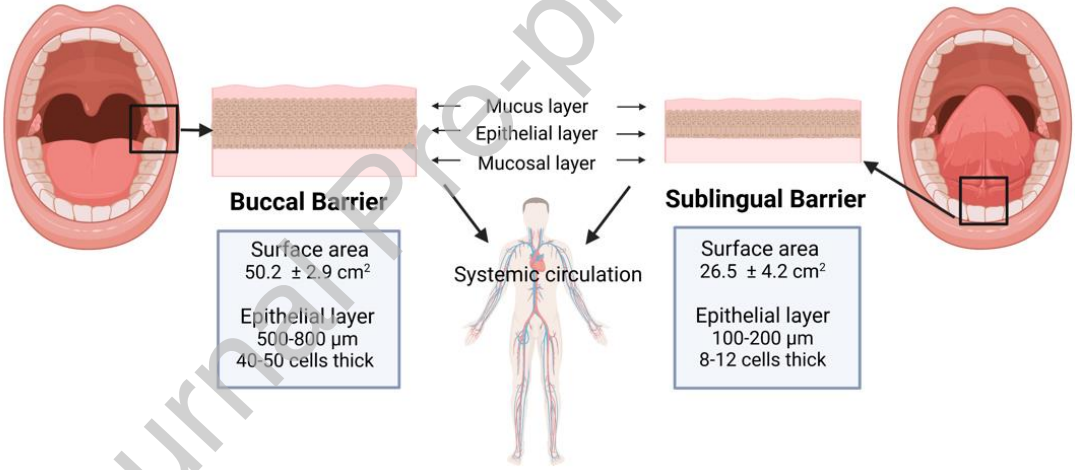

Fig 2: Barriers to the absorption of TPPs through the buccal and sublingual mucosa within the oral cavity. Drawn on Biorender.com.

Barriers to oral delivery of TPPs in the gastrointestinal tract

The oral delivery of TPPs in the GIT is very challenging given the harsh gastric environment which subjects large macromolecules to degradation due to the acidic pH of the stomach and abundance of digestive enzymes. These biological and chemical barriers cause denaturation of the molecular folding of TPPs leading to exposure of the amino acid backbone to enzymatic cleavage. Mucus can also exert several barriers to the absorption of TPPs. The main component of mucus consists of mucin glycoproteins which determines the functionality of the mucus layer by forming cross-linked networks that contribute to the selective physicochemical barrier properties by acting as molecular

sieves and/or providing binding sites (Cone, 2009). The diffusion coefficient of large molecular weight proteins has been demonstrated to decrease with increasing molecular weight, in porcine and intestinal mucus (Bernkop-Schnürch and Fragner, 1996). The diffusion of large molecular weight proteins (>12.4 kDa) is considered almost impossible, due to the brush-like scaffold structure of mucins (Boegh and Nielsen, 2015). Hindrance of TPP absorbance occurs by the non-covalent interactions between TPPs and that of mucin fibres, due to Van der Waals, electrostatic and hydrophobic interactions as well as hydrogen bonding (Cheng and Leblond, 1974, Capaldo et al., 2017, Vancamelbeke and Vermeire, 2017).

Consequently, most TPPs have very poor bioavailability and short half-lives leading to a significantly reduced therapeutic effect when administered orally. Nevertheless, the utilisation of the oral route to administer TPPs could be advantageous particularly for ongoing treatment of chronic conditions such as Type 1 and 2 diabetes. An oral pharmaceutical formulation incorporating TPPs must, therefore, be designed to protect and shield the TPP from the harsh gastric environment for successful therapeutic benefits. Several innovative approaches and technologies are under investigation to overcome the physiological and biological barriers of the GIT to enhance the absorption, penetration, and diffusion of TPPs to achieve a pharmacologically significant bioavailability (Brown et al., 2020, Zhu et al., 2021, Bruno et al., 2013).

2. Strategies for enhancing mucosal delivery of oral TPPs

An inherent drawback of delivering TPPs to the GIT is that a significant proportion will be degraded in the stomach and intestinal lumen and subsequent absorption of any intact TPP will then be subjected to hepatic first pass metabolism. However, studies have demonstrated that TPPs can cross the intestinal epithelia if they survive hydrolysis and digestion in the GIT (Pappenheimer et al., 1994). Semaglutide, the glucagon-like peptide 1 (GLP-1) agonist used for the treatment of Type 1 and 2 diabetes, has recently been formulated into an orally administered tablet (Kim and Jung, 2021, Hedrington and Davis, 2019). Previously available as a subcutaneous, once-weekly injection, semaglutide, was combined with salcaprozate sodium (SNAC), a permeation enhancer, and was found to be almost exclusively absorbed in the stomach demonstrating the effectiveness of the excipient in enhancing survival of the peptide drug in gastric conditions (Kim and Jung, 2021, Twarog et al., 2019).

The bioavailability of TPPs that show poor intestinal absorption can be improved by using the buccal and sublingual route of delivery where much lower doses are required because systemic absorption will bypass the various challenges encountered in the GIT. Experimental investigations of TPP delivery through the buccal and sublingual mucosa have demonstrated that penetration of these agents through the oral mucosa is possible, providing examples of promising strategies (Paris et al., 2021, Jin et al., 2015). Regardless of the route of administration, absorption across the mucosal epithelium is the rate-limiting step and is determined by several factors including the molecular structure of the TPP as well as the absorption site. For example, the epithelial lining of the buccal region is thicker than that of the sublingual and intestinal mucosal sites. There are several strategies that can be applied by which the absorption of TPPs can be enhanced and protection from environmental exposure can be minimised to obtain efficient and optimal delivery. Table 3 summarises some recent pre-clinical studies that have investigated the absorption of TPPs for delivery to buccal, sublingual and GIT sites, illustrating the rapidly growing interest in the oral delivery of biological material.

More detailed reviews of strategies used to design novel oral TPP formulations in order to protect them from biochemical barriers and enhance delivery across mucosal membranes, can be found in the literature (Chen et al., 2022, Caon et al., 2015, Dan et al., 2020, Brayden et al., 2020). The summary provided below covers the most important formulation strategies that are applicable to this review.

Permeation/absorption enhancers are substances that facilitate permeation through mucosal surfaces and can be co-administered with TPPs to increase their ability to cross the mucus or epithelial cell layers (Twarog et al., 2019). A suitable criterion for these agents is a good safety profile, non-toxic, pharmacologically, and chemically inert, non-irritant and non-allergic. Many potential candidates have been investigated to enhance permeability in the GIT but were found to be toxic. Some successful permeation enhancers are surfactants, bile salts, fatty acids and chelating agents (Danielsen, 2021). Permeation enhancers are specific to absorption sites as well as the co-administered API, and must be carefully selected to ensure lack of toxicity and avoid irreversible damage to the epithelial cells. TPPs such as calcitonin (Oh et al., 2011), luteinising hormone releasing hormone (LHRH) (Nakane et al., 1996) and α-interferon (Steward et al., 1994) show significantly increased bioavailability via buccal mucosa by the addition of permeation enhancers such as surfactants or bile salts. Whereas the co-administration of the GLP-1, semaglutide, with the permeation enhancer SNAC demonstrated increased absorption through the gastric mucosa. The mechanism of SNAC is not fully understood but it is believed to increase lipophilicity of macromolecules, as well as elevating pH and inhibiting pepsin activity (Twarog et al., 2021). A clinically available oral insulin formulation comprising a buccal delivery system, Oral-Lyn®(Generex Biotechnology Corporation, Toronto, Canada) utilises a propellant spray mechanism to deliver insulin to the buccal region (Heinemann and Jacques, 2009). Insulin is entrapped within surfactant-based micelles which enhances mucosal barrier permeation of insulin within the lipoidal carrier. The drawback to this drug delivery system is that ten sprays is required to achieve pharmacologically significant doses that has led to patient non-compliance (Heinemann and Jacques, 2009, Macedo et al., 2020).

Chemical modification of TPPs can enhance stability and improve membrane penetration, decrease immunogenicity, and improve resistance to proteolytic degradation. Possible ways of modifying the structure of TPPs is by modification of the amino acid side-chains, altering the carbohydrate moieties of glycoproteins or by conjugating to lipophilic molecules such as fatty acids to increase hydrophobicity, since the unionized form is the preferred form of a molecule for absorption by passive diffusion across biological barriers (Zhu et al., 2021). Desmopressin acetate, a synthetic peptide, is marketed as Minirin and is used for the treatment of central diabetes insipidus and nocturnal enuresis (Drucker, 2020). It has very low bioavailability (0.17 %) despite being chemically modified to improve its stability by resisting enzymatic degradation, in comparison to its native counterpart (Drucker, 2020). Due to high specificity and potency, desmopressin acetate is an effective therapeutic agent, despite such low bioavailability.

Enzyme inhibitors and pH modulation is an alternative to structural alteration. These agents can be co-administered with TPPs and reduce enzymatic activity or alter the pH of the microenvironment.

Enzyme inhibitors such as the protease inhibitors including bacitracin and cyclic dodecapeptide, or pancreatic inhibitors such as soybean trypsin, will increase the bioavailability of TPPs by reducing enzymatic degradation. Modulation of pH can affect the delivery of TPPs at the mucosal surfaces (intestinal and oromucosal) by ensuring the molecules are in an unionised form to maximise permeability or through enhanced stability (Brown et al., 2020).

Addition of a mucolytic agent such as *N*-acetylcysteine, cleaves di-sulphide bonds of steric components within the mucous layer such as mucins, thereby increasing permeability of TPPs (Bernkop-Schnürch and Fragner, 1996). The coadministration of *N*-acetylcysteine and a penetration enhancer was also found to enhance absorption (22.5-fold in comparison to control) of hydrophilic compounds in rat intestines (Takatsuka et al., 2006).

Mucoadhesive polymers such as hydroxypropyl methylcellulose (HPMC) (Morales et al., 2013) and chitosan (Batista et al., 2019) adhere to mucosal surfaces to provide a platform for intestinal and oromucosal drug delivery of TPPs. Polymers that adhere to mucosal surfaces are hydrophilic in nature with viscoelastic properties. They consist of numerous hydrogen-bond forming groups, displaying the ability for the long polymeric chains to interpenetrate into mucus and epithelial tissue. Hydrophilicity and high molecular weight of mucoadhesive polymers increases the solubility of poorly absorbed TPPs whilst protecting them from enzymatic degradation. By prolonging contact time with mucosal surfaces, the residence time of TPPs with absorption sites is increased. The most commonly used mucoadhesive polymers are anionic polymers, examples being carboxymethylcellulose (CMC) (Rahman et al., 2021) and sodium alginate (Szekalska et al., 2019).

Chitosan, a cationic polymer is extensively used as a mucoadhesive and permeation enhancer to deliver TPPs and small drug molecules. It is soluble in acidic conditions therefore not suitable as an intestinal permeation enhancer, however, van der Merwe et al was able to demonstrate that a derivative of chitosan, *N*-trimethyl chitosan, could decrease the transepithelial electrical resistance (TEER) in caco-2 cells thereby enhancing the permeation of hydrophilic compounds such as [14C]-mannitol across the cell monolayers. Confocal laser scanning microscopy was able to confirm the transport of large hydrophilic compounds via the paracellular route (van der Merwe et al., 2004). The most common formulation strategy employed for this biodegradable, biocompatible polymer is to load the therapeutic agents into fabricated nano- or microparticles for mucosal drug delivery (Batista et al., 2019), or prepare mucoadhesive films in which the therapeutic agent can be dispersed. Chitosan can also be conjugated or associated with additional excipients to alter its physicochemical properties for tailored or targeted delivery. For example, Kristó et al demonstrated that the fabrication of mucoadhesive buccal films with ascorbic acid enhanced the permeability properties of a buccal drug delivery system by acting as a plasticizer and reducing the brittleness of

the film (Kristó et al., 2022). As an alternative approach, Batista et al loaded a TPP into chitosan microparticles which were subsequently dispersed throughout a guar gum oral film for buccal and intestinal delivery (Batista et al., 2021). A more recent and novel study investigated the use of chitosan as a coating for liposomes encapsulating anionic albumin, where the mucoadhesive properties of chitosan increased contact time with buccal mucosa (Sahatsapan et al., 2022).

Stabilisers can provide both physical and chemical stability to TPPs. It is important to preserve and maintain the structure of TPPs as this will determine their therapeutic and pharmacological activity. Stabilising excipients ensure hydration of the TPPs by drawing water molecules to the surrounding microenvironment thereby providing stability during manufacturing and storage. Typical excipients that possess this hydration approach are amino acids such as arginine, glycine and histidine, and sugars including trehalose (Geeraedts et al., 2010), glucose and sucrose. Alternative means of conferring stability to TPPs is the addition of metal ions that can bind to sites on the proteins preventing unfolding and aggregation. Sugars such as mannitol and dextrans are also added to ODDFs as cryoprotectants and lyoprotectants (Izutsu et al., 1994, Sun and Davidson, 2001), as well as bulking agents to improve the appearance and smoothness of the final product (Jorgensen et al., 2009).

Particulate systems such as emulsions, liposomes, micro- and nanoparticles are widely investigated and are a promising approach to enhancing delivery of TPPS (Wilkhu et al., 2017, Tian et al., 2020, Jhaveri and Torchilin, 2014, Holpuch et al., 2010, Yun et al., 2013). They are colloidal systems (typically ranging between 10 nm to 10 µm) comprising of the TPP incorporated within the core, or adsorbed onto the surface, of the particulate structure. The TPP loaded particulate system can facilitate absorption by increasing the half-life and improving stability by shielding from the microenvironment within the GIT. A guar gum film incorporated with a bioactive peptide loaded into PLGA nanoparticles was shown to have enhanced permeation in porcine buccal tissue as well as porcine intestinal tissue cell lines, demonstrating the prolonged survival and stability conferred by the polymeric nanoparticles to transport the peptide across the epithelia (Castro et al., 2019). Liposomes are lipid-based carriers that in the pure form are inherently unstable in biological fluids and inefficient at encapsulating TPPs. However, to produce stable liposomes, cholesterol or bile salts are added to improve delivery characteristics (Wilkhu et al., 2014). Sahatsapan et al investigated a novel drug delivery system by constructing liposomes loaded with albumin, which were then coated with a chitosan derivative, chitosan maleimide, using a thin-film hydration technique. The coated liposomal drug delivery system was able to show improved permeation of albumin through buccal mucosa with the protein remaining stable throughout the fabrication process as well as after absorption (Sahatsapan et al., 2022). Figure 5 illustrates the encapsulation of TPPs within some particulate systems.

Table 3: Examples of current pre-clinical studies to enhance absorption of TPPs in the oral cavity and GIT

TPP	Therapeutic use	Route of administration	Formulation strategy	Reference
Insulin	Antidiabetic	Buccal	Insulin conjugated with a cell penetrating peptide (INS-PEG-LMWP conjugate) to enhance absorption	(Xu et al., 2020)
Insulin	Antidiabetic	Buccal	Gels comprising nanovesicles loaded with insulin-phospholipid complex	(Guo et al., 2022)
KGYGGVSLPEW	Antihypertensive peptide derived from whey protein	Buccal	Incorporated within PLGA nanoparticles and embedded in a guar gum matrix to form a film	(Castro et al., 2018)
Albumin	N/A	Buccal	Albumin loaded anionic liposomes coated with chitosan maleimide	(Sahatsapan et al., 2022)
Alpha-casozepine bioactive peptide	Anxiolytic	Buccal and Intestinal	PLGA nanoparticles in guar gum film	(Castro et al., 2019, Batista et al., 2021)
Liraglutide (GLP-1 agonist analogue)	Antidiabetic	Sublingual	Drug delivery system comprising Liraglutide loaded bovine serum extracellular vesicles derived from milk	(Xu et al., 2022)
Peptide epitope from *Mycobacterium tuberculosis*	Vaccine	Sublingual	Peptide forms self-assembled nanofiber structures and modified with polyethylene glycol	(Kelly et al., 2020)
Salmon calcitonin	Osteoporosis	Sublingual	Salmon calcitonin loaded hydroxyapatite nanoparticles	(Kotak and Devarajan, 2020)
Insulin	Antidiabetic	Sublingual	Insulin loaded into sodium alginate/polyethylene glycol scaffolds produced by 3D printing	(Erzengin et al., 2022)
Desmopressin	Nocturnal enuresis Diabetes insipidus	Sublingual delivery	Electrospun nanofiber-based hybrid film	(Stie et al., 2022)
Walnut derived bioactive pentapeptide PW5	Chronic diseases	Intestinal	Pure peptide solution (>99%)	(Wang et al., 2022)

3. Orally disintegrating dosage forms (ODDFs)

ODDFs can be broadly classified into three categories: Orally disintegrating films (ODF); orally disintegrating tablets (ODT); and orally disintegrating mini-tablets (ODMT).

Advantages

ODDFs have a myriad of benefits both from a patient-use perspective as well as having a pharmaceutical advantage (Comoglu and Dilek Ozyilmaz, 2019). From a patient perspective, ODDFs do not need water to be ingested and they disintegrate rapidly in the mouth making them a practical choice for taking medicines on the go and in areas where access to clean water is limited (Patil, 2014). ODDF doses do not need manipulation or measurement as compared to liquid dosage forms, thereby reducing the risk of dosing errors and caregiver interventions, if applicable. Another key advantage of ODDFs are their ease of swallowing which is especially beneficial for patients with swallowing difficulties such as patients with dysphagia following a stroke (Liew et al., 2013). This is also a key benefit to the paediatric population as they tend to struggle to swallow solid dosage forms such as tablets and capsules, with a study citing the average age to be able to swallow solid dosage forms being 6 years or above (Meltzer et al., 2006). ODDFs can therefore be classified as age-appropriate dosage forms that can be tailored to suit the need of the paediatric population. In addition, a study has shown that a third of all adults have difficulty swallowing traditional solid dosage forms (Radhakrishnan et al., 2021) and furthermore, ODDFs can also reduce risks of choking associated with difficulty in swallowing. The benefits aforementioned are mostly related to their ease of use from a patient perspective. Additionally, ODDFs have pharmaceutical benefits. Due to their fast disintegrating and rapid release of the API, the onset of action is increased, which is especially beneficial in indications requiring fast actions, such as migraine relief (Vrbata et al., 2013). Other indications that would benefit from fast onset of action include medicines for travel sickness, gastrointestinal disturbances, allergies, and medicines used as anxiolytics and antipsychotics. In addition, as the dosage forms disintegrate rapidly in the oral cavity, pre-gastric absorption is evident which further enhances the bioavailability of the drug delivered (Hannan et al., 2016).

Limitations

The fact that ODDFs disintegrate rapidly in the mouth can be both an advantage and limitation of the system when it comes to palatability. Palatability can be defined as the overall ability and willingness of a patient to take a medicine, and encompasses qualities such as taste, mouthfeel and even the handling of the dosage form (Mohamed-Ahmed et al., 2016). Mouthfeel might be an issue

as when the dosage form such as an oral film or ODT start to breakdown, a feeling of grittiness or astringency may develop which could be aversive to the patient and affect compliance in the long run (Abdelhakim et al., 2020). Nonetheless, these issues can be mitigated with the appropriate formulation strategy. Taste is another significant issue relating to ODDFs, as when the drug is released in the oral cavity, it is in contact with the taste buds on the tongue and therefore if the drug is bitter, patients would perceive this bitterness which again may be aversive and could affect compliance. Similar to mouthfeel, taste-masking strategies exist and depending on the API used these can be manipulated to formulate a taste-masked ODDF (Walsh et al., 2014).

In addition to palatability, a key limitation of ODDFs is the fact they usually do not offer dose flexibility. Liquid dosage forms can be measured into different amounts, tablets can be split in half, and mini-tablets can be adjusted to count depending on the dose, but ODDFS are usually harder to be formulated as flexible dosage forms due to their hygroscopic nature (Lopez et al., 2015).

4. Manufacturing strategies for the fabrication of oral TPPs in orally disintegrating dosage forms

The most common manufacturing methods employed for the fabrication of ODDFs are traditional, well-established technologies such as direct compression for ODTs and ODMTs (Olah et al., 2019, Lund et al., 2019) and freeze-drying (also known as lyophilization) (Wilkhu et al., 2017) or solvent-casting for ODFs (Morales et al., 2013, Heinemann et al., 2013, Tavares et al., 2011). Since the delivery system itself is a simple and straightforward technology, the incorporation of TPPs within fast-dissolving tablet formulations requires manipulation of excipients and process parameters in order to protect TPPs during manufacturing and long-term storage, to ensure the TPP does not denature or lose pharmacological activity and confer immediate disintegrating properties to the solid-state dosage form.

However, over the past few decades electrohydrodynamic (EHD) techniques have drawn a great deal of interest to encapsulate TPPs as they do not involve heat in the manufacturing process. EHD techniques such as electrospinning have demonstrated the production of TPPs in solid-state formulations such as tablets and films, with work ongoing to explore scaling up of the manufacturing process (Moreira et al., 2021, Vass et al., 2019). In contrast, 3D printed TPPs in ODDFs would offer the possibility of personalised therapeutics that can address the specific needs of individual patients and is being widely explored for the manufacturing of drug delivery systems (Samiei, 2020) including delivery of TPPs (Montenegro-Nicolini et al., 2017). Below, is an overview of manufacturing strategies for the incorporation of TPPs in ODDFs.

Orally disintegrating tablets (ODT) and mini-tablets (ODMT)

ODTs are uncoated tablets that disintegrate rapidly in the mouth without the intake of any liquid, before being swallowed. The European Pharmacopoeia (Ph.Eur.) defines a maximum disintegration time of three minutes, therefore, any manufacturing method used to fabricate ODTs needs to fulfil this requirement. On the other hand, the U.S. Food and Drug Administration (FDA) defines an ODT as a tablet with maximum disintegration time of 30 seconds, which may be more relevant from a patient perspective (Kokott et al., 2021).

Direct compression

Directly compressing powder to form tablets is one of the most traditional methods to formulate a medicine due to its low manufacturing costs and scale up potential. Direct compression (DC) allows

for the tabletting of a blend of ingredients without prior steps such as granulation. Although it is a relatively simple process, DC is directly impacted by the properties and amounts of the materials used and thus careful optimisation is needed (Thoorens et al., 2014). Adding specific superdisintegrants can give rise to ODTs, whilst changing the die punch size allows for fabrication of ODMTs. Famotidine ODTs were prepared using a single punch tablet press by direct-compression using either Ac-Di-Sol, or Primojel, or Low-substituted hydroxypropylcellulose (L-HPC) as superdisintegrants; the authors found that L-HPC showed superior performance with the highest dissolution (Abdelbary et al., 2009). A study by Hesari *et al*. (2016) explored the use of directly-compressed taste-masked ranitidine via complexation with cellulose derivatives, leading to an ODT with rapid disintegration time (Hesari et al., 2016) and an improved taste.

To overcome swallowing and mouthfeel issues, ODMTs offer a more acceptable formulation to specific populations such as the elderly and paediatrics. Enalapril maleate 2 mm ODMT tablets were fabricated using a rotary tablet press and using co-processed excipients such as Hisorad and Ludiflash, the tablets should have adequate content uniformity which is the largest challenge for low dose mini-tablets (Kokott et al., 2021).

Lyophilization

Lyophilization or freeze drying is a process of removing water from a product at a low temperature by sublimation and desorption (Tang and Pikal, 2004). The process starts with freezing the product and then subjecting it to a high vacuum for the water to sublime (Garcia-Amezquita et al., 2016). The process parameter such as freezing temperature and time, chamber pressure, container type, product composition and concentration must be optimized to maintain the product's chemical and physical stability (Franks, 1998). Cryoprotectants such as quaternary amine and glycerol is usually added to protect the solute from stressing and aggregation (Chung et al., 2012). Lyophilization has been commonly used to prepare TPPs in ODTs due to the low temperature condition of the process and high yield (Bae et al., 2018). Lyophilized ODTs are highly porous in structure, providing rapid disintegration (Vanbillemont et al., 2020). Altering the composition of the formulation by adding binders and fillers such as povidone, gelatin and saccharides is employed to optimize the mechanical properties and the disintegration time of the ODTs (Chandrasekhar, 2009, Lal et al., 2013). Although freeze drying has various advantages, it is a rigorous and time-consuming process. Optimization over the different stages of the process is critical for efficient and cost-effective production (Tang and Pikal, 2004, Lal et al., 2013).

3D printing

3D printing or additive manufacturing is an emerging concept in fabricating drug delivery systems. Various 3D printing techniques have been used to prepare ODDFs, such as fused deposition modeling (FDM) (Hussain et al., 2020), stereolithography (SLA) (Martinez et al., 2017), selective laser sintering (SLS) (Fina et al., 2018), and semisolid extrusion (SSE) (Panraksa et al., 2022). The FDA has approved the first 3D-printed fast-disintegrating tablet. SPRITAM® (levetiracetam) is 3D printed using a technology called ZipDose®, the process based on the spraying of an aqueous solution to bind layers of powders to produce a porous, rapidly disintegrating tablet (West and Bradbury, 2019). The key element in selecting the most suitable 3D printing technique is sensitivity of the API and the final characteristics of the dosage form (Fina et al., 2018, Jamróz et al., 2018). SLS is based on sintering powder by laser energy and FDM is based on extruding heated filament through a narrow nozzle (Zhang et al., 2017). Both technologies were investigated to fabricate ODTs (Allahham et al., 2020, Hussain et al., 2020). However, they generate high temperatures during the printing process that make it unsuitable for thermolabile APIs. Semisolid extrusion (SSE), 3D printing, or direct ink printing allows the fabrication of multidrug in an easily customizable manner (Annaji et al., 2020). The SSE technique is based on the preparation of extrudable gel or paste (printing ink); the ink is extruded by a pressure-assisted syringe through a nozzle at an adjusted speed to follow the printing pattern layer by layer to form the designed object (Firth et al., 2018). 'Tablet-in-Syringe' is a model based on SSE 3D printing for on-site preparation of personalized ODTs (Panraksa et al., 2022). SSE offers the feasibility of printing sensitive APIs such as TPPs while avoiding harsh conditions (Seoane-Viaño et al., 2021).

Orally disintegrating films (ODFs)

ODFs can comprise one or multiple layers that contain APIs as well as suitable excipients such as adhesives to optimise function. They disintegrate rapidly in the mouth without water intake and are favoured by special patient populations due to their ease of swallowing and ease of transportation (Musazzi et al., 2020). They are mainly composed of natural polymers such as chitosan or cellulose, or synthetic polymers such as polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP). A main limitation of ODFs is the maximum drug loading, estimated to be approximately 50 mg per film (Visser et al., 2020). Figure 3 illustrates the mechanism by which a typical ODF disintegrates and liberates the active compound.

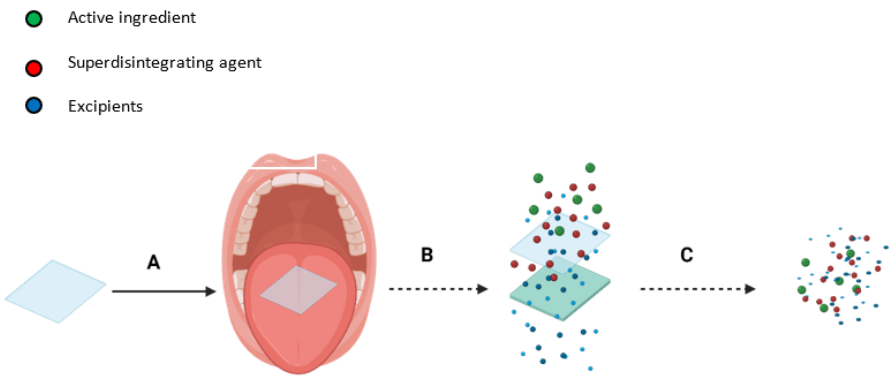

Fig 3: ODFs comprise various components, including the active compound, fast-disintegrating agents such as super disintegrants, and additional excipients such as permeation enhancers, saliva stimulators, pH modulators or mucolytic agents (A). Saliva in the mouth causes the disintegrating agent to swell creating channels for penetration of saliva (B), and the film rapidly disintegrates within 30s of contact with the tongue (C) where the active components are liberated and can be either swallowed with ease, for gastric delivery, absorbed via buccal and sublingual mucosa, or exert a local effect within the oral cavity. Drawn on BioRender.com

Solvent casting

Solvent-casting is a well-established technology that was driven by the needs of the photographic industry. In the drug-delivery arena, it has become the most traditional method for manufacturing oral films. The method entails dissolving polymers, APIs and other excipients in an appropriate solvent and then drying off the mixture. The mixture is usually dried by passing it through a drying apparatus such as an oven. The resultant dried film is then cut into strips of pre-determined sizes and individually sealed in specialised packaging. It is imperative for the solution to set without entrapped air bubbles to ensure a homogenous film is formed (Siemann, 2005). If bubbles are trapped, this could cause batch-to-batch variation which is not pharmaceutically acceptable. Solvent casting is the most widely explored manufacturing method for the formulation of TPPs in ODFs for administration to the buccal and sublingual region of the oral cavity.

One of the main advantages of the special dosage forms mentioned in this review is the improved palatability they offer. ODFs of betahistine using a cation exchange resin were successfully developed through the use of drug-resin complexes, used to taste-mask bitter drugs used to improve medicine compliance (Shang et al., 2018). Solvent cast ODFs that contained spray-dried naproxen nanoparticles were formulated to achieve an ultra-rapid dissolution and hence achieve a faster clinical response, for example for pain relief (Steiner et al., 2018).

Electrospinning

Electrospinning is based on the ultra-rapid removal of solvents from polymer-based solutions under a high applied voltage to form a nanofibrous mesh solid structure and can be used for the fabrication of ODFs in a one-step continuous manufacturing way, or further processed into other dosage forms. Figure 4 shows a schematic diagram of the principles that underpin the process and how solid structures are fabricated from polymers using the technique.

Unlike solvent casting, electrospinning does not utilise heat nor does it require the addition of a plasticizer to fabricate flexible ODFs, which offers an advantage if the formulation is intended for paediatrics where the number of excipients should be kept to a minimum (Abdelhakim et al., 2020). A current limitation of electrospinning is scaling up the process, nonetheless, there are current groups that are tackling this issue and there are specialised Good Manufacturing Practice (GMP) manufacturers' that allow for assessment of the electrospun ODFs in humans (Vass et al., 2020). Additionally, there is currently an electrospun oral patch on the market, Rivelin ® , which targets oral lesions, showing that that technology can be translated to ODFs that can be clinically marketed.

One of the main widely electrospun polymers is PVP, a biodegradable and biocompatible material. PVP has been used on numerous occasions in the electrospinning of ODFs including: amlodipine/valsartan films (Bukhary et al., 2018); paracetamol/caffeine (Illangakoon et al., 2014); meloxicam (Samprasit et al., 2015); and helicid (Wu et al., 2015). A lysozyme-incorporated electrospun mucoadhesive patch was fabricated for rapid local delivery to the oral mucosa (Edmans et al., 2020), demonstrating that this technique shows future promise for TPP-based ODFs.

3D printing

3D printing has been recently employed in the fabrication of ODFs. Several 3D printing techniques have been used to prepare ODFs. Techniques such as inkjet 3D printing, FDM, and SSE were investigated to fabricate ODFs (Elbl et al., 2020). Inkjet printing is a simple and easily customizable technique that enables the simultaneous use of drug combinations. The ink passes through a motor-controlled print head, solidifying once it reaches the printing bed, forming a thin layer (Capel, 2018, Jamróz et al., 2018). FDM is another 3D printing technique utilized to prepare ODFs (Musazzi et al., 2020, Ehtezazi et al., 2018). However, the high temperature required to extrude the filament may restrict its application with biological materials. While SSE allows the extrusion of the material at low temperature. It shows great potential in extruding biological material (Seoane-Viaño et al., 2021).

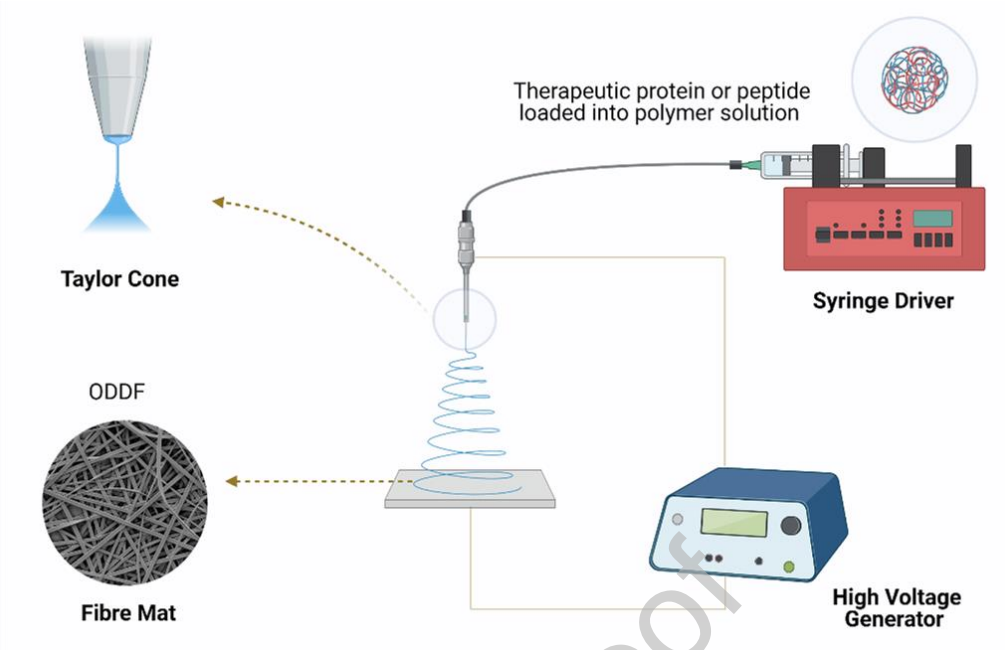

Fig 4: A schematic diagram of the underlying principles of electrospinning. The TPP dissolved or dispersed in a polymer solution is held in a syringe which is pumped at a constant flow rate. An electric field, at a specific voltage is applied between the syringe tip and the collector. As the electrostatic force builds up at the tip of the syringe, the droplets of the polymeric solution will deform into a conical shape called a 'Taylor Cone'. Further increase in the electric field results in the formation of a jet that is ejected out of the syringe-tip towards the collector. If the solution viscosity is within a certain threshold, solid fibres are generated as the solvent evaporates, resulting in the formation of a dry structure. Drawn on Biorender.com.

Hot Melt Extrusion

Hot melt extrusion (HME) is a method to produce a molecular dispersion of APIs in polymeric matrices (Censi et al., 2018). Generally, HME is based on feeding the raw materials through temperature-controlled die at high pressure. The extrudate flow through a die until the product is extruded and then solidified (Zheng and Pokorski, 2021, Cossé et al., 2017). HME is commonly used in the production of several pharmaceutical dosage forms such as tablets, implants, films, and pellets (Albarahmieh et al., 2016, Cossé et al., 2017, Gryczke et al., 2011). In the preparation of films, the polymeric melt is extruded through a thin slit and subsequently passed along cooling rolling cylinders and finally cut down to smaller pieces (Breitenbach, 2002). An ODF of Vitamin B12 was prepared by HME. The disintegration time, and the pharmacokinetic profile of the drug were comparable to the commercially available orally disintegrating strip, Quicobal® (Suryawanshi et al., 2021). The utilization of the technique in the production of TPPs is still in the early stages. TPPs are generally unstable at a temperature above 40° in the liquid state. However, studies have confirmed an improved stability at high temperatures while at solid state (Moriyama et al., 2008). HME was used to prepare biodegradable poly(lactic-*co*-glycolic acid) (PLGA) implant for sustained bovine serum albumin release (BSA) (Ghalanbor et al., 2012).

5. Formulation strategies for the delivery of therapeutic protein and peptides in orally disintegrating dosage forms

The U.S. FDA recommends that ODTs should be distinguished as a separate dosage form because of the specific, intended performance characteristics, which are rapid oral disintegration in saliva with no need for chewing or drinking liquids to ingest these products. This guideline recommends that ODTs should disintegrate within 30s of being placed in the mouth. There are two types of ODDFs that are currently being investigated for the oral delivery of TPPs targeted for absorption through the oral mucosa or gastric and small intestinal mucosal surfaces, i) orally disintegrating films (ODFs), and ii) orally disintegrating tablets (ODTs). These dosage forms are of particular interest not only due to the obvious reasons of rapid disintegration in a small volume of liquid e.g. saliva, but in addition they prove a flexible and convenient way of administering APIs that do not require excessive swallowing mechanisms and where the API can be released, dependent on the formulation, within the oral cavity targeted to specific areas such as the buccal or sublingual mucosa, or to the stomach and small intestines of the GIT, or the potential for delivery to both physiological regions. Furthermore, to enhance flexibility of these formulations, ODDFs can be tailored such that the API is protected via encapsulation within for example, nano- or microparticles, to offer stability and protection from enzymatic degradation or exposure to gastric acid. Therefore, ODDFs are highly desirable and unique platforms for the delivery of TPPs, providing multiple possibilities by which fragile macromolecules can be delivered and targeted to different mucosal sites. Below is an overview of the progress that is being made in the research and development of formulating ODDFs for the delivery of TPPs, including strategies to improve stability, permeability, and bioavailability.

Orally Disintegrating Films (ODFs)

ODFs are thin-layered or multi-layered, flexible films fabricated from natural or synthetic biodegradable polymers that disperse rapidly when placed in the oral cavity including buccal and sublingual regions of the mouth (Lai et al., 2018, Nagaraju et al., 2013, Castro et al., 2015). Selection of the polymeric backbone is based on the water-solubility and hydrophilic properties of the matrix (Li et al., 2020) where the presence of a small quantity of aqueous biological fluid (e.g. saliva) causes

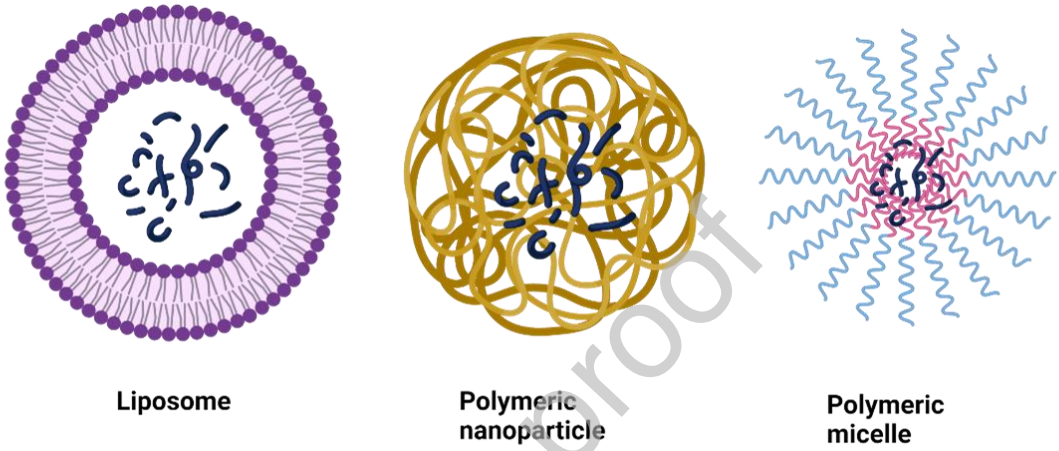

Fig 5: Different types of protein/peptide-loaded particulate structures. Liposomes have micro-vesicular structures composed of aqueous cores and amphiphilic bilayers commonly consisting of phospholipids combined with cholesterol to mimic the natural components of biological barriers. However, this alone is unstable in the GIT, but addition of bile salts has been found to increase stability. Polymeric nanoparticles consist of synthetic or natural, biodegradable polymers and are widely investigated as drug delivery systems. The API is typically dispersed or dissolved within a solution of the polymer; as the solvent evaporates, the long-chain of the polymers will entangle and wrap around the API, eventually shrinking and collapsing to form a micro/nanoparticle which then hardens to form solid, stable structures encapsulating the TPP. Polymeric micelles are self-assembling nanoconstructs consisting of ampiphilic copolymers that are under investigation for the delivery of TPPs. Drawn on BioRender.com.

the polymer within the matrix to swell or disintegrate thereby releasing the API (Borges et al., 2016, Tedesco et al., 2016). The API is dispersed within the matrix of the film and rate of release can either be immediate (Steiner et al., 2019) or controlled (Speer et al., 2019, Morales et al., 2013) which is subject to, in general, the physicochemical properties of the polymeric backbone. Various strategies and approaches are currently being developed to protect TPPs intended for delivery via ODFs to facilitate oromucosal delivery (buccal and sublingual) and are discussed below.

Strategies for enhancing the stability of TPPs in ODFs

Excipients such as polymers and sugars that are used for the fabrication of ODFs can also confer stability to TPPs (Varanko et al., 2020) and are particularly advantageous due to their dual function. Protein stability was evaluated by incorporation of three model proteins, ovalbumin, β-

galactosidase, and lysozyme, in ODFs made from blends of trehalose and pullulan (Tian et al., 2018). Both excipients are used in combination to fabricate ODFs for drug delivery via the oral mucosa, with trehalose possessing protein stabilising capacity and pullulan demonstrating good gel-forming properties (Teekamp et al., 2017). Blends of trehalose and pullulan have previously been studied in formulations to stabilise proteins incorporated into ODFs (Geeraedts et al., 2010). Trehalose is a low molecular weight disaccharide sugar with a low glass transition (Tg). It can provide a compact molecular coating around the protein, providing protection and stability. However, the low Tg can lead to crystallisation, particularly at high relative humidity. Therefore, addition of pullulan, which possesses a high Tg (Teekamp et al., 2017), confers optimal physicochemical properties to enhance protein stability. From the three model proteins investigated in this study, the most poorly stable protein, β-galactosidase, was used to assess protein stability and it was found that stability increased with increasing ratios of trehalose/pullulan blends showing promising application for future ODF formulations for poorly stable TPPs. Another smart delivery platform that is being developed to improve the stability of TPPs in ODF systems is nano- and microparticle technology (Batista et al., 2018, Holpuch et al., 2010). It can offer enhanced permeation through the mucosal epithelia, protection from enzymatic degradation and stability of TPPs with regards to processing, manufacturing and storage (Batista et al., 2018). Two independent studies have reported the development of nanoparticles loaded with insulin embedded into a chitosan film for buccal delivery (Giovino et al., 2012). Morales *et al* (2013) reported the development of lysozyme-loaded nanoparticles embedded in a polymeric film comprising HPMC and Eudragit RLPO, for buccal delivery. The study found that increasing the amount of the more hydrophilic polymer, HPMC, increased the rate of release of lysozyme and the quickest release rate was found for the film consisting of 100% HPMC, indicating the possibility of developing rapid as well as controlled release film formulations. Furthermore, the activity of lysozyme was found to be stable throughout the study (Morales et al., 2013).

CMC is another example of a polymer used to fabricate ODFs, forming hydrophilic oral films, reported to enhance the rapid disintegration of ODFs (Kim et al., 2020). CMC is an anionic water-soluble polymer derived from insoluble cellulose that possesses excellent gel-forming properties (Rahman et al., 2021). A recent study investigating the effectiveness of an alternative iron supplement derived from *Pereskia aculeata* Miller, commonly known as ora-pro-nobis (OPN), which is a native cactus plant in Brazil known for its rich source of proteins and minerals such as iron and calcium, when incorporated into a CMC-based ODF (Maciel et al., 2021). The release rates of protein-based OPN extract was assessed in an ODF. OPN-loaded chitosan-pectin microparticles were dispersed within a CMC-film matrix and compared with OPN free extract (non-encapsulated) incorporated within the polymeric film. The ODF containing OPN-loaded chitosan-pectin microparticles demonstrated maximum release after 26 mins in comparison to the unencapsulated

OPN incorporated within the ODF (50 mins). It was suggested that the presence of the microparticles within the CMC-based ODF was causing disruption of bonds within the matrix (Takeuchi et al., 2019) thereby facilitating faster disintegration and subsequent release of the OPN. Fabrication of an ODF for the delivery of probiotic species to the oral cavity also utilised CMC but as a mixture with starch and gelatin (Heinemann et al., 2013). It was found that formulations containing probiotics incorporated with the three polymers showed better survival of the organisms after 3 months storage (>10^7 cfu/mL) in comparison to ODFs containing only CMC and gelatin (<10^6 cfu/mL). Although the study did not determine disintegration time for the probiotic-loaded ODFs, it managed to demonstrate the advantages conferred upon stability of the probiotic species when incorporated within a film matrix consisting of a mixture of polymers suitable for ODF formulations (Heinemann et al., 2013).

Mechanical and tensile strength is equally important for the ODFs to protect and maintain integrity of TPPs during processing, manufacture and storage conditions to produce flexible and non-brittle films (Yoo et al., 2006, Garcia et al., 2020). The mechanical properties of ODFs are dependent on both the polymer backbone used to fabricate ODFs as well as the concentration of TPP (Al-Hassan and Norziah, 2012). Strong interactions between the two can result in weakening of polymer chains during formation of the film. A recent study characterised ODFs, based on gelatin and HPMC combinations, incorporating peanut skin extract (PSE) as a therapeutic phenolic compound. The study revealed that the presence of gelatin prolonged the disintegration time of the film due to complex formations between amino acid residues of gelatin and tannins from the PSE (Tedesco et al., 2017). The formation of these complexes resulted in increased disintegration times for HPMC/gelatin blended films (25.94s, 50% blend) in comparison to a film containing HPMC alone (16.95 s). Faster disintegration times were attributed to the greater hydrophilic nature of HPMC, which has been noted in other studies (Ding et al., 2015, Satyanarayana and Keshavarao, 2012), and the lack of undesirable interactions between HPMC and PSE (Tedesco et al., 2017).

Strategies for enhancing buccal delivery of TPPs in ODFs

Delivery through the buccal mucosa can be exploited to increase the systemic absorption and subsequent bioavailability of TPPs (Morishita and Peppas, 2006). This region of the oral cavity has been investigated for the systemic delivery of TPPs by formulating mucoadhesive films intended for rapid disintegration (Edmans et al., 2020) as well as controlled release (Mortazavian et al., 2014). Batista et al developed and characterised an ODF for buccal delivery of a bioactive peptide with antihypertensive properties. The optimal film formulation had a thickness of 136µm and consisted of chitosan, sorbitol (as plasticiser to prevent film brittleness), and citric acid (saliva stimulator). The plasticiser molecules interpose between the polymer chains within the film matrix by interacting with the functional groups, thereby increasing film flexibility and mechanical strength. Furthermore,

the bioactive peptide was first encapsulated within chitosan microparticles, demonstrating increased stability of the peptide, and the microparticles were subsequently loaded into chitosan-based films which enhanced delivery through the buccal mucosa (Batista et al., 2019).

Strategies for the incorporation of vaccines in ODFs

Buccal and sublingual routes of administration for vaccine antigens formulated in ODFs is an emerging field being explored as an alternative to conventional parenteral injections (Trincado et al., 2021, Uddin et al., 2019). Mucosal immunisation via the buccal and sublingual route has many advantages over parenterally administered routes which includes the potential for patients to self-administer, reduced need for trained personnel required for parenteral administration, and the ease of use in infants and children. Initial exposure and first line of defence against invading pathogens in the host is at the mucosal surfaces. It has been widely reported that systemic immunisation induces very poor mucosal immunity, whereas mucosal immunisation has been known to provide immune protection far from the site of administration, including induction of antigen-specific systemic responses (Gallichan and Rosenthal, 1996, Holmgren and Czerkinsky, 2005).

Mucosal associated lymphoid tissue (MALT) in the oral cavity includes the Waldeyer's ring and palatine tonsils (Kraan et al., 2014). Buccal and sublingually administered antigens are captured by local antigen-presenting cells (APCs) such as Langerhan and dendritic cells and migrate to proximal draining lymph nodes where the antigens are presented (see Fig. 6), leading to the induction of strong cellular and humoral immune responses resulting in the production of secretory IgA, the predominating immunoglobulin along mucosal surfaces (Suárez et al., 2021, Kraan et al., 2014). Vaccine immunisation research via the buccal and sublingual route is very much in its infancy. Preclinical studies in animals, such as mice, have revealed promising results showing potential for further development of buccal and sublingual routes of administration for vaccine antigens. Immunisation of a single dose of measles virus nucleoprotein (MV NP) administered to the murine buccal mucosa demonstrated the recruitment of dendritic cells and uptake of the antigen leading to priming of both B and T cells resulting in an MV NP-specific immune response. In this study, the induction of MV NP-specific cytotoxic T lymphocyte (CTL) response was mediated by major histocompatibility class I (MHC I) restricted $CD8^+$ effector T cells (Etchart et al., 2001). Furthermore, a similar study evaluated the response of an exogenous antigen, hapten 2,4-dinitrofluorobenzene (DNFB) to buccal immunisation in BALB/c and C57B1/6 mice and observed a contact specific response mediated by CD8+ effector T cells associated with rapid migration of DC cells to the buccal epithelium and induction of costimulatory molecules on the surface of some DCs leading to T cell priming and subsequent proliferation (Desvignes et al., 1998).

More recent studies conducted by Gala et al evaluated an ODF formulation of a buccally administered measles vaccine in juvenile pigs. The measles vaccine antigen was incorporated into a solution of bovine serum albumin (BSA) and processed by spray-drying to form microparticles. The microparticles were subsequently embedded into an ODF fabricated from Lycoat RS720, Neosorb P60W and Tween 80. After buccal administration, a significantly higher induction of the innate and adaptive immune response (*in-vivo*) was found with a significant increase in serum IgG antibody

levels evaluated from collected blood samples, post-immunisation, in comparison to blank. This exploratory study established proof-of-concept that the measles vaccine can elicit a robust immune response when formulated into an ODF formulation and administered to the buccal mucosa of juvenile pigs (Gala et al., 2017).

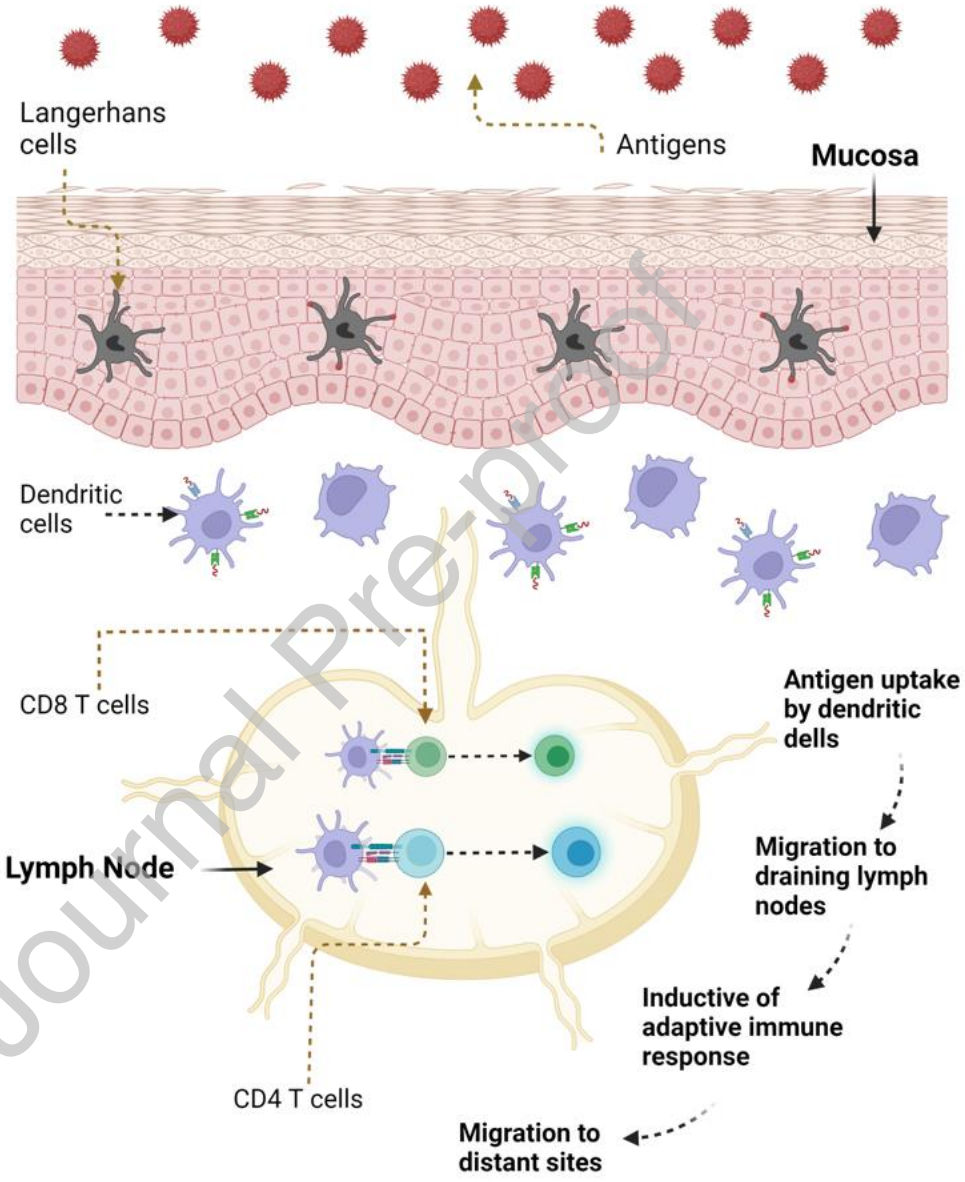

Fig 6: The uptake of antigens from the buccal and sublingual mucosa. Antigens are captured by local APCs such as Langerhan and dendritic cells that reside in the mucosa. The antigen is processed on the surface of the APCs and the complex migrates to nearby lymph nodes where the antigens are presented to T cells and an adaptive immune response is induced. The naïve T cells leave the lymph nodes and migrate to distant effector sites. Drawn on Biorender.com.

The sublingual route has also been described as an effective method of immunisation that is painless, provides ease of administration and, can stimulate both systemic and mucosal immunity (Trincado et al., 2021). In addition, this route of administration has been suggested to be safer than intranasal application of live virus vaccine antigens (Cuburu et al., 2007, Song et al., 2008). A study examined the effectiveness of sublingual immunisation against *Helicobacter pylori* infection to stimulate immune responses and provide protection in the stomach and small intestine of mice. The mice received two sublingual vaccine doses, two weeks apart, of *H. pylori* lysate antigen in combination with the potent adjuvant, cholera toxin, and the immune response levels were compared to pre-immunisation markers. Sublingual immunisation of the vaccine induced immune protection against a challenge of live *H. pylori* bacteria by induction of strong antibody responses in the serum (IgG) as well as the stomach and small intestinal mucosa (IgA) demonstrating a broad immune response and protection distant from the site of administration of the vaccine (Raghavan et al., 2010). These findings are supported by other preclinical studies (Huang et al., 2011) where stimulation of the mural common mucosal immune system as well as peripheral lymph nodes was demonstrated when administered with sublingual administration of OVA as a model antigen with cholera toxin (adjuvant). Induction of the systemic humoral immune response (IgG isotype) was reported as well as high mucosal IgA antibody titres seen in saliva, nasal washes of mice and lung mucosa, indicating a broad-based systemic and mucosal immune response (Cuburu et al., 2007).

Long-term storage and stability considerations for vaccines in ODFs

Concerns regarding the long-term storage of influenza vaccines, due to the instability of the antigen, has led to investigations into seeking more appropriate formulations using safer processing and manufacturing methods that will not hinder antigenic activity (Geeraedts et al., 2010, Tian et al., 2020). Other than Flumist®, a live attenuated influenza vaccine administered intranasally, all other influenza vaccines on the market are injectable. Various studies have demonstrated the successful delivery of influenza vaccine via the sublingual route of administration (Oberoi et al., 2016, Murugappan et al., 2014). An effective influenza-specific immune response was demonstrated when the influenza antigen was co-administered with an adjuvant, CRX-601, encapsulated within polyethylene glycol modified liposomes (Oberoi et al., 2016), and applied to the sublingual tissue. When the modified liposomes were coated with methylglycol chitosan, a mucoadhesive agent, a robust systemic and mucosal immune response was observed. The whole inactivated virus (WIV) influenza strain, formulated into sublingual tablets, and administered as a primer dose 4 weeks prior to an intramuscular (i.m) booster vaccination of the same strain, found that sublingual priming enhanced both lung and nasal IgA responses, whereas i.m booster dose alone, only produced significant IgA responses in the respiratory tract (Murugappan et al., 2014). A recent study attempted to evaluate the stability of WIV in ODF formulations with the aim of protecting antigenic integrity during processing and maintaining activity of WIV throughout long-term storage of the

formulation (Tian et al., 2020). WIV was stabilised in a solution comprising a blend of trehalose and pullulan with the ODF fabricated as a blank film, consisting of predominantly HPMC as the major component forming the backbone of the film with the addition of carbomer 974P, disodium edetate, trometamol and glycerol 85%. Specific volumes of the WIV solution were added onto the surface of the blank ODF and either air- or vacuum dried, and then samples were stored at 60^{o}C/0% RH or 30^{o}C/56% RH for four weeks. The activity of WIV, determined by haemagglutination assay, remained the most stable for the WIV/trehalose/pullulan/ODF in comparison to all other controls including WIV/trehalose/pullulan solution alone suggesting that HPMC in the ODF contributed to stability of the influenza antigen.

Orally disintegrating tablets (ODTs)

Most ODFs are designed specifically to attach to mucosal surfaces in the oral cavity, however ODTs can be targeted to both the GIT, as well as buccal and sublingual sites. For gastrointestinal delivery, when placed on the tongue, ODTs will rapidly disintegrate and can be ingested using normal swallowing reflexes that require little or no effort (Nagar P, 2011). The formulation of fragile TPPs in ODTs is particularly advantageous because the minimal moisture content within the formulation (< 5%) can protect them from shear stress and compaction during manufacturing, denaturation during long-term storage particularly in sub-tropical climates, and a reduced cold-chain footprint during transport (Wilkhu et al., 2017, Lal et al., 2013). Up to 80% of the cost of vaccination programmes is due to maintaining cold conditions for storage (Pelliccia et al., 2016). Vaccines as ODT formulations offer cheaper alternatives and are cost-effective since the main aim of their design is to provide a replacement for parenteral liquid formulations that are bulkier with a shorter shelf-life due to sterility measures, potential hydrolysis and instability of the TPPs in aqueous media, as well as contamination. Most importantly, from a health and welfare prospective, the development of ODTs as life-saving therapies is humane because cheaper manufacturing, storage and transport costs allows wider proliferation and use world-wide, particularly for low-resource countries in need of more cost-effective vaccines and 'on-demand' treatments for medical emergencies. Innovative and novel uses of the principles underpinning ODTs have also been applied to veterinary vaccines, where a fast-dissolving tablet (FDT) for the protection of poultry against a contagious viral infection, Newcastle disease, was developed. The FDT was designed to be reconstituted in a small amount of water and administered as an eye-drop. Orally disintegrating formulations are also used to treat patients for breakthrough cancer pain in the form of effervescent buccal and sublingual tablets containing the opioid pain killer, fentanyl (Jandhyala and Fullarton, 2012)(Jandhyala and Fullarton, 2012).

Although there are very few investigations reported, studies so far on the formulation of TPPs in ODTs have shown encouraging and positive outcomes indicating the inevitable eventuality of the

expansion of orally administered TPPs that will consequently require innovative and simple methods of delivery, manufacturing, storage and transport systems needed to reach the wider patient population.

Formulation strategies for gastric and intestinal delivery of TPPs in ODTs

TPPs in conventional tablet formulations

There are minimal studies in the literature that have investigated the incorporation of TPPs into an ODT formulation for targeted delivery to the GIT; a reflection, no doubt, of the complexities and difficulties surrounding the traversing of intact macromolecules across the epithelial lining of the stomach and small intestine. Examples of TPPs that have undergone clinical trials for oral administration using conventional tablet formulations are the parathyroid hormone (PTH1-34) (Hämmerle et al., 2012, Mannstadt et al., 2013), a hormone fragment that is currently licensed to be administered as a daily subcutaneous injection for the treatment of osteoporosis, and salmon calcitonin for the treatment of acute hypercalcaemia and osteoporosis, available as a parenteral injection and nasal spray (Chesnut et al., 2000). An oral formulation of salmon calcitonin containing citric acid to enhance protease resistance and paracellular transport, has been investigated in phase III clinical trials showing significant improvement in bone density in post-menopausal women (Binkley et al., 2012). The glucagon-like peptide-1 (GLP-1) receptor agonist, oral semaglutide, is the first therapeutic peptide licensed for the treatment of Type-2 diabetes mellitus as an alternative to insulin treatment and was approved by the FDA in September 2019; previously only available as a once-weekly subcutaneous injection, along with several other long-acting and short-acting GLP-1 receptor agonists, also administered parenterally. Oral semaglutide is formulated as a tablet with the addition of the permeation enhancer, salcaprozate sodium (SNAC) (Twarog et al., 2019, Kim and Jung, 2021). Recent studies have shown that peptides co-formulated with SNAC are absorbed by paracellular diffusion in the stomach rather than what was previously thought was the small intestine (Buckley et al., 2018). The above examples demonstrate that investigations into conventional tablet formulations of TPPs have progressed significantly.

Critical quality attributes for TPPs in ODTs

For the development of ODTs containing TPPs, optimisation studies have been performed to evaluate the combination of excipients required to produce an ODT with the necessary critical quality attributes including, disintegration time, dissolution profile and activity of the TPP. Olah et al studied the effect of superdisintegrants and processing conditions on ODT formulations incorporating lysozyme enzyme (Olah et al., 2019). Lysozyme enzyme is often used as a model protein as proof-of-concept studies for the delivery of TPPs in ODTs. Recent studies have suggested that it may be effective against chronic inflammatory bowel conditions (Rubio, 2014) and is widely used as a food additive due to its antibacterial properties (Proctor and Cunningham, 1988). The ODT

formulations were produced using the DC method which is the simplest and most cost-effective method of manufacturing, allowing the utilisation of conventional tabletting equipment and inexpensive excipients. However, heat generated during the tabletting process and the shear stress applied can be irreversibly damaging to the stability of lysozyme and indeed, most TPPs. An important observation made in this study was that harder tablets provided better stability for the enzyme despite having to apply a higher compression load. The advantage of a high tablet density was attributed to increased contact between the protein and the stabilising agent, consisting of sugars such as mannitol and trehalose, which were easily deformable allowing the protein to evenly distribute between the small molecules, with reduced pore formation, conferring protection and stability to the lysozyme enzyme. A range of formulations containing several superdisintegrants were investigated with the optimal formulation yielding fast disintegration times with liberation of lysozyme in less than 30 secs.

Oral vaccines in ODT formulations targeted to the intestinal mucosa

Since the oral route is the most desirable route for therapeutic drug administration, research into the development of oral vaccines retains its significance in terms of healthcare research. The GIT is rich in lymphatic tissue, mainly concentrated in the Peyer's patches present under the epithelial lining of the small intestine but with isolated lymphoid patches situated throughout the GIT. Upon entering the intestinal lumen, antigens are transcytosed across the intestinal epithelium by specialised M cells that are constantly sampling the contents of the lumen; antigens are processed and presented by APCs such as DCs to naïve T cells in the germinal centre located in the Peyer's patches. The T cells will go through a process of selection called 'priming' and will activate B cells which then leave the germinal centre and migrate to effector sites, mature, and produce antigen-specific antibodies. The GIT presents many challenges to the delivery of oral vaccines including degradation of antigens, poor uptake and the development of tolerance to antigens. Oral vaccines and the associated mechanisms of the immune response is beyond the scope of this review and can be found in greater detail in the literature (Vela Ramirez et al., 2017, Coffey et al., 2021). Wilkhu et al investigated the optimisation of a formulation suitable for oral delivery comprising a recombinant sub-unit vaccine, H3N2, as a model antigen, encapsulated within bilayer vesicles called niosomes, and formulated into an ODT (Wilkhu et al., 2017). Research into nano-systems as carriers to improve permeability and bioavailability of poorly soluble, small molecule drugs for incorporation into ODTs has shown promising results (Anup et al., 2018) and attempts have been made to translate this technology to the delivery of vaccines. Niosomes are bilayer vesicles constructed from non-ionic surfactants and cholesterol, that can encapsulate APIs, such as TPPs and other antigens for delivery to the intestinal tract, and are known to be stable in gastric acids (Ge et al., 2019). The bilayer vesicles have been shown to be taken up by M cells in the lumen of the small intestine and to stimulate an immune response (Wilkhu et al., 2014). In this study, a suitable protocol was developed

to obtain freeze-dried ODTs containing the model antigen encapsulated within the noisome vesicles, where the aim was to ensure stability and integrity of H3N2 subunit vaccine/bilayer vesicle complex during the freeze-drying process (Wilkhu et al., 2017). Different cryoprotectants comprising of sugars such as mannitol and dextran were investigated, in varying ratios, and an optimal freeze-drying protocol was determined by measuring the disintegration time and mechanical strength of the final product. Vesicle size and zeta potential were used to determine the integrity of the vesicles before and after freeze-drying. Lal et al also explored freeze-drying and packaging conditions for a fast-dissolving tablet formulation of a live attenuated enterotoxigenic *Escherichia coli* vaccine (ACE527) (Lal et al., 2013). The model vaccine used in this study (ACAM2027) was the most labile component of the trivalent live attenuated vaccine under development for the treatment of diarrhoea induced by enterotoxigenic *E. coli* and has already shown encouraging results in Phase I and II clinical trials in humans (Harro et al., 2011, Darsley et al., 2012). ACE527 vaccine was intended to be developed into an oral vaccine for infants and children in developing countries therefore formulation into a fast-dissolving tablet would be particularly advantageous for ease-of-administration, packaging and minimal cold chain footprint during the lifetime of the formulation. For this formulation sucrose and trehalose were included as cryoprotectants; phosphates and glutamate salts as buffer to mitigate acidic degradation; and Natrosol®, PVP and mannitol as binders. The formulations were lyophilised as this is the suggested recommended method of manufacturing stable, heat sensitive biological products and vaccines (O'Ryan, 2007, Saha et al., 2011). The study demonstrated that standard freezing prior to lyophilising caused the least loss in viability of bacterial organisms, whereas 'fast-freezing' in liquid nitrogen resulted in high process loss. Slower freezing processes prevent formation of ice-crystals within the intracellular space of the bacterial organism (Seth, 2012). Long-term storage testing demonstrated that 4^{o}C for at least 54 weeks produced no significant loss in viability of the antigen however there was a significant loss when stored for 1 week at 37^{o}C. It was shown that the final tablets disintegrated in less than 10 sec. These preliminary studies provide proof-of-concept that it is possible to formulate successful vaccine delivery systems in an easy-to-use ODT format that is cost-effective and possible to scale-up for mass production.

Formulation strategies for the delivery of oral TPPs in ODTs to oromucosal sites

TPPs in ODTs for topical application

A strategy for the topical treatment of oral diseases associated with bacterial aetiology including gingivitis, dental caries and periodontitis, is the application of probiotic treatments. Probiotic species are being developed as ODTs for buccal administration (Hoffmann and Daniels, 2019). The greatest challenge for APIs delivered to the oral cavity is the possibility of salivary wash out (Jin et al., 2015, Caon et al., 2015). This issue was addressed in a study that used a mucoadhesive agent, HPMC, in

combination with Eudragit L100-55, a pH sensitive polymer – with solubility >pH 5.5 - that would readily dissolve in the oral cavity releasing the probiotic species, *Lactobacillus plantarum* and *Lactobacillus paracasei*. The inclusion of HPMC, increased contact time of the organisms when evaluated *ex-vivo* on porcine buccal mucosa but with the resultant ODT formulation still showing disintegration times of less than 30 sec (Hoffmann and Daniels, 2019).

TPPs in ODTs for 'on-demand' or emergency therapy

The delivery of TPPs in ODT formulations targeted for buccal and sublingual mucosal sites are being developed for 'on-demand' treatments and have shown promising results. Recent investigations include developing an ODT for the treatment of neurogenic bladder dysfunction arising from damage to the central nervous system (CNS) which results in symptoms of urinary retention. The condition is traditionally treated in hospital settings where catheterisation is required for bladder emptying however multiple use is not practical for long-term therapy. A study evaluating the efficacy of a lyophilised ODT sublingual tablet containing the heptapeptide, [Lys5,MeLeu9,Nle10]-NKA(4–10) (LMN-NKA) was administered to anaesthetised rats with spinal cord injury (Bae et al., 2018). The formulation contained gelatin, glycine and sorbitol making it highly soluble with a disintegration time of less than 30 s. It was found that the onset of action of the ODT was slightly longer (0.5 mins) in comparison to intramuscular administration of the heptapeptide. A heat-stable oxytocin-loaded rapid ODT formulation for sublingual administration is also under development for the treatment of post-partum haemorrhaging (PPH) (Zhu et al., 2018). PPH occurs in pregnant women where ≥500mL of blood is lost from the genitals within 24 hrs after childbirth and is a major cause of morbidity and mortality in low-resource countries. The recommended treatment is the administration of intravenous or intramuscular injections of oxytocin by skilled health workers usually requiring treatment in hospital settings. A rapid-acting sublingually administered oxytocin ODT tablet would save lives by ensuring that isolated or rural areas that are distant from hospitals would be able to store and use the medication for on-demand emergencies. In this study, administration of this formulation to female mini-pigs demonstrated good pharmacokinetic and bioavailability profiles, as the formulation contained the permeation enhancer, sodium taurocholate, a bile salt, that facilitated rapid absorption of oxytocin.

TPPs in ODTs for allergy immunotherapy tablets

In contrast to vaccine therapy that elicits an immune response, the aim of allergy immunotherapy treatment for allergy-based respiratory conditions is to induce immune tolerance in subjects to reduce hypersensitivity to various allergens including pollen and house dust-mites (Bahceciler et al., 2014, Nolte and Maloney, 2018). Allergen-specific immunotherapy treatment was commonly performed by repeated subcutaneous injection. However, due to the inconvenience of this route of administration for patients as well as reports of rare but severe and fatal systemic reactions (Roberts

et al., 2006), sublingual immunotherapy tablets (SLITs), an ODT formulation, have been developed as an alternative to the parenteral injections and are available to use in clinics (Nolte and Maloney, 2018). SLITs contain standardised allergens that were initially developed for systemic absorption. However, biodistribution studies with radio-labelled allergens in humans revealed that systemic absorption through the oral mucosa was negligible (Bagnasco et al., 1997, Bagnasco et al., 2001). Consequently, the clinical effect has been ascribed to a local interaction with the immune system. The SLITs are administered daily and although the mechanism of action is not fully understood it has been suggested that frequent contact of the allergen with the sublingual mucosa causes development of oral tolerance that involves innate immune components embedded within the mucosal tissue (Nolte and Maloney, 2018). The greatest advantage of the SLIT is that allergic patients can self-administer daily, and it has been shown that the treatment is well tolerated. In addition, phase III clinical studies have demonstrated good safety profiles with few adverse events occurring which were related to only local reactions, rather than, more concerning, broad-based systemic effects (Demoly et al., 2021). SLIT has been proven efficacious against allergic rhinitis and allergic asthma (Bahceciler et al., 2014, Demoly et al., 2021) indicating that sublingual administration of the allergen induces immune tolerance in distance mucosal surfaces. A study that compared two different SLIT formulations – a freeze-dried and compressed formulation – found that the freeze-dried SLIT tablet provided rapid and complete release of the allergen in a small volume of solvent (Lund et al., 2019). However, the study did not provide details of the excipients included in each formulation. Table 4 summarises the studies that have been investigated using various pharmaceutical strategies for the fabrication of ODDFs involved in the delivery of TPPs.

Table 4: Current studies investigating therapeutic proteins and peptides (TPPs) in ODDFs. HPMC = hydroxypropyl methylcellulose, CMC = carboxy methylcellulose, NPs = nanoparticles, MPs = microparticles, BSA = bovine serum albumin

Dosage form	Therapeutic protein- or peptide based active ingredient	Target site for delivery	Delivery strategy	Manufacturing method	References
Orally disintegrating film (ODF)	Ovalbumin β-galactosidase Lysozyme	Oral mucosa	Incorporation of proteins within film matrix	Solvent-casting	(Varanko et al., 2020, Tian et al., 2018, Teekamp et al., 2017)
	Lysozyme	Buccal mucosa	Protein-loaded NPs incorporated within HPMC and Eudragit RLPO film matrix	Solvent-casting	(Morales et al., 2013)
	Ora-pro-nobis	Oral mucosa	Chitosan-pectin microparticles dispersed within CMC film	Tape-casting technique	(Maciel et al., 2021)
	Lactobacillus acidophilus, Bifidobacterium	Local delivery to oral	Probiotics dispersed within film matrix comprising CMC,	Solvent casting technique	(Heineman n et al., 2013)

	animalis subsp. lactis	mucosa	starch and gelatin		
	Bioactive peptide with antihypertensive properties (sequence: KGYGGVSLPEW)	Buccal mucosa	Chitosan MPs incorporated within chitosan film with sorbitol (plasticiser) and citric acid (saliva stimulator)	Solvent casting technique	(Tavares et al., 2011)
	Peanut skin extract (phenolic compound)	Buccal mucosa	Incorporation within HPMC and gelatin film matrix	Solvent casting technique	(Tedesco et al., 2017)
	Measles vaccine	Buccal mucosa	Incorporated into BSA-based-MPs and embedded into a film matrix comprising Lycoat RS720, Neosorb P60W and Tween 80	Spray-dried NPs embedded in an ODF using the solvent casting method	(Gala et al., 2017)
	Influenza vaccine	Oral mucosa	Vaccine stabilised with trehalose and pullulan, and incorporated into film matrix comprising a backbone with HPMC including carbomer 974P, disodium edetate, trometamol and glycerol 85%	Antigen solution was pipetted onto plain ODFs (made from the solvent casting method) and then either air- or vacuum-dried	(Tian et al., 2020)
Orally disintegrating tablet (ODT)	Lysozyme	GIT	A range of superdisintegrants with addition of protein stabiliser/filler such as trehalose and mannitol	Direct compression	(Olah et al., 2019)
	Recombinant subunit vaccine, H3N2	GIT	Antigen encapsulated within niosomes, and incorporated in 10% mannitol and 10% dextran as cryoprotectants and bulking agents	Lyophilisation	(Wilkhu et al., 2017)
	Escherichia coli vaccine	GIT	Sucrose and trehalose as cryoprotectants; phosphates and glutamates salts as buffers to minimise acid degradation; Natrosol®, PVP, mannitol as binders	Lyophilisation	(Lal et al., 2013)
	Lactobacillus plantarum and Lactobacillus paracasei	Buccal mucosa for delivery in oral cavity	HPMC and Eudragit L100-55	Granulation	(Hoffmann and Daniels, 2019)
	Heptapeptide	Sublingual	Gelatin, glycine and	Lyophilisation	(Bae et al.,

(LMN-NKA)		sorbitol		2018)
Oxytocin	Sublingual	9% sucrose, 1.5% HPMC, 9% mannitol, 4% dextran, 1% carbomer, 1% sodium taurocholate	Lyophilisation	(Zhu et al., 2018)
Standardised protein-based allergens	Sublingual	Excipients not discussed	Lyophilisation or direct compression	(Lund et al., 2019)

Future perspectives

The robustness and flexibility of ODDFs as delivery vehicles have opened exciting possibilities to deliver a wide spectrum of TPPs, including vaccines that are mostly limited to delivery through invasive routes such as parenteral injections. It is apparent from the review presented above that the formulation of TPPs in ODDFs is still in the early stages with the only marketed formulations being the SLIT formulation and desmopressin acetate (Minrin® Melt) sublingual tablet, but the 'wave' of research is intensifying. The translation of TPPs already marketed in a conventional tablet can be switched to an ODT formulation with ease. For those macromolecules that are vulnerable to enzymatic degradation, the option of delivery in the oral cavity can be considered or encapsulation within nano/microparticles or liposomal structures to enhance stability and absorption. From an economic viewpoint, a highly desirable option to extend the shelf-life, reduce packaging and minimise cold chain footprints of TPPs is to formulate them within ODTs or ODFs that are compact in size, allowing ease of transportation particularly for life saving therapeutic treatments in low-resource countries. Studies have demonstrated that careful selection of excipients to stabilise and protect TPPs from heat and energetic processes during manufacturing, as well as the addition of permeation enhancers if systemic absorption is required, and soluble biosynthetic polymers to confer rapid release properties to the formulation, can lead to successful outcomes. ODDFs clearly have a firm place in delivering TPPs for emergency or 'on-demand' medical situations. Additionally, with the new advancement in manufacturing techniques, 3D printing is offering greater potential for the fabrication of TPPs in simple, personalized, cost effective ODDFs. This approach enables the formation of advanced multilayer dosage forms in a single step process. EHD methods of fabricating TPPs in ODDFs have been found to be highly suitable for heat labile APIs and the possibility of mass production of protein-type drugs is achievable by incorporation into electrospun fibres (Vass et al., 2019, Vass et al., 2020).

As research into ODDFs has accelerated, attention is now being focussed on TPP-based treatments for the paediatric population (Gleeson et al., 2021). The World Health Organisation (WHO) recommends a shift of paediatric oral medication from liquid to solid oral dosage forms (Lajoinie et

al., 2017) including powders, multiparticulates and orodispersible dosage forms. As a result of this report, ODMTs are now being considered as a suitable strategy for development of an appropriate child-friendly dosage form for infants as young as 1 month old (Stoltenberg and Breitkreutz, 2011). Similar to liquid formulations, ODMTs can allow flexible dosing options and administration with added taste-masking properties that are beneficial for paediatric drug formulations (Gleeson et al., 2021, Stoltenberg and Breitkreutz, 2011). Furthermore, TPP-based ODMTs are particularly applicable to target paediatric populations in low-resource countries for treatment of diseases such as HIV (Schlatter et al., 2016). Mini-tablets are defined as having a diameter of 4mm or less and are considered the most appropriate method of administering medicines to young children and infants (Comoglu and Dilek Ozyilmaz, 2019). Human clinical trials were conducted with drug-free ODMTs on neonatal subjects between the age of 2-28 days. When the minitablet was placed between the gum and inner cheek, it was shown that acceptance of the dosage form was comparable or even better than a syrup (Klingmann et al., 2015). Although there are some studies that are in the process of developing ODMTs for small molecule drugs specifically for paediatrics (Khan et al., 2021), it will only be a matter of time before this work will translate to the incorporation of TPPs especially when all the advantages of using this type of formulation are considered. Mini-tablet formulations incorporating dextran-4000 have been shown to improve the absorption of the large macromolecule across porcine intestinal tissue in *ex vivo* studies. Dextran-4000 was first formulated into beads and then compacted, along with different permeation enhancers, into mini-tablets. The smallest sized beads (0.5mm) was shown to have the fastest absorption (Bodenstein, 2020). In a clinical trial that evaluated the preference of young children, suffering from primary nocturnal enuresis, for sublingual tablet vs conventional oral tablets containing desmopressin acetate, it was reported that the sublingual tablet would be more suitable for young children, particularly between 5-11 years of age (Lottmann et al., 2007). Patient-centric therapy and treatment is now the way forward in healthcare research as it is being widely acknowledged that treatments must be tailored for distinct patient groups to obtain optimal satisfactory health outcomes with considerations for age, gender, geographical location, and ethnicity.

6. Conclusions

The research and development of oral TPPs is a rapidly expanding field that has demonstrated success as increasing numbers of TPPs are being licensed for use in clinics. As a result of this, there is a need to target specific patient populations who can often be overlooked, including paediatrics, geriatrics, patients with dysphagia and those in low-resource countries. It is important to ensure that these groups are given wider options and choices suitable for their particular needs, thereby maximising the effectiveness of treatment therapies to improve overall health benefits.

ODDFs are rapidly disintegrating dosage forms that dissolve in the oral cavity without the need for water. Successful ODDFs that have entered the medicinal market contain small drug molecules that are aimed at treating 'on-demand' or 'when required' medical conditions such as pain relief for migraine. Treatments that require TPPs to be rapidly absorbed into the systemic circulation are now being studied by incorporation into ODDFs for administration via the sublingual and buccal routes. Examples of this approach is the administration of oxytocin for the treatment of PPH in pregnant women within 24hrs of childbirth, where studies have exploited the fact that ODDFs are highly suitable for rapid absorption via the oral cavity and can therefore be used in the treatment of life-saving medical conditions. ODDFs have also been identified as desirable alternatives to liquid formulations where the stability and longevity of TPPs are extended when formulated in ODDFs since solid dosage forms contain significantly reduced moisture content. The resultant benefits are; minimal sterility requirements in comparison to liquid preparations, the potential for extended storage periods after manufacture, a reduced cold chain footprint and reduction in packaging requirements. All these advantages can allow cheaper production costs and ease of transport resulting in the development of much needed medicines for specific patient populations including those in low-resource countries.

Vaccine-based ODDFs can provide the potential to mass immunise vulnerable patients at reduced cost, as well as very young children who can benefit from non-invasive methods of administration. The potential for eliciting a robust immune response in the oral cavity and GIT by next generation vaccine antigens, such as subunit proteins, has been demonstrated in several studies. Subunit vaccines are vulnerable to degradation in the harsh microenvironment of the stomach but have shown a good immune response when administered through sublingual and buccal routes. This has led to investigations exploring the incorporation of antigens in ODDFs as buccal and sublingual vaccines. However, research into the delivery of TPPs, including vaccine antigens, to sites within the GIT is currently ongoing and various approaches and strategies are being used to increase stability of the actives, and enhance absorption.

By careful consideration of the physicochemical characteristics exhibited by a TPP molecule, various strategies and approaches can be applied for incorporation of these actives in ODDFs to enhance stability during manufacturing, storage and transport, as well as *in vivo* protection and enhanced absorption. Although a challenging task that is in the early stages of progress, a thorough understanding of factors including appropriate excipients and manufacturing processes can facilitate and fulfil the criteria of an optimal design to formulate TPP loaded ODDF systems.

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Graphical Abstract