Review Article

Theme: Preclinical Peptide Developability Assessment

Guest Editors: Annette Bak and Weiguo Dai

Physicochemical and Formulation Developability Assessment for Therapeutic Peptide Delivery—A Primer

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Abstract. Peptides are an important class of endogenous ligands that regulate key biological cascades. As such, peptides represent a promising therapeutic class with the potential to alleviate many severe disease states. Despite their therapeutic potential, peptides frequently pose drug delivery challenges to scientists. This review introduces the physicochemical, biophysical, biopharmaceutical, and formulation developability aspects of peptides pertinent to the drug discovery-to-development interface. It introduces the relevance of these properties with respect to the delivery modalities available for peptide pharmaceuticals, with the parenteral route being the most prevalent route of administration. This review also presents characterization strategies for oral delivery of peptides with the aim of illuminating developability issues with the drug candidate. A brief overview of other routes of administration, including inhaled, transdermal, and intranasal routes, is provided as these routes are generally preferred by patients over injectables. Finally, this review presents formulation techniques to mitigate some of the developability obstacles associated with peptide delivery. The authors emphasize opportunities for the thoughtful application of pharmaceutical science to the development of peptide drugs and to the general advancement of this promising class of pharmaceuticals.

KEY WORDS: developability; oral peptide delivery; parenteral peptide delivery; peptide therapeutics.

INTRODUCTION

Peptides occupy a therapeutic niche between small molecules and large biologics and have been the focus of intense research over the past few decades. Compared with small-molecule active pharmaceutical ingredients, peptides can exhibit increased potency and selectivity on account of specific interactions with their targets (1). As a result of this improved selectivity, peptides have the potential for decreased off-target side effects and decreased systemic toxicity. Despite these distinct advantages of peptide therapeutics, several key challenges still limit their widespread application

including low oral bioavailability, poor membrane permeability, and metabolic liabilities (1,2). Hence, peptide administration is largely limited to injectable routes such as subcutaneous (SC), intravenous (IV), and intramuscular (IM) administration. However, since injections are associated with pain and low patient compliance, patients prefer alternative routes of administration (3,4) such as oral, transdermal, intranasal, pulmonary, and ocular. In general, the oral administration of peptides is limited by poor bioavailability due to enzymatic degradation in the gastrointestinal tract, the inability to cross the epithelial barrier, and the presence of efflux pumps (2). As outlined in Table I, alternative delivery routes offer their own advantages and limitations for peptide drug delivery.

Peptides have significant potential to impact the lives of patients by addressing unmet medical needs. Recent market analysis supports this potential (5), with actual and projected revenues for several peptide therapeutic delivery routes given in Table II. Injectable peptides are projected to have the largest market share in 2018; however, the compound annual growth rate (CAGR) for peptides delivered by alternative routes, including oral, pulmonary, and transdermal, is projected to exceed that of parenterals (5), underscoring a preference among patients for these less invasive routes.

This review is intended to be a primer for scientists new to the field of therapeutic peptide discovery and early

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Table I. Peptide Delivery Route Advantages and Limitations

Route	Advantages	Limitations
Oral	Easy access, convenient to dose; patient compliance; high dose delivery	Epithelial barrier of the small intestine; chemical and enzymatic degradation; efflux pumps; first-pass gut and hepatic metabolism
Transdermal	Easy access, convenient to dose; patient compliance; large surface area for treatment	Low dose delivery; difficult barrier to penetrate; toxicity/irritation at site of application
Pulmonary/inhalation	Large surface area for absorption; thin epithelial barrier (moderate permeability); very well perfused; bypasses first pass metabolism; low proteolytic activity	Limited dose, dose volume; reproducible deposition; safety and lung function; taste liability; device development required; tight and complex respiratory mucosa
Intranasal	Highly permeable epithelia; convenient dosing; commercially available devices; bypasses first pass metabolism	Limited dose, dose volume; rapid clearance; taste liability; irritation potential
Ocular	Easy access, convenient to dose (eye drops); good patient compliance (eye drops)	Limited dose, dose volume; limited to local delivery; irritation potential; low permeability barrier; may require surgery (injections or implants)

Adapted from Reference (105)

development and a reliable reference to those with more experience in the field. As such, this article first summarizes the physicochemical and formulation developability criteria for parenteral and oral delivery of peptides. For the purpose of this article, “oral delivery” is defined as administration via the gastrointestinal tract; intraoral delivery via the sublingual or buccal routes is distinct and is covered briefly in the “[Other Routes of Administration](#)” section of this article. Next, the challenges and opportunities associated with emerging delivery routes including transdermal and pulmonary are discussed in brief. Lastly, the authors provide a perspective on developability strategies at the discovery-to-development interface for peptides, highlighting their similarities and differences relative to small molecules and biologics. There are several definitions of “therapeutic peptides” in the scientific literature. This article defines them as peptides similar or smaller in size to insulin (51 amino acids), as the authors’ experiences suggest that these entities share strong commonalities, particularly with respect to biophysical characterization.

PARENTERAL PEPTIDE FORMULATIONS

Due to typically poor permeability across biological membranes, most peptide drugs are delivered by injection. The general challenges and risks associated with developing peptides for common injection routes including SC, IV, and IM are similar to those for developing any injectable dosage form. Formulations need to be stable, sterile, and pyrogen- and particulate-free. They must fall within reasonable bounds

of pH and tonicity to prevent local irritation and hemolysis, and the injection volume must be appropriate to the route of administration. However, peptides have unique physicochemical properties that can make meeting the above criteria more challenging than with small molecules. A few of the most important developability concerns include self-association and aggregation, adsorption to surfaces, and a strong dependence of solubility and stability on pH. In addition, the smaller size of peptides compared with larger biologics gives rise to different formulation challenges, particularly with respect to chemical and conformational stability (6,7), as elaborated below. These unique aspects result in challenges of achieving sufficient solubility in the formulation and maintaining chemical and physical stability through the manufacturing process and in the final dosage form.

A discussion of parenteral peptide developability assessment is not complete without acknowledging the role of long-acting delivery approaches. It is important for scientists at the discovery/development interface to carefully consider the impact of peptide half life on frequency of administration, and the potential need for strategies to mitigate short half life and frequent administration that may compromise efficacy, safety, and patient adherence. This challenge can be addressed through both molecular design to prolong half life (e.g., PEGylation, acetylation, fusion to albumin, or the Fc region of an antibody) as well as formulation (sustained release dosage forms). This is well beyond the scope of this article, and the reader is encouraged to consult recent articles on the topics, for example (8).

Table II. Actual and Projected Peptides Therapeutics Revenue (US\$ Million) for Peptide Delivery Routes (2012–2018) and CAGR (2012–2018)

Application	2010	2011	2012	2013	2014	2015	2016	2017	2018	CAGR% 2012–2018
Parenteral	11,537	12,201	13,141	14,152	15,239	16,408	17,666	19,017	20,600	7.8
Oral	774	869	978	1,098	1,233	1,382	1,548	1,733	1,907	11.7
Pulmonary	375	427	483	546	617	695	782	880	1,017	13.2
Mucosal	383	428	512	607	715	836	974	1,130	1,144	13.5
Others (intradermal and nasal)	132	212	260	316	380	453	533	627	763	19.3
Total	13,200	14,138	15,374	16,719	18,183	19,775	21,504	23,386	25,432	8.8

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Peptide Solubility and Its Influence on Formulation Composition

Parenteral peptide products are preferentially formulated as solution dosage forms. Thus, the solubility of a peptide in the formulation is a primary concern. A successful formulation requires sufficient solubility to allow for a dosing volume suitable for the selected route of administration. IV formulations may be introduced in larger volumes via continuous infusion, whereas SC and IM formulations may have more limited dosing volumes and thus require a drug substance with higher solubility. The aqueous solubility of a peptide is highly dependent on the pH and is at a minimum at the peptide's isoelectric point (pI), the pH at which its net charge is zero. Adjusting the pH of the dosing formulation away from the pI can vastly improve the solubility of the peptide, so it is critical to evaluate the solubility of the peptide across the tolerable range for IV administration, typically considered pH 3–10 (9). However, adjusting pH to improve solubility may also increase the risk of acid- or base-catalyzed hydrolysis or deamidation (10). Thus, an optimum pH region is one in which solubility is maximized and chemical instability (see the next section for more details) is minimized. Indeed, the solubility of a peptide as a function of pH is one of the key considerations that should be evaluated at the lead optimization stage to ensure solubility at a physiologically relevant pH. The final pH of the dosing formulation can be precisely controlled through the use of buffered vehicle; however, the buffer components and their relative concentrations can also impact the solubility of the peptide (11–14). Additionally, the choice of counter-ion for the ionized peptide can also affect its solubility. Typically, higher concentrations of buffer salts can help improve pH control through increased buffer capacity, but the resulting increase in ionic strength can result in a decrease in solubility (via “salting out” or increasing hydrophobic interactions) or increased risk of local irritation at the injection site. Alternatively, excipients such as surfactants, co-solvents, and cyclodextrins may be used to help solubilize the peptide. As with buffers, the identities and concentrations of the excipients may impact the stability of the peptide and thus must be carefully chosen (10).

While solution formulations are widely preferred, suspension formulations may be necessary when peptide solubility is insufficient to achieve a fully soluble formulation. In specific cases, suspensions may exhibit improved stability or give a preferred *in vivo* release and absorption profile relative to solutions. This is the case for a number of insulin suspension formulations developed to enable a long-acting pharmacokinetic response (15,16). When considering suspension formulations, the solid-state form and particle size of the suspended particles must be carefully characterized to ensure reproducible behavior (17). Similar to small molecules, crystalline forms are often preferred over amorphous forms in suspension formulations, as the former may be isolated in highly pure form, may exhibit stable and reproducible particle sizes and morphologies, and often provide improved physical stability relative to the latter. However, crystallization conditions for peptides may be difficult to identify (18). If that is the case, amorphous forms may be considered, though they tend to have higher risk of physical instability via aggregation, flocculation, and phase conversion.

Chemical and Physical Stability

The development of an appropriate parenteral formulation requires a thorough understanding of the physical and chemical stability risks of the peptide in solution (19–21). Typically, in clinical development and the commercial space, a satisfactory peptide drug should remain chemically and physically stable over a minimum of a 2-year shelf-life. Identifying stability issues early during the discovery or development of a peptide drug candidate will allow time to mitigate these risks and potentially avoid costly interventions later in development. The International Conference on Harmonization (ICH) recommends criteria for stability and testing conditions (22), and these guidelines may be implemented in designing a formulation with minimal stability risks (23,24). A few of the chemical and physical degradation liabilities common for peptide therapeutics are summarized in Table III; these are routinely evaluated at the preformulation stage (10).

The formulation compositional variables described in the previous section, including buffer salts, surfactants, and pH, can all impact chemical stability just as they influence solubility. Additionally, peptides are sensitive to oxidative degradation, particularly peptides containing cysteine, methionine, histidine, tyrosine, and tryptophan residues. Oxidation may be exacerbated by residual metal impurities or by exposure to light (10,25–27). Furthermore, peroxide

Table III. Common Peptide Stability Risks and Strategies for Mitigating These Risks Through Formulation (67)

Stability risk	Formulation mitigation strategy
Solubility	pH modification and salt formation Optimization of ionic strength Addition of solubilizing excipients (i.e., surfactants, co-solvents)
Hydrolysis	Evaluation of stability across pH 3–10 range Addition of buffer excipients to control pH
Oxidation	Low-temperature storage Addition of antioxidants Addition of chelating agents Maintenance of pH <7 Anaerobic processing Protection from light
Aggregation	Low-temperature storage Lower peptide concentration pH modification and salt formation Addition of buffer excipients Optimization of ionic strength Addition of solubilizing excipients (i.e., surfactants, co-solvents)
Adsorption	Addition of surfactant and polymer excipients Addition of albumin Appropriate container selection or surface modification
Denaturation	Addition of salts or metal ions Appropriate pH Low-temperature storage
Microbial contamination	Addition of preservative excipients Low-temperature storage

contaminants in excipients such as polysorbates can accelerate oxidative degradation (28–30). Antioxidants may be added to protect the peptide, though their benefit must be carefully weighed against potential liabilities. For example, ascorbic acid has been used as an anti-oxidant, but in some cases has been found to form highly reactive oxidative species (31). Chelating agents can also be introduced to inhibit metal-catalyzed oxidation. Generally, oxidation propensity lessens at lower pH (32). Handling the peptide under an inert atmosphere, such as nitrogen or argon, can also mitigate oxidation (33). Photosensitive peptides require the added precaution of protection from light throughout the processing workflow and in the final product packaging (34).

In addition to chemical stability risks, the physical stability of peptides must be carefully monitored to ensure a consistent formulation, which can otherwise lead to differences in bioperformance and to immunogenicity safety concerns. These risks include aggregation and self-association, changes in secondary and tertiary structure, and adsorption to surfaces (10). Peptides are smaller than proteins and, as a result, may lack a single stable secondary structure. Instead, the peptides may exist as multiple conformations of similar energy in solution. However, certain peptide conformations may be less stable, leading to loss of function, aggregation, and/or precipitation (35–39). Changes in peptide conformation can potentially be determined using techniques such as circular dichroism or calorimetry, although these approaches may be difficult if multiple conformations are in rapid equilibrium. Changes in conformation may increase solvent exposure of the hydrophobic regions of the peptide sequence, leading to self-association, particularly at higher concentrations. If the aggregates become sufficiently large and insoluble, precipitation may result. Changes in the particle size of the peptide species can be monitored through techniques such as light scattering, as well as size exclusion chromatography. Ionization of the peptides through adjusting the pH of the formulation media can increase the overall zeta potential of the peptide, typically increasing colloidal stability. The addition of salts can stabilize the native conformation through non-specific binding to the peptide surface, and solubilizing excipients such as polyethylene glycol (PEG) can also be used to reduce peptide aggregation.

Processing Stability

Peptides may also display sensitivity to an increased variety of manufacturing processing conditions, including freeze/thaw cycles, lyophilization, and shear mixing (40,41). The evaluation of peptide stability during filtration and terminal sterilization is of critical importance. Preservative excipients may also be required for multidose products (42–44). Compatibility with primary containers/closures must be evaluated, as the amphiphilic nature of peptides render them prone to adsorption to surfaces including glass, rubber, and plastic, resulting in loss of material during processing and storage (45–50). Formulation and manufacturing processes should be evaluated to minimize loss via adsorption, and appropriate containers must be used for peptide storage and delivery. Surface binding sites are generally finite and saturable; as a consequence, excipients such as human serum albumin or surfactants can be added to the formulation to

competitively bind to the surface and prevent binding of the active peptide (51,52). Surfactant excipients can also be added to stabilize peptides against denaturation and can bind to the exposed hydrophobic surfaces of peptides to reduce their adsorption tendency (49). However, certain surfactants can compromise peptide stability (i.e., polysorbate surfactants may contain oxidative impurities), so the formulation must be carefully monitored and options thoughtfully weighed.

Parenteral Peptide Developability Case Studies

Recent formulation efforts to improve the stability of oxytocin formulations exemplify a thoughtful approach toward mitigating peptide stability risks. Oxytocin is a cyclic nonapeptide that plays an important role in the function of the central nervous system and has been indicated by the World Health Organization as an effective treatment for postpartum hemorrhage, a major cause of childbirth-related mortality in the developing world (53,54). It has been traditionally formulated as a solution for parenteral administration, but the acute instability of oxytocin at elevated temperatures requires a refrigerated formulation, making its distribution and utilization in tropical climates challenging (55).

Under stressed conditions, chemical and physical degradation of oxytocin were observed (56,57), with deamidation products observed below pH 2 and above pH 9. Based on kinetic analysis, pH 4.5 was identified as the most stable solution condition (58). Further stabilization has been achieved with the addition of divalent metal ions such as calcium, magnesium, and zinc in combination with buffers such as citrate or aspartate (59–61). One hypothesis is that the interaction of oxytocin, the metal ion, and the acidic buffer may contribute to stabilization of the peptide conformation, thereby reducing the risk of degradation (59). In this case, identification of a composition likely to remain stable for 2 years under ICH Zone IV (i.e., hot and humid) conditions remains elusive, and alternative approaches, such as use of synthetic analogs with improved intrinsic stability (i.e., carbetocin) represent a clearer path toward an ICH Zone IV-stable product (62). However, this example illustrates how a thorough evaluation of peptide stability can help guide and optimize the formulation development strategy.

ORAL DELIVERY OF PEPTIDES

Drug Substance and Drug Product Characterization

Oral delivery, although typically the preferred administration route, represents a significant challenge for peptide therapeutics for the reasons cited earlier in this review. Despite many years of research and development effort, oral delivery of peptides has met with limited commercial success. Still, the medical and commercial potential of orally bioavailable peptides remains significant, and the knowledge accumulated over time suggests a number of strategies for formulating peptides for oral delivery. For these reasons, oral delivery can be considered a high-risk, high-reward approach to peptide administration. The potential for oral peptide therapeutics is exemplified by cyclosporine A, a natural product. Its physicochemical, formulation, ADME and PK

(pharmacokinetic) properties have been extensively described in the literature and are summarized in Table IV (63–65). Characteristics of this molecule include a cyclic structure, modified peptide bonds, and unnatural amino acids that help stabilize cyclosporine A against classical peptide degradation routes (i.e., exo- and endo-peptidases). The molecule degrades primarily via oxidative metabolism (63), a route typically seen for small molecules and peptidomimetics. Metabolism of orally administered linear, chemically unmodified peptides is usually dominated by peptidases and proteases. But even cyclosporine A has its liabilities. The structural features that render cyclosporine A stable against peptidases yield a compound with very poor aqueous solubility which is classified as a Biopharmaceutical Classification System (BCS) class IV compound (64) (i.e., low solubility, low permeability). PK variability on account of its limited solubility has limited the application of cyclosporine A for the drug's primary indication of transplant rejection prevention. Some patients on Sandimmune® experienced classical cyclosporine A kidney toxicity (i.e., too high exposure to cyclosporine A), while others suffered from transplant rejection (too low exposure to cyclosporine A). Neoral® (launched in 1994) was able to reduce this variability by more effectively solubilizing cyclosporine A in the small intestine. Formulation optimization subsequent to the launch of Neoral® has aimed at further reducing PK variability (64). Therefore, the cyclosporine A case study highlights, in line with the authors' own experiences, the important interplay between structural features and delivery considerations when designing and developing a peptide for oral delivery. A balanced approach is needed between eliminating the liabilities of peptides (i.e., limited permeability and peptidase metabolism) while also avoiding issues classically seen with small molecules (i.e., solubility limited absorption).

The physicochemical properties and formulation aspects necessary for oral delivery are mainly driven by the

gastrointestinal barrier biology, and drug substance and product quality concerns. They can therefore be viewed as similar among small molecules and peptides and have been reviewed elsewhere (66). Accordingly, the criteria for oral delivery encompass five categories: (1) physicochemical properties, (2) biophysical stability, (3) chemical stability, (4) biopharmaceutical properties, and (5) formulation characteristics and strategies (with the formulation strategies covered separately in the next section). Drug properties within each of these categories and methods for evaluating them are detailed in Table V.

As described above, the aqueous solubility of a peptide can be limited, particularly in a solution maintained near the peptide pI (67). Therefore, the solubility of a peptide in orally biorelevant fluids (e.g., simulated gastric and intestinal fluids) is one important developability criterion. Another, but related, consideration is the biophysical stability of the peptide. Physical stability limitations for peptides manifest as aggregation, fibrillation, and micelle formation. These phenomena can adversely impact solubility in the formulation and in gastrointestinal fluids (68–70) and consequently also affect oral absorption. As such, physical stability should be incorporated in candidate selection criteria. Due to their low permeability and slow oral absorption (2,3), peptides may have a significant residence time in the gastrointestinal (GI) tract. Therefore, it is recommended that oral peptide therapeutics be evaluated for chemical stability in biorelevant fluids (71–73).

In the Biopharmaceutical Classification System, peptides are often classified as either low-permeability, high-solubility (BCS III) or low-permeability, low-solubility (BCS IV) compounds. Thus, significant effort should be directed toward assessing and de-risking biopharmaceutical aspects such as permeability. *In vitro* studies may be used to generate partition coefficients early in drug discovery or development, with the log(heptane/ethylene glycol) partition coefficient serving as a more predictive measure for peptides compared

Table IV. Physicochemical, Formulation, ADME, and PK Properties of Cyclosporine A

Property	Cyclosporine A
Number of amino acids	11
Molecular weight	1,202.61 g/mole
Structural features	Cyclic, 1 D-amino acid, N-methyl amide bonds, intramolecular hydrogen bond
Log P	3.64
Polar surface area	278.8 Å ²
Solubility	Poor in water (0.004 mg/ml), good in protic organic solvents, modest in non-protic organic solvents
Chemical stability	Stable in the solid state, modest acid instability (t _{1/2} at 37°C=63 h at pH 1.1 and 79 h at pH 3)
Physical state	Crystalline needles with Mp of 148–151°C
Absorption	BCS IV. Absorbed in the ileum and jejunum passively, actively, and via lymph system (Peyer's patches); formulation and meal dependent food effect
Distribution	Widely throughout the body due to lipophilic nature
Metabolism, transport	Cytochrome P540 3A4, P-glycoprotein, liver uptake transporters
Excretion	Biliary system, mainly as metabolites
PK	Variable (10–80% F). Variability is higher with Sandimmune® (macroemulsion relaying on lipid digestion) than with Neoral® (self-emulsifying micro-emulsion)
Formulation: Sandimmune®	Cyclosporine A, alcohol, corn oil, Labrafil M 2125 CS, gelatin capsule shell, coloring agents
Formulation: Neoral®	Cyclosporine A, DL- α -tocopherol, maize oil, propylene glycol, gelatin capsule, coloring agents (also available as solution)

Adapted from References (63–65)

BCS Biopharmaceutical Classification System, PK pharmacokinetic

Table V. Physicochemical, Chemical, and Physical Stability and Biopharmaceutical and Formulation Characterization Conducted for Oral Peptide Candidates

Category	Property	Assay	Criteria/ consideration	References
Physicochemical	“Rule-of-five”	MW, LogP, pKa, HBD, HBA	Impact on solubility and permeability	(125,126)
Physicochemical	Conformation, secondary structure	NMR, CD	Impact on solubility and permeability	(126,127)
Physicochemical/ chemical stability	Solubility/stability	Stability/solubility in SGF, FaSSIF w/o enzymes	Impact on absorption	(71–73)
Chemical stability	Chemical degradation	HPLC, LC-MS	Impact on DS, DP and <i>in vivo</i> stability	(128)
Chemical stability	Water sorption	Moisture sorption, Tg	Water in the DS/DP can impact chemical stability	(129)
Physical stability	Aggregation	FTIR, DSC	Can affect DS and DP performance and bioperformance	(68–70)
Physical stability	Fibrillation	Thio-T		(68)
Physical stability	Micelles	DLS, TEM		(70)
Physical stability	Particle size	DLS, Zeta potential, microscopy	Batch variability and DS/DP stability, performance	(70–73,78,130)
Biopharmaceutical	Food effect	PK study	Decreased exposure and increased variability with food	(77)
Biopharmaceutical	Permeability prediction	PAMPA	Artificial/Caco-2 membrane permeation assay	(75)
Biopharmaceutical	Permeability prediction	Log heptane/ethylene glycol	May predict GI permeability better than LogP	(74)
Formulation	Assays claim	HPLC	Dose strength confirmation	(69,71)
Formulation	Drug release	Dissolution	Dissolution QC and bioperformance	(71,78)
Formulation (emulsion)	Formulation performance	Viscosity, turbidity, conductivity, RI, microscopy	Batch-to-batch variability	(69,87,130)

CD circular dichroism, DLS dynamic light scattering, DP drug product, DS drug substance, DSC dynamic scanning calorimetry, FaSSIF fasted state simulated intestinal fluid, FTIR Fourier-transform infrared spectroscopy, HBD/HBA hydrogen bond donors/acceptors, HPLC high-pressure liquid chromatography, LogP octanol/water partition coefficient, MW molecular weight, NMR nuclear magnetic resonance, PAMPA parallel artificial membrane permeability assay; pKa acidity constant, QC quality control, RI refractive index, SGF simulated gastric fluid, TEM transmission electron microscopy, Tg glass transition temperature, Thio-T thioflavin-T assays

with a classical octanol/water logP screen (74) typically used for small molecules. Studies measuring permeability across artificial membranes (i.e., PAMPA) and Caco-2 cell monolayers have also been used (75) for screening purposes and to optimize the peptide structure. Compounds that display permeability-limited absorption are more prone to exhibiting a negative food effect (76). Therefore, not surprisingly, the systemic exposure of calcitonin when delivered orally was reduced by as much as 75% in a fed state relative to a fasted state in clinical trials (77). Thus, additional developability criteria must be established to evaluate food effects.

To date, most research into oral peptide delivery has taken place in discovery and preclinical development. The characterization and stability testing for oral peptide formulations described in the scientific literature reflects the requirements in terms of duration and rigor for this stage of research and development (69,71,72,78). As more oral peptides move into clinical development, additional criteria will have to be established. It is anticipated that researchers will look at experiences with oral small molecules to guide this development. However, due to the unique delivery challenges with peptides, the excipients and formulations will likely be diverse and complex.

Oral Peptide Formulation

Strategically, oral formulation development for peptides should address two aspects: (1) drug product quality aspects

such as excipient performance/quality and storage stability and (2) drug product bioperformance aspects such as *in vivo* stability and appropriate excipient selection to overcome drug delivery barriers. Since drug availability is frequently limited at this early stage, these screens must be conducted in a targeted fashion.

With respect to drug product quality aspects, initial characterization of the physical and chemical stability liabilities of the peptide is typically completed via preformulation work-up and hence known at the start of formulation development, though aggressive manufacturing processes can introduce unforeseen challenges in development. Bio-physical or chemical storage stability can often be improved by judicious excipient selection. For example, excipients can stabilize the secondary structure or crystalline form of a peptide through interactions with metal ions (68) or amino acids. Acidic or alkaline excipients can be selected to modify local pH (79) to mitigate hydrolytic issues. If oxidation is determined to be a liability, peroxide-containing excipients should be identified and avoided, and antioxidants or metal chelators can be included to minimize degradation of the peptide in the dosage form. Physical and chemical stability of the peptide in the formulation in appropriate packaging should also be assessed.

As mentioned, an important objective of oral formulation development is to ensure appropriate bioperformance, and there are several barriers to be overcome for oral peptide delivery. Once the dosage form is swallowed, it encounters an

acidic, peptidase-rich environment in the stomach which can rapidly metabolize the peptide. To mitigate this problem, the dosage form or its component granules can be enclosed in an acid-resistant enteric coating to minimize release in the upper GI (80). Often, peptidase inhibitors and pH modifiers are included (81) to provide additional protection for peptides against the harsh stomach environment. The success of these approaches can be confirmed by *in vitro* assessment of the peptide formulation stability with respect to acidic and enzymatic degradation (82,83).

As the dosage form or its components progresses to the small intestine, the peptide must be released from the formulation and solubilized. Enteric coatings will dissolve at the higher pH of intestinal fluid, resulting in the disintegration and dispersion of the dose. Many peptides are relatively insoluble in the GI environment, and a diversity of drug delivery options has been investigated to improve solubility and permeability. These include approaches commonly used for small molecule delivery, including the use of surfactants (84), polymer micro-/nanoparticles (85), solid lipid micro-/nanoparticles (73,86), microemulsions (87), liposomes (88), micelles (89), and combinations of these approaches (90).

When systemic delivery is desired, the peptide must exit the GI into the bloodstream as an intact chemical species. This may be the most challenging barrier to oral peptide delivery. Some native or modified peptides interact with endogenous transporters such as transferrin (91,92) and biotin (93) or glucose (94) transporters which will facilitate transport across the gastrointestinal mucosa. Others can be absorbed through GI-associated immune tissues into the lymphatic system (95) or translocate cell membranes (cell penetrating peptides) (96). However, the most prevalent strategy is to facilitate peptide entry into the bloodstream via transient disruption of paracellular tight junctions by the application of transient permeation enhancers (97,98). Select evidence suggests that some surfactants, lipids, and polymers may act as permeability enhancers, but, among these, only a few are effective and safe enough for application.

Alternatively, the residence time of the peptide on the wall of the intestine can be enhanced with mucoadhesive excipients that promote peptide absorption by retaining them in the upper part of the small intestine. Examples of bioadhesive polymers (99) include carbomer (100), chitosan (101), and modified chitosan (102,103). Thiolated polymers or 'thiomers' are typically chitosan-based and induce effective mucoadhesion by *in situ* formation of disulfide bonds (104). Ultra-pure preparations of chitosan have enabled evaluation of its commercial viability in this application, though thiomers are not yet available with the required safety testing for pharmaceutical use or at commercial scale.

Despite the many challenges to oral peptide delivery, clinically relevant bioavailability can be achieved in some cases by the application of emerging drug delivery systems. A subset of these technologies is summarized in Table VI. A noteworthy example is an oral tablet formulation of salmon calcitonin (sCT) which progressed to phase III studies after demonstrating a 10× increase in exposure and improved efficacy over the marketed nasal spray with approximately 24× the dose. In this case, the dosage form contained (8-(*N*-2-hydroxy-5-chloro-benzoyl)-amino-caprylic acid), an *N*-acetylated amino acid that reversibly complexes with sCT to

facilitate passive transcellular permeation of the compound (77).

In summary, intelligently designed formulations based on a thorough understanding of peptide characteristics can overcome many of the barriers to oral peptide delivery. While it is unlikely that a single technology addressing one or all of these biological barriers will be equally suitable for all peptides, significant progress in this arena has increased the feasibility of oral peptide therapeutics.

OTHER ROUTES OF ADMINISTRATION

Alternative delivery routes including nasal, pulmonary, transdermal, intraoral (i.e., through the buccal and sublingual mucosa), ocular, vaginal, and rectal have been pursued for peptide therapeutics and present compelling therapeutic and strategic advantages, as mentioned previously. In this section, we will discuss transdermal, intraoral, and respiratory delivery of peptides.

Selection, early developability, and risk assessment of alternative routes of peptide administration are well-documented (with advantages and limitations of available routes outlined in Table I) (105). As with oral delivery, this process should include consideration of peptide physicochemical properties and evaluation in relevant *in vitro* and *in vivo* model systems to assess projected bioavailability and associated variability. Ideally, peptides should be selected for delivery via these routes based on potency, large therapeutic window to accommodate an increased risk for PK variability, and physicochemical properties appropriate to the route and delivery technology of interest. The developability assessment strategy for each peptide delivery route will differ slightly.

Enzymatic degradation is less prevalent in the skin compared with other delivery routes. However, peptides can still undergo enzymatic degradation while traveling through skin layers during transdermal delivery (106), and therefore skin-specific (especially epidermal) protease stability should be examined *in vitro* as part of the developability assessment strategy (107). Optimization of the transdermal formulation design includes examination of the application area (patch size), the rate of epidermal transport, and the incorporation of specific enzyme inhibitors to aid in maintaining the peptide's integrity. In addition, the transdermal route is a challenging option for peptides, owing to the low permeability of large, polar molecules through the stratum corneum, the primary barrier to transdermal flux. Opportunities for transdermal delivery of peptides have, however, increased in recent years with the advent of delivery technologies capable of disrupting this layer through mechanical or other means. Examples of these include microneedles, thermal ablation, iontophoresis, electroporation, and sonophoresis (2,108,109). The PassPort™ (Nitto Denko) is an example of such a system that has been investigated for delivery of insulin and other peptides (Fig. 1a). Microneedles are small projections typically less than 1 mm in length which can be composed of insoluble or soluble/biodegradable solids. They can be arrayed on a patch and coated with a drug formulation or, if hollow, used to inject a solution, thus facilitating the intradermal administration of drugs and vaccines (110). Microneedles have been successfully applied for delivery of insulin and are being explored for administration of GLP-1

Table VI. Oral Peptide Delivery Technologies

Company/website	Technology
Chiasma http://www.chiasmapharma.com/	Transient permeability enhancer, TPE® (emulsion, permeation enhancer)
Aegis http://aegisthera.com/	Intravail® (permeation enhancer, stabilizer)
SDG http://sdgpharma.com/	Phospholipid nanoparticle
Generex http://www.generex.com/	ORAL-LYN™ aerosolized buccal delivery
Sigmoid Pharma http://www.sigmoidpharma.com/	SmPill® (mini-spheres, solubility, stability, permeability enhancer)
Biocon http://www.biocon.com/	Alkyl PEG conjugates
Diabetology http://www.diabetology.co.uk/	Dry powder (enteric coated capsule, permeation enhancer, solubilizer)
Merrion http://www.merrionpharma.com/	GIPET® (enteric coated tablet, permeation enhancer)
Enteris http://enterisbiopharma.com/	LLC permeation enhancer
Emisphere http://www.emisphere.com/	Eligen® (carriers, permeation enhancer)
BioSeranTach http://www.bioserantach.co.jp/e-index.html	GI-MAPS® mucoadhesive patch
Catalent http://www.catalent.com/	Permeation enhancers
Ceramisphere http://www.ceramisphere.com/	Porous silica nanoparticles
Entrega http://www.entregabio.com/	Coated mucoadhesive wafer
Monosol http://www.monosolrx.com/	Sublingual thin film

TPE transient permeability enhancer, PEG polyethylene glycol

receptor agonist using Zosano's transdermal microprojection delivery system (Fig. 1b) (111,112). Evaluating the transdermal formulation *in vivo* should also be completed as part of formulation development, and several models are available for energy driven methods (such as iontophoresis (113), electroporation (114), and sonophoresis (115)), and minimally invasive systems (including velocity-based technologies) (116) that can also aid in predicting the human clinical outcome.

Owing to easy accessibility and acceptance by patients, the buccal and sublingual mucosal routes have been extensively investigated for the delivery of peptides such as oxytocin, insulin, salmon calcitonin, GLP-1, and others. Unfortunately, low permeability and limited surface area combine to make this a challenging route for peptide delivery (2,117,118). Two examples of intraoral formulations that have been applied for insulin are Ora-lyn (Generex) and gold nanoparticles (Midatech/Monosol) (119). Several commercial peptide products are formulated for intranasal delivery including salmon calcitonin, desmopressin, lypressin, oxytocin, buserelin, and nafarelin, and still others are under investigation (105,120). Delivery of peptides by inhalation has also been approved for insulin (Afrezza, Exubera) and

lucinactant, a surfactant approved by the FDA in 2012 to treat respiratory distress syndrome in premature infants (111,121,122). *In vitro* (123) and *in vivo* (123,124) aspects of the formulation should be assessed in pulmonary models developed to support inhaled peptides. Regardless of the delivery mode selected, a thorough assessment of the formulation will minimize risk and maximize the overall success for that particular formulation.

CONCLUSION

This review has described and discussed preformulation and formulation assays and activities intended to be carried out as part of a developability assessment of potential peptide candidates. Conducting such an assessment at the discovery-to-development interface is productive, as it will allow researchers to flag major issues with potential candidates early and enable mitigation strategies via further design efforts before investing in clinical development. The go/no-go criteria will depend on delivery route but also risk-adversity according to the potential of the target in question.

a Nitto's PassPort® System **b Zosano's Transdermal Microprojection Delivery System**

Fig. 1. a The PassPort™ advanced drug delivery system combines thermal microporation and patch technology (<http://www.nitto.com/jp/en/press/2012/0425.jsp>). **b** Zosano's transdermal microprojection delivery system (<http://www.zosanopharma.com/index.php/20091103117/Research/Research-General/Technology-Platform.html>)

An overview of delivery routes available for peptides was presented in this review. Parenteral delivery is the most common administration route used for peptides, and the main preformulation characteristics to consider when assessing developability are solubility, chemical, and biophysical stability. These aspects are important both from a drug substance and product quality and performance perspective, and assessing them early will reduce this risk of issues appearing later in development.

Significant hurdles exist for the oral delivery of peptides, but cyclosporine A has set a precedent for success. The structural features of this cyclic 11-amino-acid natural product render it permeable and resistant to degradation, illustrating that both design and delivery aspects are needed to overcome the challenges of oral peptide delivery. Formulation strategies for oral delivery of peptides should be based on the unique characteristics of the peptide in question. These may include selection of excipients to solubilize peptides (i.e., surfactants and polymers), inhibit proteases, or increase permeability. Drug delivery routes for peptides, other than parenteral and oral, include transdermal, intranasal, intraoral, and pulmonary. These routes are usually explored for local use, patient compliance or convenience needs, or due to issues with parenteral or oral administration of the peptide in question. Several products in late-stage clinical development or on the market utilize these alternative routes of administration.

In conclusion, significant advances in characterization methodologies have been made to accurately illuminate developability concerns of peptide candidates at the discovery-to-development interface. In addition, formulation technologies have helped overcome the drug delivery barriers and have facilitated the progress of several peptide candidates into the clinic and the commercial space. These advancements will help peptide therapeutics realize their full potential against severe and debilitating diseases and help improve patients' lives. In this way, peptides are well-positioned to become an increasingly important therapeutic modality, presenting unique value and impact for addressing unmet medical need.

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