The formulation and delivery of a novel peptide drug via the buccal route by an orally disintegrating tablet.

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Abstract

There is a growing interest in the delivery of peptide drugs by non-intravenous routes. This study attempted to determine the feasibility of delivery of a peptide drug via the buccal route with an orally disintegrating tablet. To accomplish this we determined an acceptable proportion of ingredients to make a tablet that would meet the FDA guidelines for orally disintegrating tablets, be palatable to a potential patient, and deliver the drug to the patient. Our study showed a 3/2/1 mixture of gelatin/glycine/sorbitol when combined with the active pharmaceutical delivered an acceptable orally disintegrating tablet formulation which passed the FDA disintegration test. The stability of the drug in this formulation was also tested over three months with <5% of the initial drug having degraded. In summary, we were able to formulate an orally disintegrating tablet for a peptide drug that passed the FDA criteria for disintegration and was stable over a three-month time period.

Background

Peptide derived medications are potentially excellent candidates for therapeutic application and their role in the clinic is expanding rapidly. (1) However, the delivery of peptide medications has always been a problem. They are usually delivered via intravenous or intramuscular injections which are not convenient routes for outpatient or chronic medications. Peptides are not stable in the acidic environment of the stomach, and are susceptible to enzymatic degradation in the GI tract. (2) These limitations of conventional administration routes allow buccal delivery to be a desirable method of administration.

Buccal delivery is being increasingly utilized to administer medications via orally disintegrating films (ODF) and orally disintegrating tablets (ODT). The buccal tissue is highly vascularized and absorption via this route avoids the GI tract and first pass metabolism. (3) Delivery via the buccal route helps to avoid the use of needles, which require special training, bring a risk of infection, and require the patient to overcome their fear of injecting themselves. Buccal delivery allows a patient to take a product several times a day in a manner similar to oral tablets or capsules. The disadvantages of buccal delivery via ODT include: the patient mistakenly swallowing the tablet before it dissolves in the oral cavity, formulating the drug to not leave behind an unpleasant residue or taste, and formulating the drug to overcome the highly-keratinized tissue in the buccal cavity. The FDA recommends: that an ODT should disintegrate within 30s, weigh less than 500 mg, an ODT should be pleasant to the patient, masking any bitterness of the active drug, and not leave an unpleasant residue after disintegration. (4)

Formulations of an ODT vary based on the active pharmaceutical ingredient involved and the method of tablet formation. Freeze-drying is a proven method to generate a pleasant tablet without degrading the active ingredient. (5)

The matrix material is an important base for the ODT as it provides a porous structure in a low humidity environment while also quickly dissolving in water, gelatin is a preferred matrix. (6) Glycine was included based on its ability to increase the water solubility of the ODT and aid disintegration. (6) Sorbitol was utilized to also aid in disintegration and to allow for a more firm and pleasant appearing tablet. (7) The addition of the active pharmaceutical ingredient can have many impacts on the formulation of the ODT by altering the solubility, matrix structure, compressibility, and hygroscopicity. These factors were considered with the addition of DTI-100, but due to it being a hydrophilic peptide it aided in disintegration of the final ODT formulation without negatively affecting the tablet properties.

The research presented here focuses on the formulation, manufacture, and stability of an ODT for delivery of a novel peptide drug, DTI-100, which was developed to help contract the muscles of the bladder and void the contents therein. This drug will help those without voluntary control of their bladder avoid life-threatening urinary tract infections and decrease the number of catheterizations needed to provide relief from a full bladder.

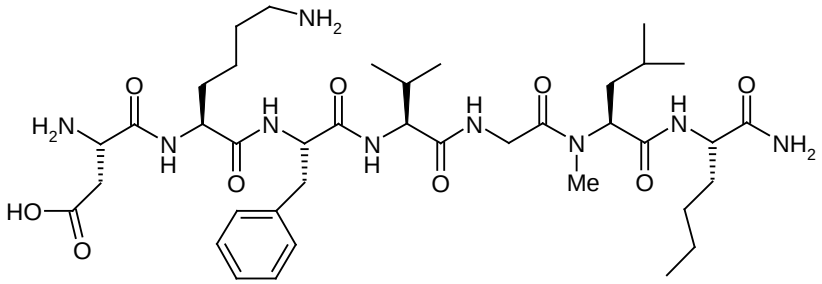


Figure 1 – Structure of DTI-100

Materials and Methods

Materials

Gelatin, Glycine, Sorbitol, TFA, and acetonitrile were purchased from Fisher Scientific. DTI-100 was presented to us by Dignify Therapeutics, Durham, NC.

Preparation of Orally Disintegrating Tablets

ODTs were prepared using an experimental design approach where by more than one formulation parameter was adjusted in each experiment and the effect on tablet composition and disintegration time were measured to help select the best formulations. Stability under accelerated conditions and ability to incorporate DTI was then assessed to determine which formulation was suitable for our purpose. Based on the literature gelatin, glycine and sorbitol were selected as the main components for ODT preparation. Gelatin provides the matrix of the ODT, while glycine and sorbitol increase the water solubility of the ODT. All tablets were

prepared by combining all the components in a 10 mL glass vial before bringing the volume up to 2 mL with DI water. If required, some formulations were heated to 40 ^{o}C for 5 minutes to encourage all excipients to dissolve. When active drug was to be added, the solutions were allowed to cool to room temperature before the addition of the active drug. The final solutions were then added as 200 uL aliquots into blister pack molds and frozen at -80 ^{o}C prior to lyophilization under vacuum (200 Torr) using the step gradient method shown in Appendix 1 using a VirTis aDvantage wizard 2.0. Final lyophilized product was stored in a HDPE vial under nitrogen.

Evaluation of Orally Disintegrating Tablets

Following their preparation ODTs were visually inspected and their firmness graded from 1 (Soft) to 5 (Hard). The disintegration rate was assessed by placing the tablets in 2 mL of DI water and recording the time required for disintegration to occur. Disintegrated ODTs were analyzed via HPLC for content.

HPLC Analysis Method

Analysis was performed on a prominence HPLC (Shimadzu Corporation, Kyoto, Japan) equipped with a PDA detector. A reverse phase gradient separation was performed using a YMC Pack Pro C18 column 150 x 4.6 mm^2 internal diameter with 3 µm particle size (YMC) at 45 ^{o}C and a flow rate of 1.0 mL/min. The mobile phases were composed of water with 0.1% trifluoroacetic acid (A) and 0.1% trifluoroacetic acid in acetonitrile (B). The mobile phase followed a gradient from 95:5 to 90:10 for 5 min, then to 85:15 for 3 min, to 65:35 over 20 min after which the gradient remained at 65:35 for 2 min prior to re-equilibration of the system at 95:5 for 5 min (Total 35 min). DTI-100 concentrations were analyzed at a wavelength of 220 nm across a range of 10 – 500 µg/mL.

Stability of Orally Disintegrating Tablets

The stability of DTI-100 ODTs were evaluated under accelerated stability conditions at both 25^{o}C/65%RH and 40^{o}C/75%RH. Before the study was initiated various storage container conditions were evaluated to determine the most appropriate material and desiccated HDPE bottles were selected. Tablets were placed in the HDPE bottles and at 0, 0.5, 1, and 3 months the DTI content and dissolution performance of the tablets was evaluated in triplicate.

Results

Optimization of ODT formulation

Initially twelve formulations were prepared utilizing different ratios of excipients, and their properties were evaluated using DOE (Figures 1 & 2). Analysis of the data was performed in JMP to determine which components had the greatest effect on both the disintegration time and firmness of the tablets. The results show that gelatin had the greatest effect on both the firmness and disintegration time of the tablets. The glycine concentrations used had no effect on tablet performance and increasing sorbitol concentrations seem to play a minor role in firmness and

decreasing disintegration times. In general, as firmness of the tablet increases the disintegration time increases, though these are not directly correlated.
From this point forward DTI was incorporated into the ODTs to ensure that its effects on the formulation were also assessed. Initially two concentrations of DTI were investigated: low dose (50 ug/tablet) and high dose (150 ug/tablet). These doses were selected based on preliminary *in vivo* efficacy data utilizing IV administration in a rat model. Based on the DOE analysis, a final component ratio was determined to be 3/2/1% gelatin/glycine/sorbitol.

The DOE analysis was repeated to determine the effect of DTI-100 on the formulations. In general, DTI-100 has no effect on the firmness of the tablets and the disintegration times were increased slightly but still in the desired range of <30s.

Based on the disintegration rate (<30s), final appearance (firm, white tablet with no deformities), and physical characteristics of the tablet, the 3/2/1% gelatin/glycine/sorbitol formulation was selected for further investigation. This formulation would be carried forward for stability and final product testing.

Disintegration test
Disintegration testing was performed to determine if the tablet would meet the FDA recommended criteria for an ODT. The final formulation of the ODT disintegrated in <30 seconds and did not leave a residue. The disintegration process required gentle agitation to help mimic the movement of saliva in the mouth. These were also conducted at room temperature and not at physiologic temperature.

Stability of Orally Disintegrating Tablets
The stability of the final ODT formulations was measured for 3 months under two different storage conditions of 25C/65% RH and 40C/75% RH. Three tablets per dosage strength from each storage condition was assessed at each time-point to determine the performance of the tablet via disintegration and test the content of the tablet to measure stability of the peptide in the formulation (Table 1). No decrease in performance was observed. The content of each tablet was measured by HPLC and there was no significant degradation observed over the first 90 days (Table 1).

Conclusions
This project was initiated to help develop an alternative delivery system for DTI-100, a novel peptide designed to stimulate the bladder to help with voiding. ODT was chosen as the delivery system to allow the patient to control the delivery of the drug and to avoid degradation in the GI tract. The formulation of the ODT led to a 3/2/1% gelatin/glycine/sorbitol final product. These were found to be the optimal ratios of the components and allowed for easier manufacturing of the ODT. This formulation met the FDA guidelines for disintegration recommended for an ODT.

The stability of the formulation was verified over a three-month period using accelerated conditions. A storage condition needed for the tablets is bottling under nitrogen with calcium carbonate desiccant as the peptide is susceptible to hydrolysis if left exposed to room air for an extended period of time. This storage condition also helps to mimic a commercial manufacturing operation as ODTs are typically packaged in blister packs under a vacuum.

However, early *in vivo* testing of the ODT showed that 20 times more of the drug was needed than initially calculated to achieve efficacious levels in the plasma of the rat model due to the highly-keratinized nature of the buccal cavity. This increase in the amount of drug contained in each ODT helped to decrease the disintegration time due to the hydrophilic nature of the peptide. This barrier to delivery is the focus of future work for this ODT formulation as overcoming the penetration issue while leaving the buccal mucosa intact would be an ideal method to help increase the bioavailability of the drug.

In summary, this project developed the manufacturing process for the delivery of DTI-100 via the buccal route by an ODT formulation. The formulation was guided by DOE analysis and meets the criteria for disintegration set forth by the FDA. The formulation was also shown to be stable up to 90 days from manufacture under two storage conditions, and retained its performance and physical characteristics.

Table 1 – Stability of DTI-100 under accelerated conditions

Condition (°C/ %RH)	Strength (mg)	**Day 14**		**Day 28**		**Day 90**	
		Concentration (mg)	% Label Claim	Concentration (mg)	% Label Claim	Concentration (mg)	% Label Claim
25/60	1.5	1 – 1.63 2 – 1.58 3 – 1.58 **1.59 □□□□□□**	1 – 108.7 2 – 105.3 3 – 105.3 **106.4 □ 2.0**	1 – 1.55 2 – 1.55 3 – 1.57 **1.56 □□□□□□**	1 – 103.3 2 – 103.3 3 – 104.7 **103.8 □□□□□**	1 – 1.47 2 – 1.43 3 – 1.48 **1.46 □□□□□□2**	1 – 97.8 2 – 95.4 3 – 98.5 **97.2 □□1.33**
25/60	5	1 – 5.09 2 – 4.96 3 – 5.05 **5.03 □□□□□□**	1 – 101.8 2 – 99.2 3 – 101.0 **100.7 □□□□□**	1 – 5.29 2 – 5.19 3 – 5.22 **5.23 □□□□□□**	1 – 105.8 2 – 103.8 3 – 104.4 **104.7 □□□□□**	1 – 5.13 2 – 5.10 3 – 5.10 **5.11 □□□□□1**	1 – 102.6 2 – 102.0 3 – 102.1 **102.2 □□0.26**
45/75	1.5	1 – 1.64 2 – 1.49 3 – 1.63	1 – 109.1 2 – 99.3 3 – 108.7	1 – 1.57 2 – 1.56 3 – 1.58	1 – 104.6 2 – 104.0 3 – 105.3	1 – 1.48 2 – 1.45 3 – 1.45	1 – 98.6 2 – 97.0 3 – 96.5

		1.59 □□□□□□	**105.7** □□□□□	**1.59** □□□□□□	**104.6** □□□□□	**1.46** □□□□□**1**	**97.4** □□□□**90**
45/75	5	1 – 4.89 2 – 4.98 3 – 5.00 **4.96** □□□□□□	1 – 97.8 2 – 99.6 3 – 100.0 **99.1** □□□□□	1 – 4.75 2 – 4.65 3 – 5.08 **4.82** □□□□□□	1 – 95.0 2 – 93.0 3 – 101.6 **96.5** □□□□□	1 – 4.90 2 – 4.82 3 – 5.04 **4.92** □□□□**09**	1 – 98.0 2 – 96.4 3 – 100.9 **98.4 □□1.88**

**For day 0 we used the content uniformity analysis as our starting concentrations. L- 1.6 mg and H- 5.4 mg. This was due to the quantity of tablets that could be manufactured in one day.

Figure 2 – DOE Results for dependence of disintegration time on ODT components

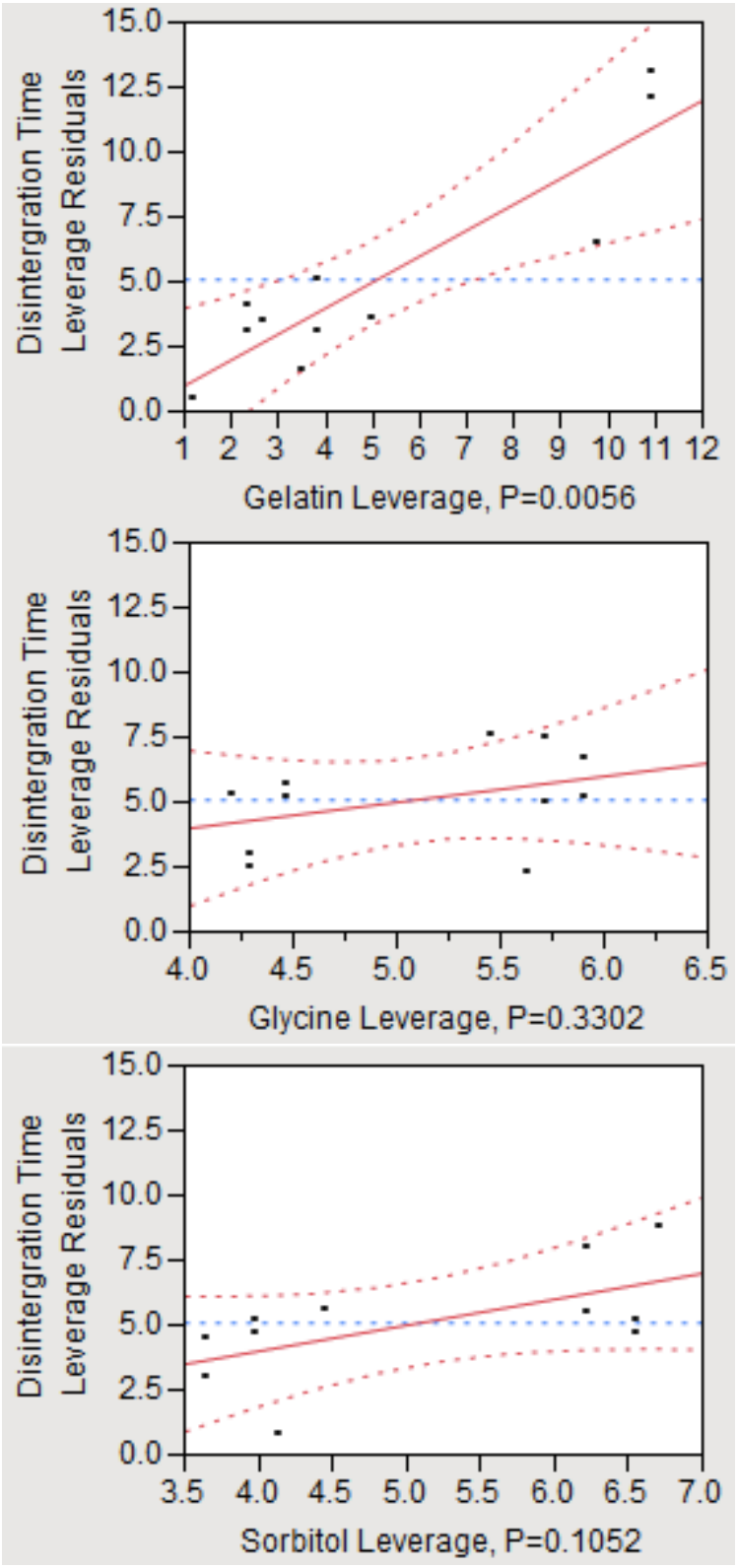
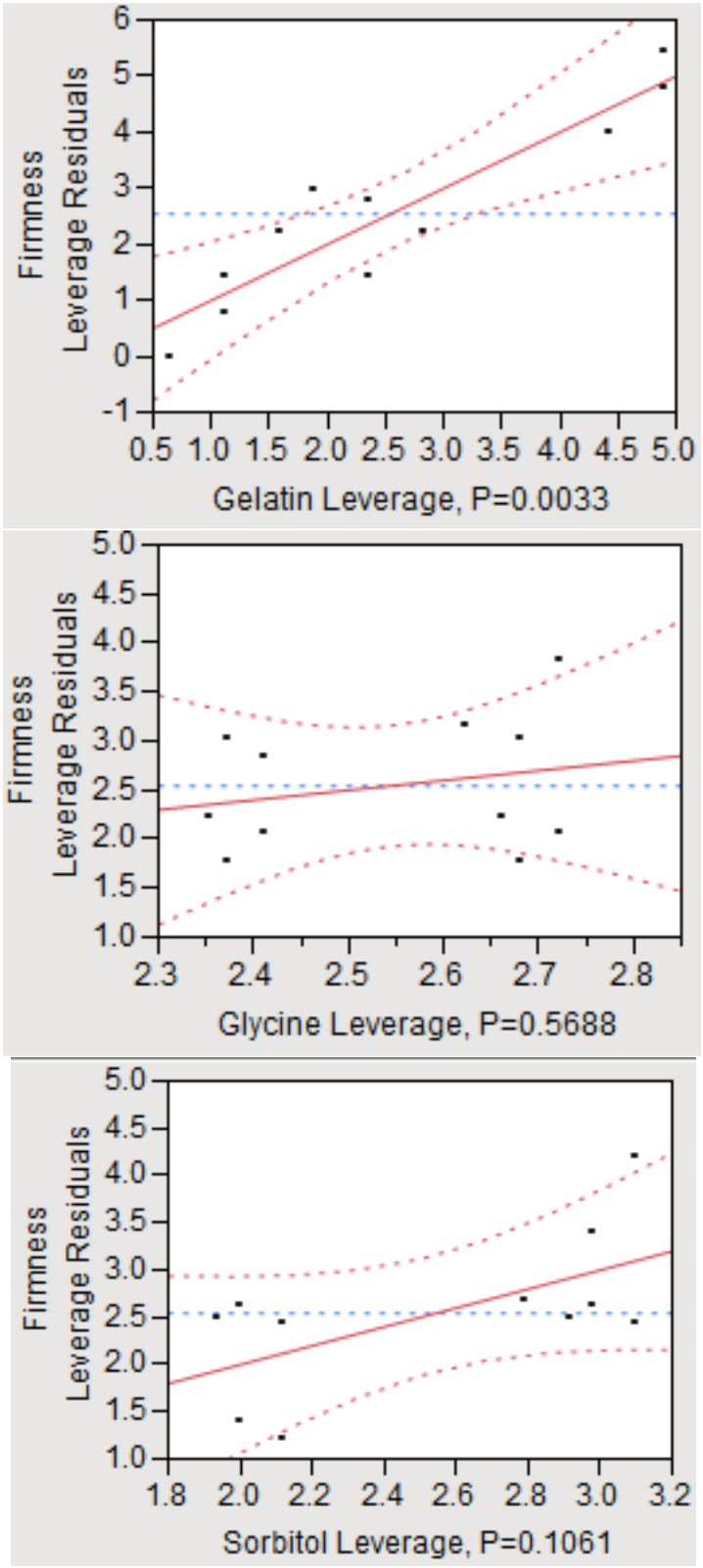


Figure 3 – DOE Results depicting the dependence of ODT firmness on its components



APPENDIX I -Lyophilization Temperature Gradient

Table 1 – Lyophilization Protocol

Temperature (ºC)	**Time (Min)**
-50	30
-45	10
-40	5
-35	5
-30	5
-25	5
-20	240
-15	5
-10	5
-5	5
0	5
5	5
10	5
15	5
20	90
25	30

References

1. Insight of current technologies for oral delivery of proteins and peptides. Drug Discov Today Technol. 2012;9(2):e71-e174.
2. Choonara BF, Choonara YE, Kumar P, Bijukumar D, Du toit LC, Pillay V. A review of advanced oral drug delivery technologies facilitating the protection and absorption of protein and peptide molecules. Biotechnol Adv. 2014;32(7):1269-82.
3. Smart JD. Buccal drug delivery. Expert Opin Drug Deliv. 2005;2(3):507-17.
4. FDA guidance for industry: Orally disintegrating tablets. Food and Drug Administration. December 2008.
5. Shukla D, Chakraborty S, Singh S, Mishra B. 2009. Mouth dissolving tablets I: An overview of formulation technology. Scientia Pharmaceutica 77(2):309–326.
6. Shoukri RA, Ahmed IS, Shamma RN. In vitro and in vivoevaluation of nimesulide lyophilized orally disintegrating tablets. Eur J Pharm Biopharm 2009;73(1):162–171.
7. Chandrasekhar R, Hassan Z, AlHusban F, Smith AM, Mohammed AR. 2009. The role of formulation excipients in the development of lyophilised fast-disintegrating tablets. Eur J Pharm Biopharm 72(1):119–129.