Solid-state heat stability of peptides: kinetics and mechanisms

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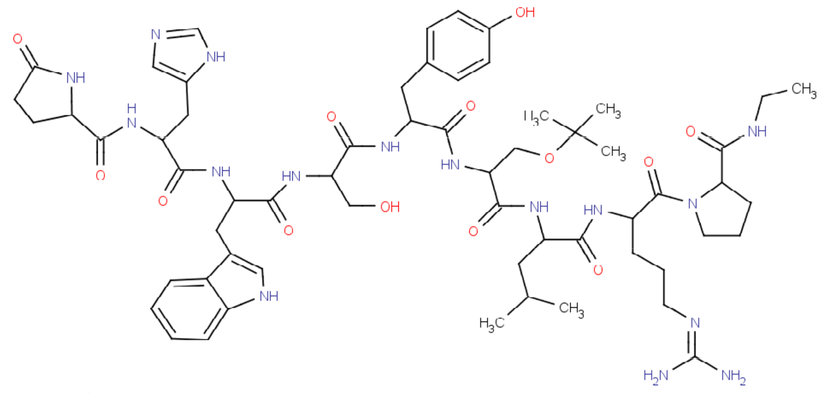
INTRODUCTION

Biologically active peptides
Therapeutics, *e.g.* oncology (buserelin)

Processing hot melt extrusion (HME)

1. **High temperature exposure ← OBJECTIVE**
2. Mechanical shear stress
3. Polymer/matrix influence



pGlu-His-Trp-Ser-Tyr-D-Ser(tBu)-Leu-Arg-Pro-NHEt
(mono acetate form)

EXPERIMENTAL

Stability indicating UPLC method:

Acquity BEH300 C18 1.7µm (2.1 ×100 mm)
MF A: 95/5 H_2O/ACN + formic acid
MF B: 5/95 H_2O/ACN + formic acid

1.5 min isocratic hold at 100% A
linear gradient from 0 to 21% B in 9.5 min
7 min isocratic hold

Dry heat conditions

T (℃)	150	157.5	165	172.5	180
Time (t) (min)	40	25	15	10	10
	80	50	30	20	20
	120	75	45	30	30
	160	100	60	40	40

Detection

•DAD-UV (kinetics via normalized areas)
•MS/MS (degradant identification)

Kinetic data evaluation

•Statistical evaluation of 17 solid-state kinetic models:
- ✓ Nucleation (7)
- ✓ Geometrical contraction (2)
- ✓ Diffusion (4)
- ✓ Reaction-order (4)

•Extrapolation to HME-related conditions

RESULTS and DISCUSSION

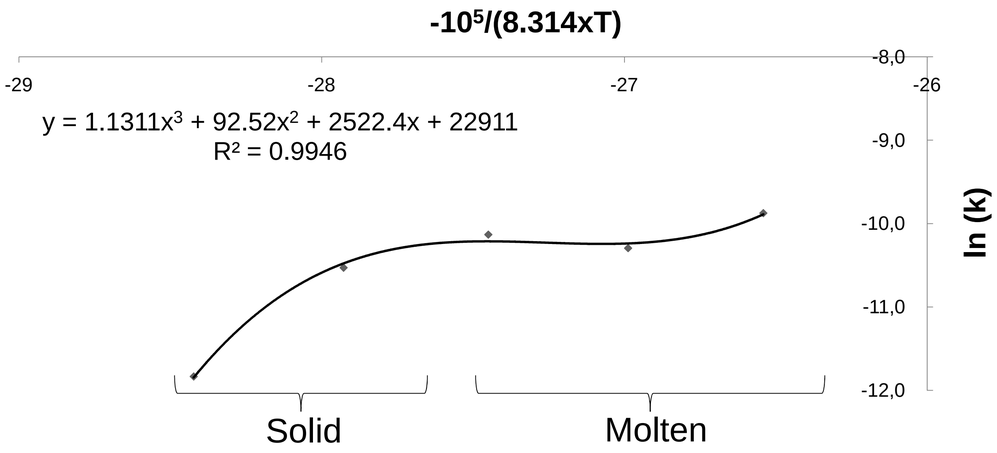
Kinetic data evaluation per temperature

Ginstling-Brounshtein (Diffusion model): minimal AIC values

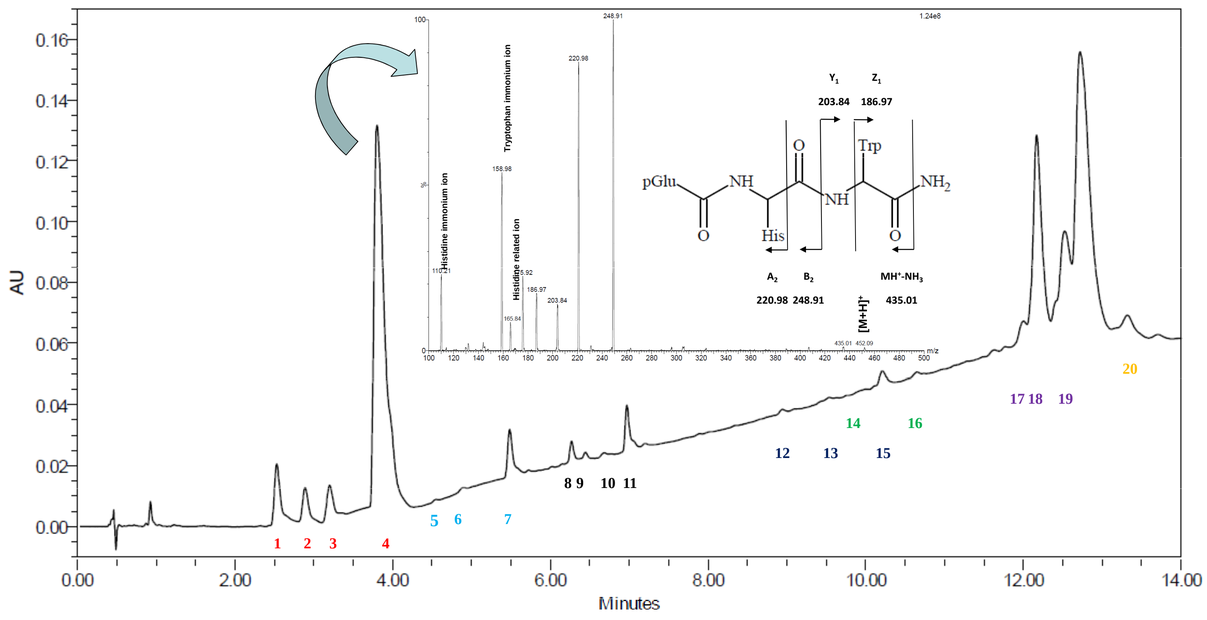
$$1-(2\alpha/3)-(1-\alpha)^{2/3}=k\times t$$

α=fraction degraded

5 degradation constant k ⇔ temperature T



Degradant identification



Degradation mechanism

β-elimination + fragmentation	**pGlu-His-Trp-NH_2 ***
	pGlu-His-Trp-Ser-Tyr-NH_2 *
	pyruvoyl-Tyr-Ser(tBu)-Leu-Arg-Pro-NH-Et
Backbone hydrolysis	**pGlu-His-Trp ***
	Tyr-Ser(tBu)-Leu-Arg-Pro-NH-Et *
	Ala-Tyr-Ser(tBu)-Leu-Arg-Pro-NH-Et *
Isomerisation*	**pGlu-His-Trp-Ser-Tyr-Ser(tBu)-Leu-Arg-Pro-NH-Et**

Predicted degradation at HME-related conditions

	Polynomial regr.	Linear regr. solid state
5 min 100°C	<0.01%	0.10%
5 min 125℃	<0.01%	1.33%

CONCLUSIONS

(1)Kinetics: Ginstling-Brounshtein degradation model: **HME** ✓

(2)Degradant profiling:
1. β-elimination
2. Backbone hydrolysis
3. Isomerisation

On-going:
(1) Manufacturing HME implant
(2) Characterisation, incl. dissolution
(3) Stability
(4) Pharmacokinetics *in-vivo*: (mice subdermal)

REFERENCES

M. D'Hondt et al. Dry heat forced degradation of buserelin peptide: kinetics and degradant profiling, International Journal of Pharmaceutics, 2014, 467, 48-59.
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