Accelerated Stability Modeling for Peptides: A Case Study with Bacitracin

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Abstract

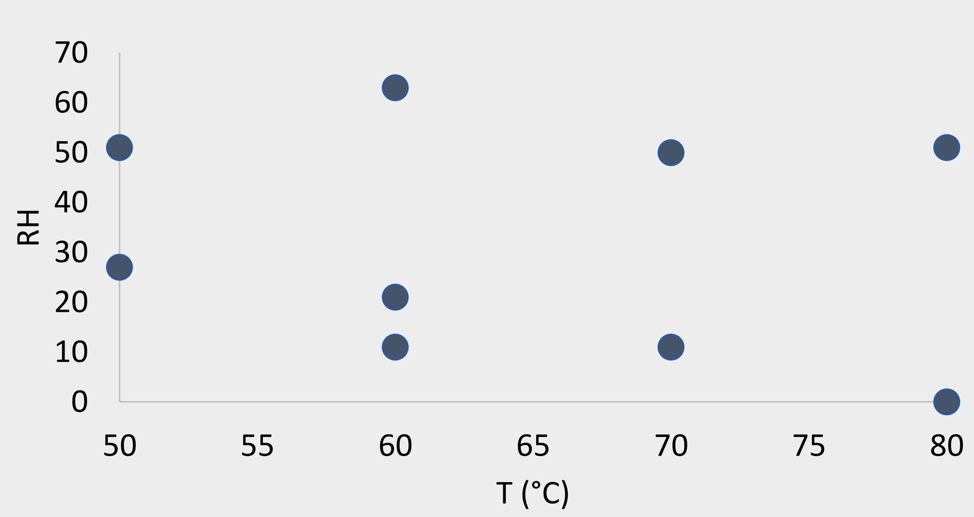
- Peptides can be used as active pharmaceutical ingredients and as such, share some stability properties of small molecules
- Solid bacitracin and bacitracin Zn were used as model peptides to examine the applicability of the Accelerated Stability Assessment Program (ASAP) to rapidly model shelf-life
  - Times to specification limits were determined (isoconversion times) with the complexity of overlapping isoforms
  - The modified Arrhenius equation was applied with appropriate statistics
  - The accelerated model-based predictions for ambient shelf-life and specific degradation product formation matched those observed in real time
  - Modeling provides insight into the impact of zinc complexation

Experimental Design

- Bacitracin (native and Zn complex) was exposed (open) to a range of T, RH (RH controlled by saturated salts) and times
- HPLC analysis was performed on samples to determine loss of active (isoforms A, B1, B2) and formation of degradant (form F)
- Data fit to following equation:

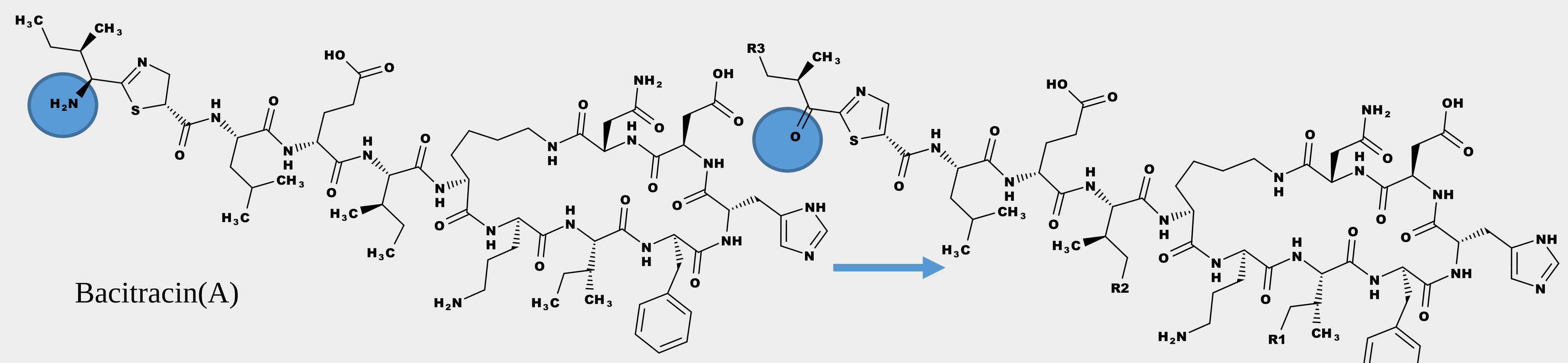
$$\ln\left(\frac{1}{failure\ time}\right) = lnA - \frac{E_a}{RT} + B(RH)$$

- 3 and 6 month data collected at lower T's for comparison

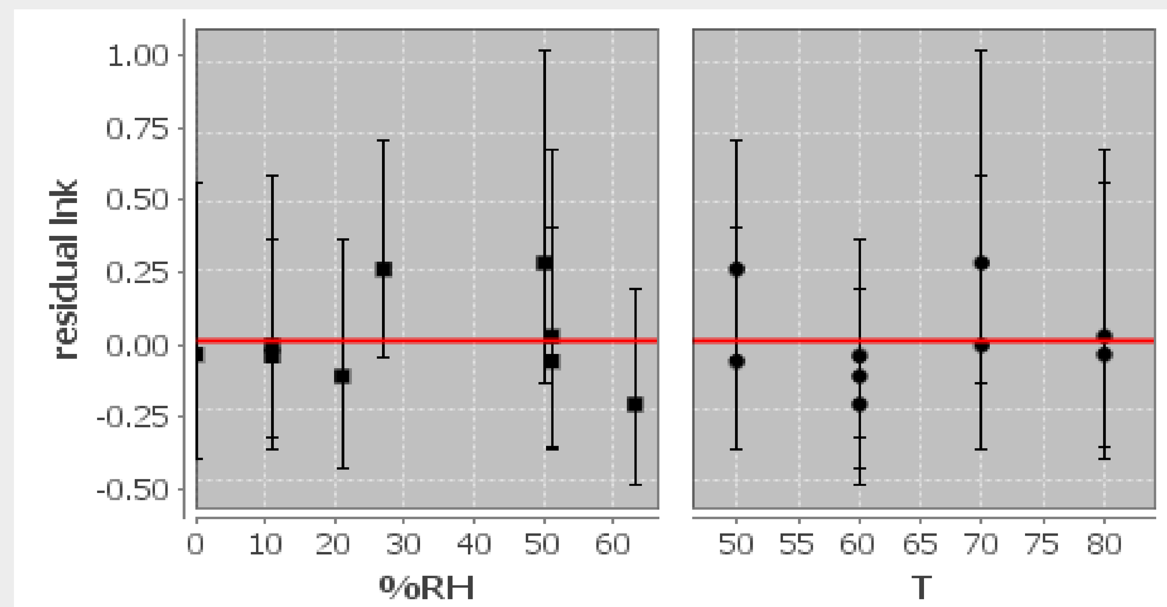


Exposure conditions for accelerated stability modeling of bacitracin

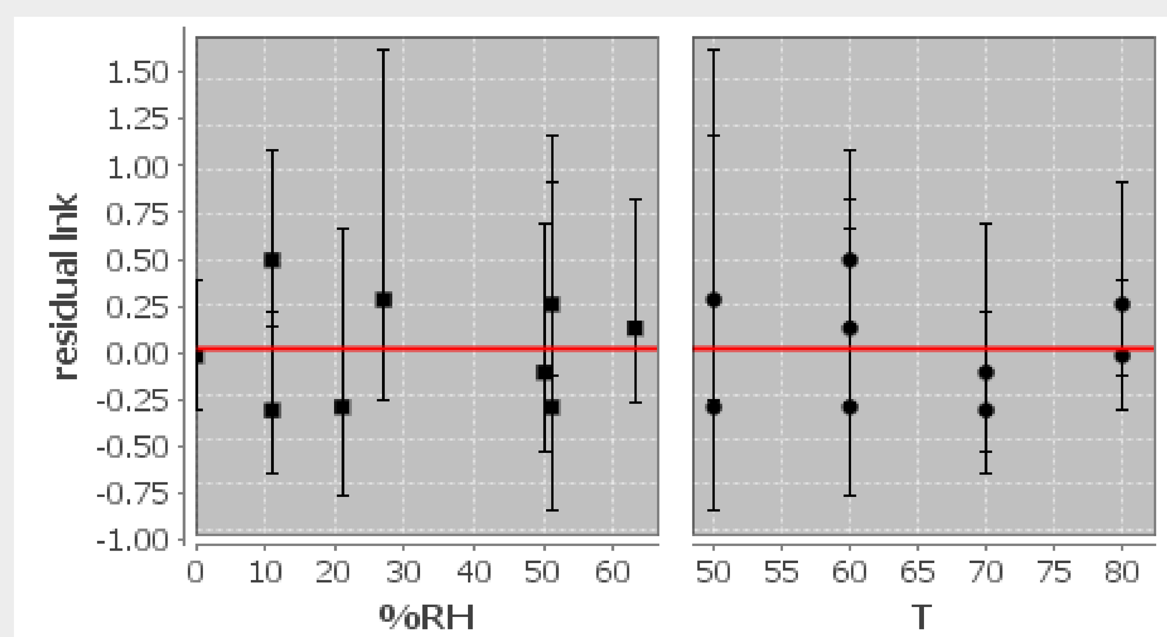
Peptide form	Stability-indicating parameter	ln A	E_a (kcal/mol)	B	R^2	Mean predicted (open) shelf-life at 25°C/60%RH
Bc	Loss of A	38.6±5.9	25.7±4.0	0.031±0.009	0.984	0.5 yrs
BcZn	Loss of A	34.3±7.6	24.2±5.2	0.010±0.010	0.938	10.4 yrs
Bc	Formation of F	44.6±4.9	29.2±3.3	0.021±0.007	0.950	1 mo
BcZn	Formation of F	45.8±2.2	32.0±1.5	0.008±0.003	0.981	6.6 yrs
Bc	Loss of B1 isoform	38.3±7.0	25.8±4.7	0.044±0.011	0.957	5 mos
Bc	Loss of B2 isoform	46.2±7.4	30.9±5.0	0.036±0.009	0.970	1.1 yrs



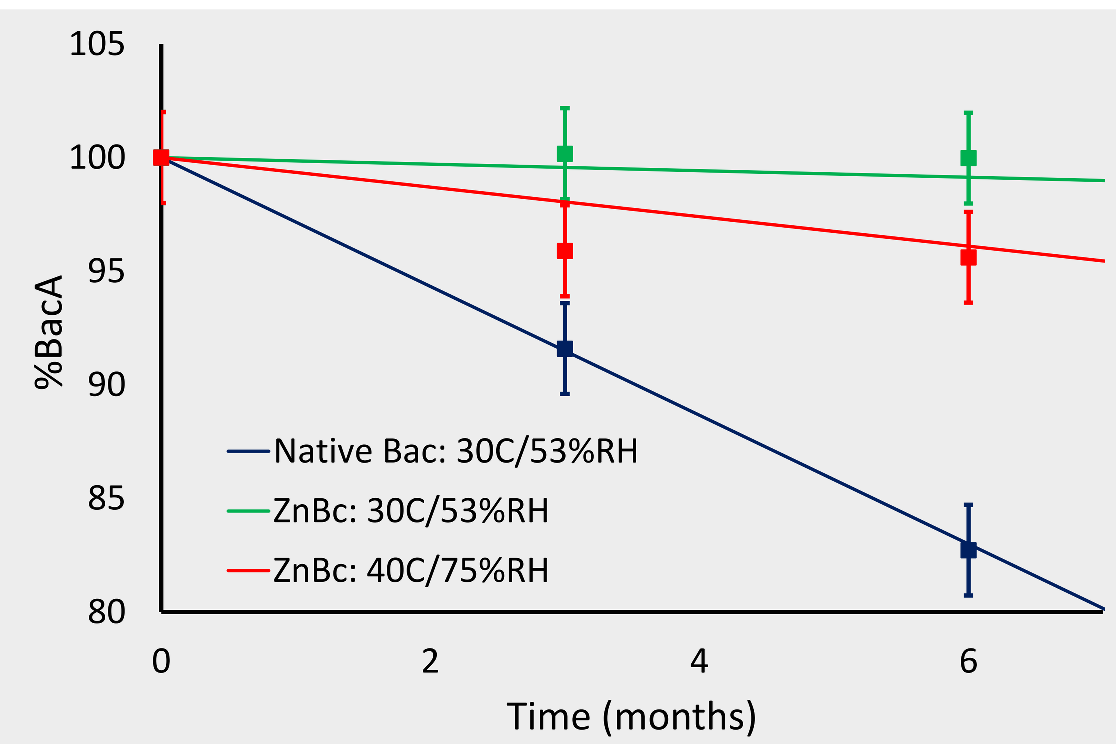
Bacitracin(A) Bacitracin(F)



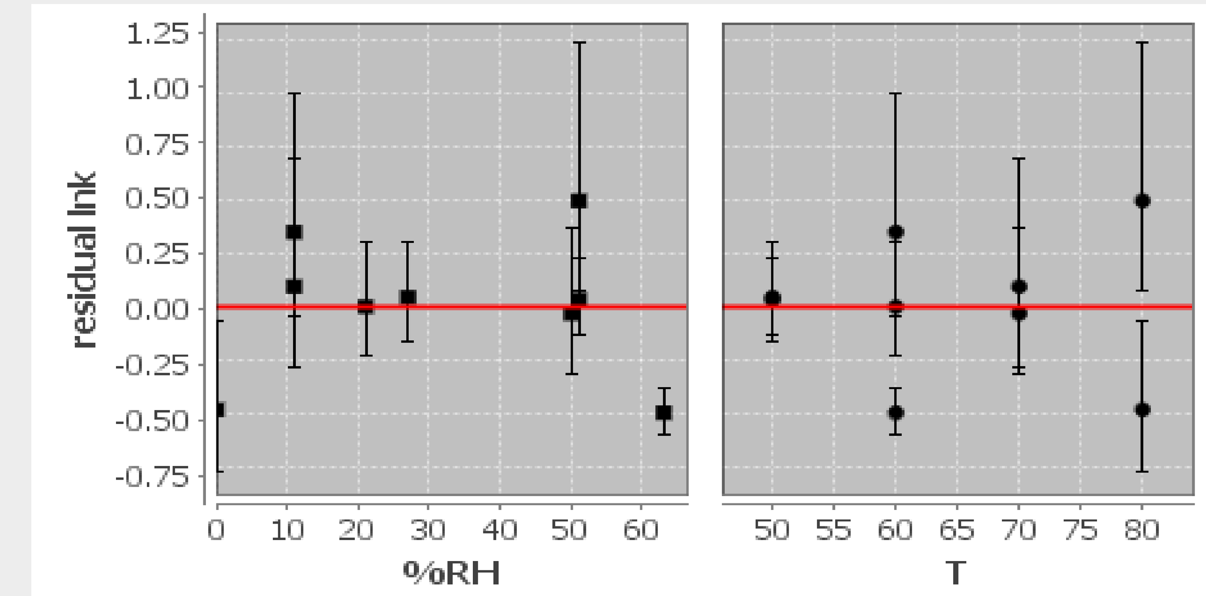
Residual graph of fitting for loss of Bacitracin(A) from native peptide plotted as ln(1/failure time) vs T, RH



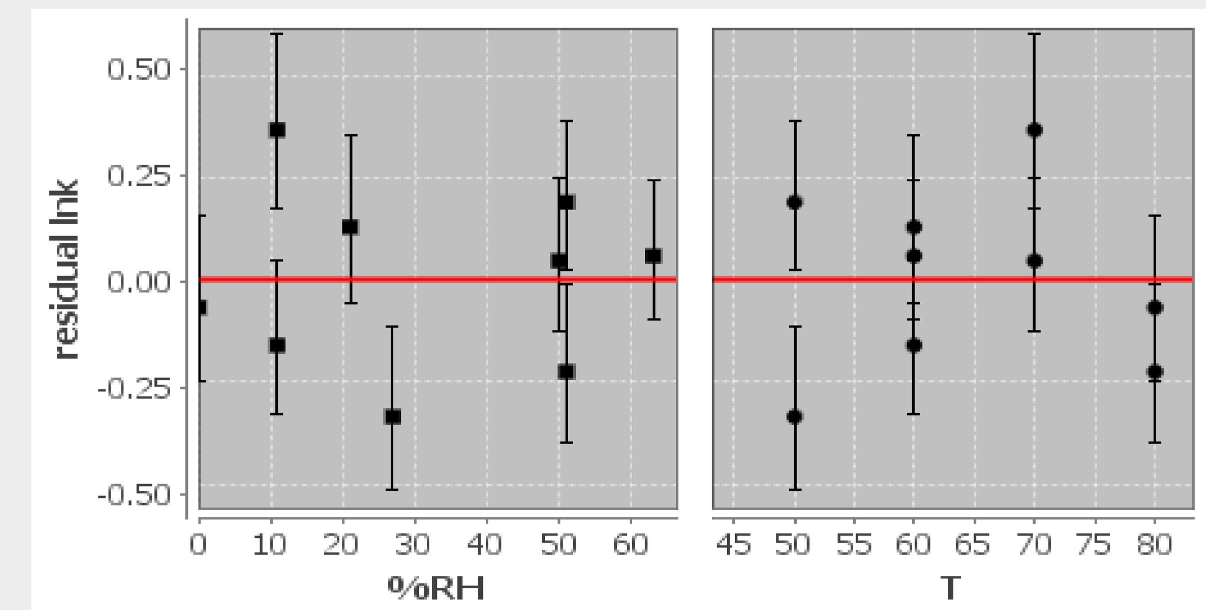
Residual graph of fitting for loss of Bacitracin(A) from Zn complex plotted as ln(1/failure time) vs T, RH



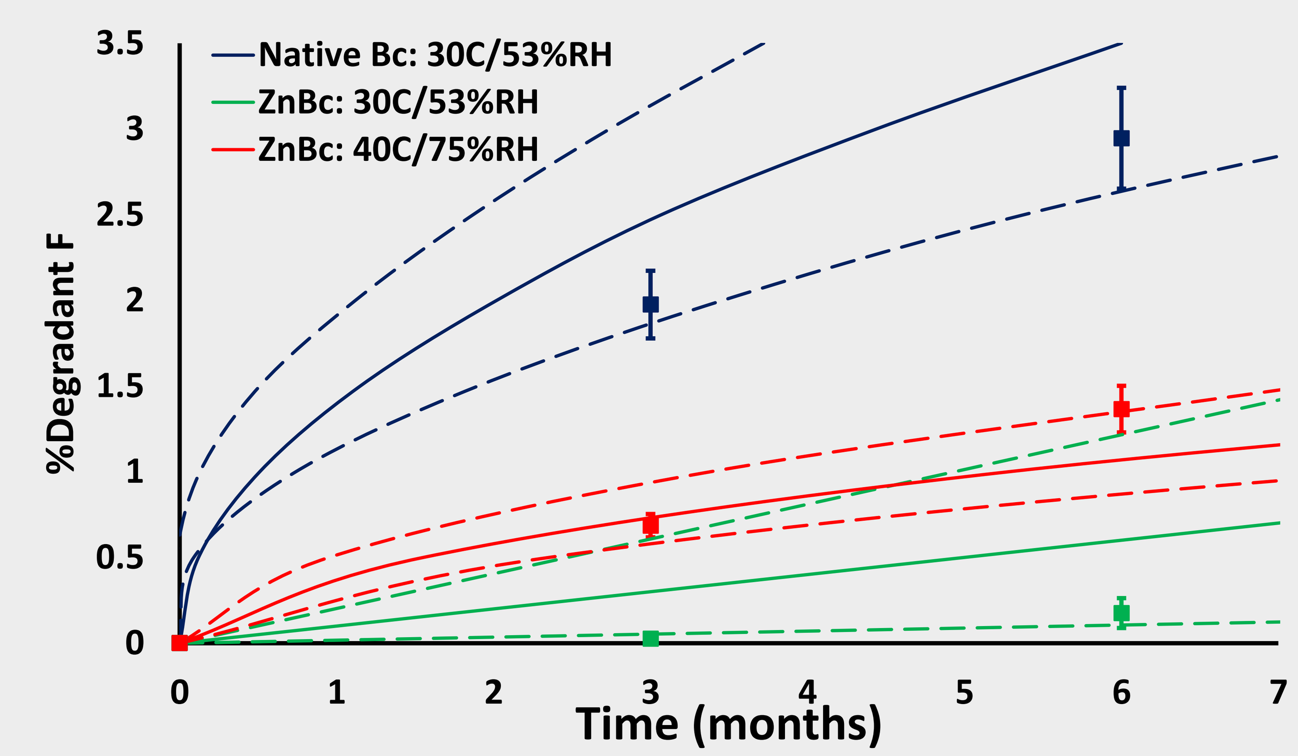
Comparison of long-term (squares) and modeling predictions (lines) for level of bacitracin(A) at two conditions



Residual graph of fitting for formation of Bacitracin(F) for native peptide plotted as ln(1/failure time) vs T, RH



Residual graph of fitting for formation of Bacitracin(F) for Zn complex plotted as ln(1/failure time) vs T, RH



Comparison of long-term (squares) and modeling predictions (lines) for level of bacitracin(F) at two conditions

Conclusions

- The Accelerated Stability Assessment Program (ASAP) was successfully applied for the first time to a peptide
- Bacitracin (native and Zn complex) was exposed for up to 21 days from 50-80°C/0-63%RH and both loss of active and formation of degradant were analyzed by HPLC
- Formation of degradant F fit a diffusion model (time$^{1/2}$) at each condition
- Model fitting to the humidity-corrected Arrhenius equation was good
- Based on fitting, the dramatic stabilization by zinc complexation is due to lower mobility (decreased ln A and B) rather than activation energy (E_a)
- Predictions matched long-term data