Multivariable Individual Heating Conditions Tested in Parallel Provide Rapid Process Optimization on *Prelude® X*

DANIEL MARTINEZ, JAMES P. CAIN, ELIZABETH RESTITUYO-ROSARIO, KATYA KARANKEVICH, NATHANIEL COSPER
Protein Technologies, Inc. 4675 South Coach Drive, Tucson, Arizona, 85714, U.S.A. Tel: +1-520-629-9626, Website: www.ptipep.com, Email: info@ptipep.com

INTRODUCTION

The application of heat represents a useful tool to optimize the production of challenging synthetic peptides, and a new technology, induction heating, has been introduced on the Prelude X. Induction heating allows for independent, simultaneous and rapid heating of multiple reactors with increased efficiency. Three different heating conditions, with coupling at 25°C, 60°C and 90°C were utilized for the synthesis of the difficult JR-10 mer peptide.

METHODS & ANALYSIS

The JR-10 mer peptides were synthesized on the *Prelude X* peptide synthesizer at 50 µmol scale using Rink Amide resin (loading 0.32 mmol/g). Deprotection was performed with 20% piperidine in DMF for 1 min at RT. Couplings were performed at a final concentration of 250 mM AA (10 eq.), 250 mM HCTU (10 eq.) and 500 mM NMM (20 eq.) for 2 min. Cleavage cocktail used was TFA/Anisole/H_2O/EDT and the reaction was performed for 2 h at RT. Triplicates were performed for each peptide.

The resulting crude peptide was dissolved in water and analyzed on a Varian ProStar HPLC using a C18, 180 Å, 5 um, 250 x 4.6 mm column (Agilent Polaris), over 60 minutes with a flow rate of 1 mL/min, and using a gradient of 5-95% B, where Buffer A is 0.1% TFA in water, and Buffer B is 0.1% TFA in acetonitrile. Detection was at 214 nm. Mass analysis was performed on a Shimadzu LCMS-2020 Single-Quad mass spectrometer, equipped with a C18, 100 Å, 2.6 um, 50 x 2.1 mm column (Phenomenex Kinetex), over 7 min with a flow rate of 1 mL/min and using a gradient of 5-50% B where Buffer A is 0.1% formic acid in water and Buffer B is 0.1% formic acid in acetonitrile

RESULTS

The synthesis of JR-10 using induction heating was found to provide the best purity at 90°C. Lower purities were observed at 60°C or 25°C (Table 1).

Temp	Min/Peptide	Purity
25°C	41.6 min	15.0%
60°C	41.6 min	48.4%
90°C	41.6 min	65.6%

Table 1. Effect on peptide crude purity of different temperature protocols during coupling for JR-10 mer peptide

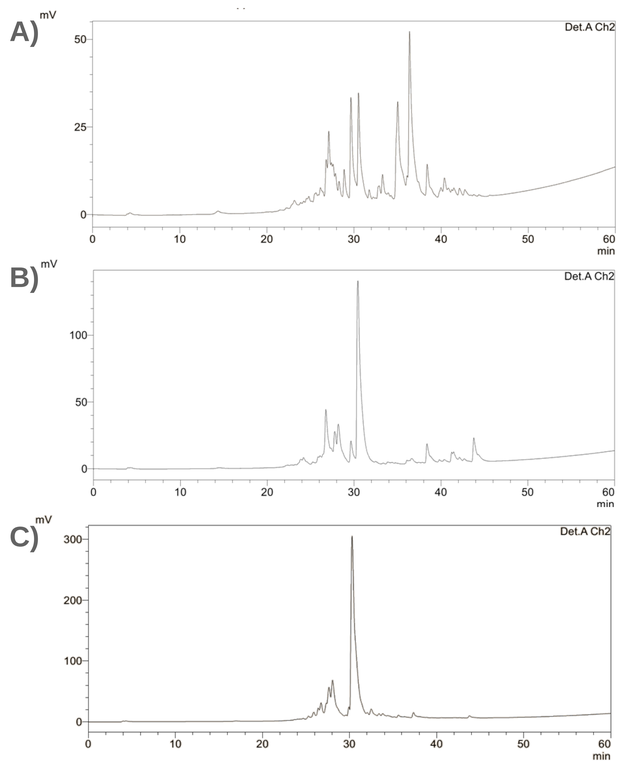

Figure 1. HPLC Traces at three different temperatures
(A) JR-10 mer peptide at 25 °C
(B) JR-10 mer peptide at 60 °C
(C) JR-10 mer peptide at 90 °C

CONCLUSION

- Heating JR-10 at 90 °C vs. RT improved crude purity by more than 4 fold
- Rapid process optimization attained through the use of independently heated parallel reaction vessels
- Ability to set unique temperatures at any step on each reaction vessel simultaneously allows for greatest flexibility
- Faster synthesis per peptide than any conventional single channel heated instrument available

PRELUDE X

- 6 parallel independent heated reaction vessels
- 3 vessels with preactivation chemistry
- 30 seconds ramp up time to 90°C from RT of 20 mL DMF
- Real time UV monitoring
- Single Shot™ additions with almost no dead volume

REFERENCES

(1) Redemann, T.; Jung, G. In *Peptides 1996, Proc. of the 24th European Peptide Symposium*; Ramage, R., Epton, R., Eds.; Mayflower Scientific Ltd: Kingswinford, UK, 1998; p 749