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Comparison of Microwave and Conduction Heating for Solid Phase Peptide Synthesis

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Abstract

Solid phase peptide synthesis (SPPS) has become a standard approach for synthesis of peptides, especially in a laboratory setting. Heating the reactions in SPPS could significantly reduce the coupling and deprotection times. One of the heating methods is to use microwave which is becoming increasingly popular because it not only dramatically reduces the synthesis times, but also increases the crude peptide purity [1]. However, microwave peptide synthesizers are relatively expensive. In this study, we investigated whether SPPS using conduction heating can achieve similar result as microwave irradiation. CSBIO II and CEM Liberty Blue were used as heating resource of conduction and microwave heating, respectively. Four peptides with length of 18mer, 19mer, 20mer (Bivalirudin) and 39mer (Exenatide) were selected as examples. The peptides were synthesized using the same synthesis protocol at 90 ºC including identical coupling, deprotection and washing cycles. The differences between the two approaches are the temperature of washing DMF (90 ºC vs 23 ºC for conduction and microwave heating, respectively) and overall synthesis cycle time (17 min vs 13 min in conduction and microwave heating, respectively). Both conduction and microwave heating generated comparable results with crude purity of 52.0% vs 51.7%, 49.0% vs 57.3%, 62.8% vs 57.1%, 37.0% vs 30.5% for 18mer, 19mer, 20mer and 39mer, respectively. One of the advantages of conduction heating is the uniformly and consistently delivered temperature during the synthesis which could minimize racemization and side reactions caused by spikes and hotspots typically associated with microwave heating. In addition, conduction heating is also a more cost-efficient heating method when compared to expensive microwave heating technology.

Methods

Materials and general methods

Solid phase peptide synthesis

The peptides were synthesized on Rink amide resin (loading 0.43 mmol/g) using Oxyma/DIC coupling method on 0.1 mmol scale. The same resin and amino acid lots were used for all synthesis. Fmoc deprotection was achieved with 20% 4-methyl piperidine. The peptides were cleaved off the beads at room temperature for 3 hours with a cocktail containing trifluoroacetic acid (TFA), 5% phenol, 5% water, 5% thioanisole and 2.5% triisopropylsilane.

Instruments used for peptide synthesis

CEM Liberty Blue

CSBIO II

Selected peptides for the study

- Four different peptides (18mer, 19mer, 20mer and 39mer) were used for the evaluation.

Sequences of selected peptides

#	Name	Sequence	Length
1	N/A	TMEDKIYDQQVTKQCLCF	18
2	N/A	YSYPETPLYMQTASTSYYE	19
3	Bivalirudin	FPRPGGGGNGDFEEIPEEYL	20
4	Exenatide	HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS	39

HPLC analysis

Gradient program of HPLC for analysis of 18-20mer

Time, min	% B
0	10
22	80
23	100
27	100
28	10
32	10

Gradient Program of HPLC for analysis of 39mer

Time, min	% B
0	30
25	50
26	100
30	100
31	30
35	30

HPLC was done in Shimadzu LC2030-C. Solution A: water with 0.1% TFA; Solution B: acetonitrile with 0.1% TFA. Flow rate: 1.0 mL/min, UV detection: 214 nm.

Peptide synthesis protocols

Standard protocols in microwave and conduction heating

0.10 mmol CEM Standard Single Coupling

Cycle	Step	Volume (mL)
1	Deprotection @ 1min	3
2	Wash	2
3	Wash	2
4	Wash	3
5	Coupling @ 2 min	4*

Total time: 5 min. Temperature: 90ºC

* Solution contains 0.5 M DIC (1.0 mL), 1.0 M oxyma (0. 5 mL) and 0.2 M Fmoc-amino acid (2.5 mL). Amino acid to beads: 5 eq

0.10 mmol CSBIO II Standard Single Coupling

Cycle	Step	Volume (mL)
1	Deprotection @ 1 min	6
2	Deprotection @ 3 min	6
3	Wash	5
4	Wash	5
5	Wash	5
6	Wash	5
7	Wash	5
8	Wash	5
9	Coupling @ 30 min	4*
10	Wash	5
11	Wash	5
12	Wash	5

Total time: 47 min. Temperature: 60ºC

Modified equivalent protocol

0.10 mmol Modified Single Coupling

Cycle	Step	Volume (mL)
1	Deprotection @ 1 min	6
2	Wash	5
3	Wash	5
4	Wash	5
5	Wash	5
6	Wash	5
7	Wash	5
8	Coupling @ 5 min	4*
9	Wash	5
10	Wash	5

Temperature: 90ºC

	Temperature	Time per cycle*	Note
Conduction heating (CSBio II)	90 ºC throughout the entire synthesis protocol. 90 ºC for both the reaction vessel, as well as the transfer vessels (pre-solvent delivery of the deprotection and wash solvent)	17 min	CSBio synthesizer has a heating block that can maintain heating throughout synthesis, as well as a heating block for pre-solvent delivery
Microwave (CEM)	90 ºC only during deprotection and coupling	10 min	CEM synthesizer system only allows microwave during deprotection and coupling steps

* Delivery and transfer speeds are slower in the CSBio synthesizer, the difference in time is largely during the wash steps

Results

Comparison of crude purity of peptides made with microwave in CEM and conduction heating in CSBIO II using a standard protocol (baseline)

Peptide	Instrument	Total synthesis time	Total waste	Crude purity
18mer	CSBio II	14h 16m	1140 mL	52.1%
	CEM	1h 35m	351 mL	36.0%
19mer	CSBio II	15h 3m	1201 mL	34.4%
	CEM	1h 40m	366 mL	25.6%
20mer	CSBio II	15h 50m	1262 mL	59.8%
	CEM	1h 48m	392 mL	47.4%
39mer	CSBio II	30h 43m	2421 mL	32.6%
	CEM	4h 16m	714 mL	A big and broad peak*

* This is probably because the delivered volume of coupling, deprotection and washing solutions was not enough to allow efficient mixing with all beads due to increased volume of beads, resulting in incomplete reactions.

Comparison of crude purity of peptides made with microwave in CEM and conduction heating in CSBIO II using an equivalent and optimized protocol

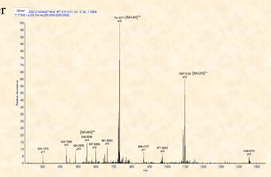
Peptide	Instrument	Total Synthesis Time	Total Waste	Batch	Crude purity
18mer	CSBIO II	5h 16m	971 mL	1	47.6%
				2	54.1%
				3	54.2%
	CEM*	3h 15m	966 mL	1	52.8%
				2	52.5%
				3	49.7%
19mer	CSBIO II	5h 33m	1023 mL	1	45.1%
				2	51.0%
				3	50.9%
	CEM*	3h 26m	1013 mL	1	56.4%
				2	56.8%
				3	59.2%
20mer	CSBIO II	5h 50m	1075 mL	1	58.1%
				2	66.0%
				3	64.3%
	CEM*	3h 42m	1077 mL	1	51.6%
				2	62.9%
				3	56.9%
39mer	CSBIO II	11h 13m	2063 mL	1	34.5%
				2	38.2%
				3	38.2%
	CEM*	7h 34m**	2049 mL	1	28.2%
				2	31.7%
				3	31.5%

* The initial Fmoc-deprotection was done twice because the 1st deprotection temperature was unable to reach and maintain 90ºC (±10 ºC) for 1 min

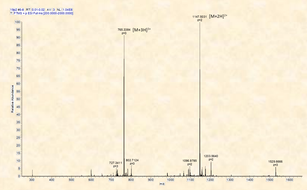
** 39mer peptide was split into two syntheses due to the volume limit of the bottle for main solvent DMF

ESI-MS of four peptides

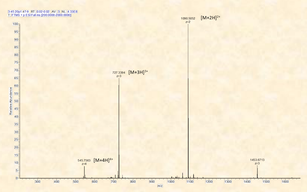
18mer



19mer



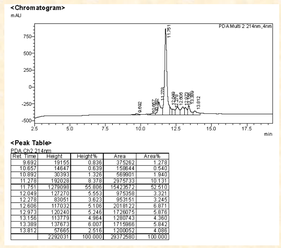
20mer



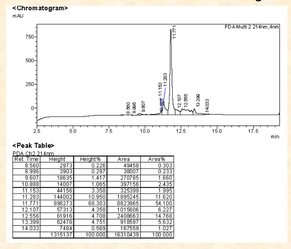
39mer

HPLC Profiles of peptides (selected examples)

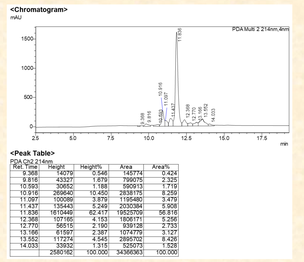
18mer from CEM microwave



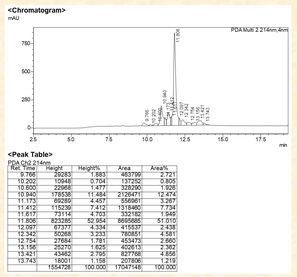
18mer from CSBIO II conduction heating



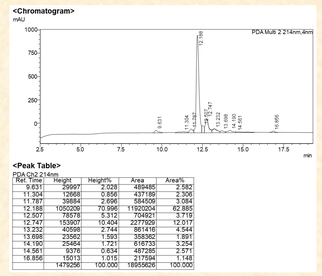
19mer from CEM microwave



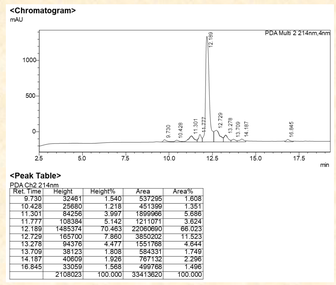
19mer from CSBIO II conduction heating



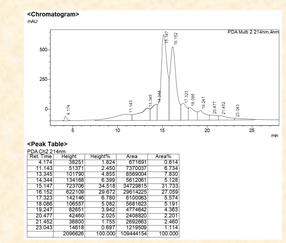
20mer from CEM microwave



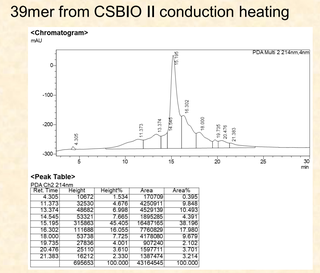
20mer from CSBIO II conduction heating



39mer from CEM microwave



39mer from CSBIO II conduction heating



Conclusions

We have developed an optimized synthesis protocol that is capable to synthesize long peptide such as 39mer with good crude purity. Both conduction and microwave heating gave comparable results with a very good crude purity as seen in the case of four peptides, when the same synthesis protocol was used.

References

[1] Pedersen SL, Tofteng AP, Malik L and Jensen KJ. ***Chem. Soc. Rev.***, 2012,**41**, 1826-1844

Acknowledgments

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