DEVELOPMENT OF NOVEL AUTOMATED SOLID PHASE PEPTIDE SYNTHESIZERS ENSURING ECONOMIC, EFFECTIVE, SCALABLE AND GREEN SYNTHESIS

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Introduction

Peptide-based pharmaceutics are at the forefront of pharmaceutical innovation and academic research. However, the high costs associated with solid phase peptide synthesis pose a significant financial challenge to this research domain, due to the costly instruments involved and the extensive use of expensive raw materials. To tackle these challenges, further optimization of synthesizers is crucial to reduce the costs, time, and manual labor of peptide synthesis. These ideal instruments should be efficient, flexible, economical, scalable, robust, and aligned with green chemistry principles. Such advancements could accelerate peptide-based pharmaceutical research in academic and in industrial R&D settings.

Custom doser

- ► **Fast and efficient dosing**
- ► **Reagent handling with disposable syringes:**
  - **Cleaning free**
  - **Minimal waste of raw materials**
- ► **36 places for reagents**
- ► **3 special places for large quantity and even heated liquid dosing from external reservoirs**
- ► **User friendly**
- ► **EXTREMELY REDUCED FOOTPRINT**
- ► **We developed a new synthesizer adapted to the new doser:**
  - **1 to 6 synthesis vials**
  - **Optional heating/cooling**
  - **Optional UV readout**
  - **Synthesis monitoring**

New reagent dispensing platform: a revolving doser

Flow based synthesizer

- ► **Though our batch technology based syntesizers fulfiled our requirements, we thought that FLOW techology could offer new opportunities:**
  - **Better heat transfer and very effective mixing**
  - **Radically decreased synthesis time**
  - **Straightforward scale-up possibility**
  - **Continuous flow-through UV readout for reaction monitoring**
- ► **We implemented a recirculation based flow system, with high (>500mL/min) flow-rates**
- ► **Reagent dosing with our doser or even with Tecan liquid handler or manually**

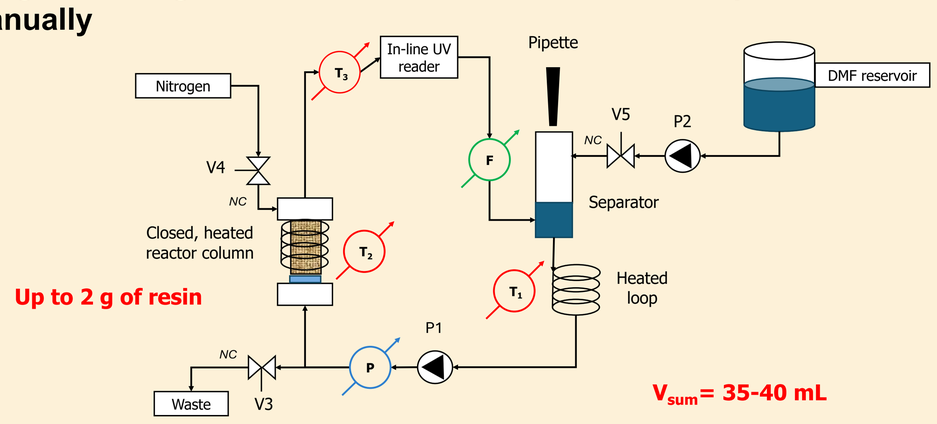
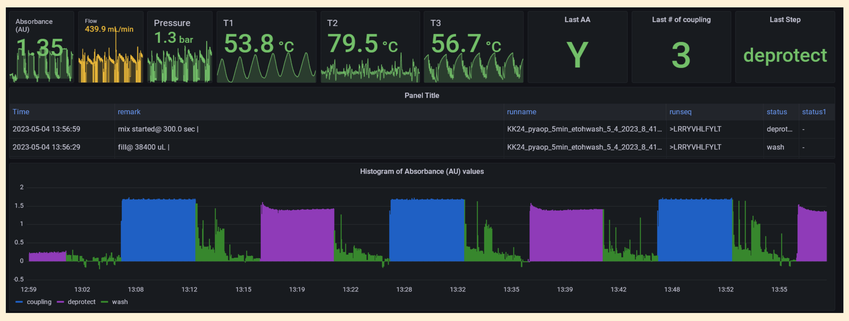


Table 3: Reproducing the results of a marketed synthesizer with our system

Sequence	Our custom FLOW synthesizer	CEM Liberty 2.0
	Yield (%)	
YSYPETPLYMQTASTSYYE	37	47
ALAVLSNYDA	84	84

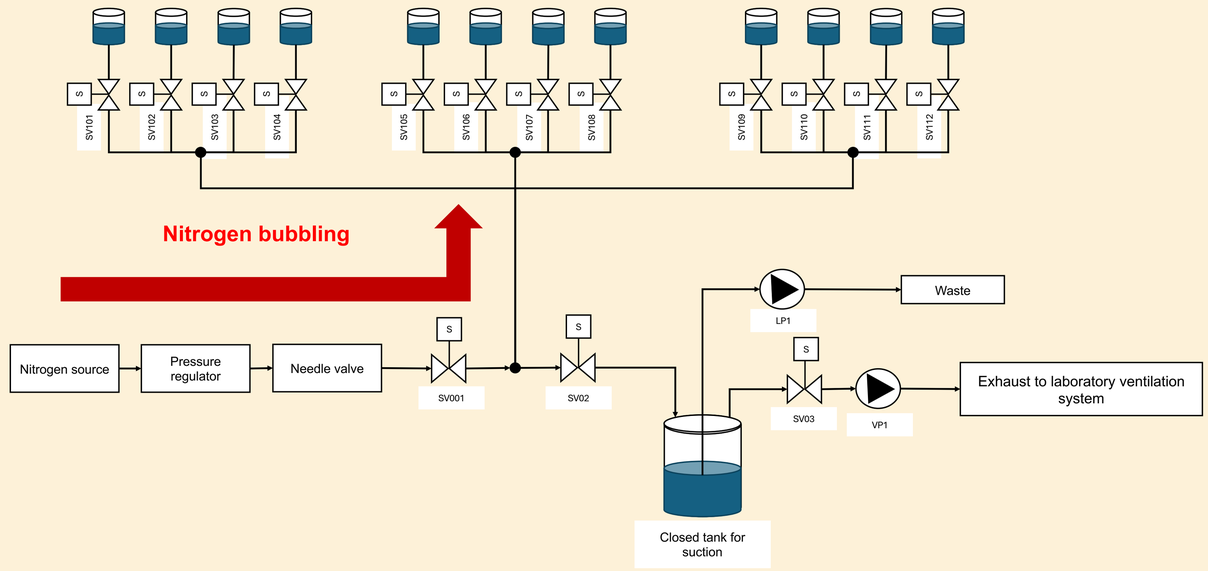
- ► **Recirculation offers longer cycle times (if needed), without the extensive use of raw materials**
- ► **Despite the high flow-rates, the pressure in the system is still low (<3bar), resulting that all types of resin is applicable in the system**
- ► **All synthesis parameters are monitored and stored in database**

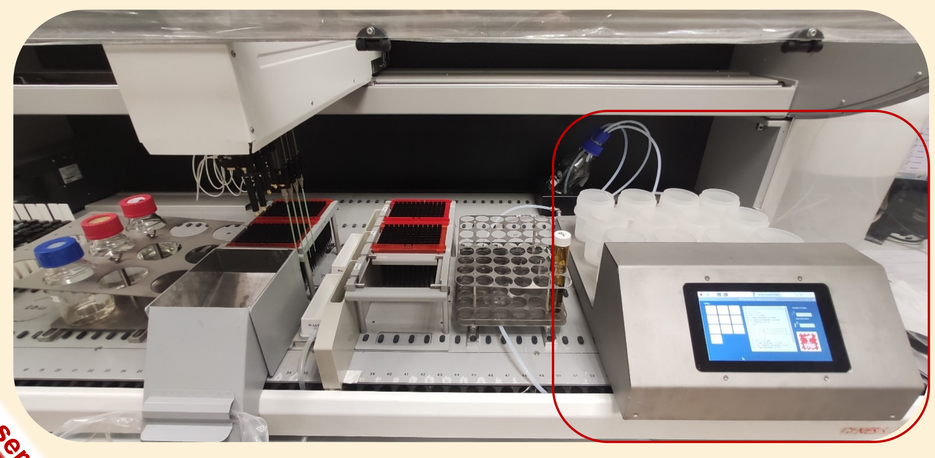


Coupling Deprotect Wash

Classical batch technology

- ► **Classical SPPS methodology – no pressure or heat**
- ► **Fully automated**
- ► **1 to 12 reactor – 1 to 12 parallel synthesis**
- ► **Scale: 0.1-1.0 g resin with capacity of 0.2-0.9 mmol/g**
- ► **Inert atmosphere**
- ► **Constant good mixing**
- ► **Minimal solvent usage – Total solvent usage per steps 3.5-4.0 mL**
- ► **Relatively fast synthesis (compared to manual synthesis): 30-60 min cycle time**





*First iteration as a **Tecan add-on***

***Tecan Worktable** was mandatory which slowed down and limited some functions*

	Procedure	Solvent usage (mL)	Time (min)
0. Initialization step	Resin swelling	8	30-60
	4 x Fmoc-removal (deprotection)	4 x 3.5	4 x 1 (4 x 2 if 20% pip. was used)
	6 x Wash after deprotection	6 x 4	6 x 1
1. step	AA Coupling	3.5	15-30
2. step	3 x Wash after coupling	3 x 4	3 x 1
3. step	2 x Fmoc-removal (deprotection)	2 x 3.5	2 x 1 (2 x 2 if 20% pip. was used)
4. step	6 x Wash after deprotection	6 x 4	6 x 1

Table 1: Typical synthesis parameters

Coupling reagent		AA	*t*	Solvent	Crude Yield (%)	Purity (%)	Yield (%)	Cost (EUR/mg)
PyAOP/DIPEA	2/4 eq	2 eq	30 min	DMF	96.59	80.71	77.96	0.18
PyAOP/DIPEA	3/6 eq	3 eq	30 min	DMF	91.21	81.01	73.90	0.25
PyAOP/DIPEA	3/6 eq	3 eq	15 min	DMF	89.98	81.25	73.11	0.25
PyAOP/DIPEA	3/6 eq	**1.2 eq**	30 min	DMF	88.95	72.90	64.85	**0.19**
Oxyma/DIC	3/6 eq	3 eq	30 min	DMF	82.35	84.83	69.86	0.13
PyAOP/DIPEA	2/4 eq	2 eq	15 min	DMF	86.53	62.72	54.27	0.27
Oxyma/DIC	3/6 eq	3 eq	30 min	**GVL**	61.48	72.00	44.27	0.47
PyAOP/DIPEA	1.2/2.4 eq	1.2 eq	30 min	DMF	80.28	55.03	44.18	0.18

Table 2: Synthesis of ipAoa-LRRY-VHLFYLT-NH2 on RinkAmide MBHA (0,65 mmol/g) resin

Color coding: Hybrid ratio in coupling step, Synthesis with green solvent (GVL)

Summary

With our recent developments, we aimed to address the challenges highlighted in the introduction: reducing costs, time, and manual labor associated with peptide synthesis. By eliminating expensive components such as microwave heating from our synthesizers, the total cost of ownership for these products becomes manageable even for peptide research labs with limited budgets.