TN0008 Scaling up CIM® trypsin immobilized enzyme reactors for industrial scale production of whey proteins hydrolysate

Coupling trypsin directly onto a Convective interaction media (CIM®) monolithic chromatographic support makes it possible to reuse the enzyme for multiple process cycles and to automate hydrolysis processes. BIA Separations offers CIM trypsin immobilised enzyme reactors (trypsin IMERs) based on monoliths with volumes from 0.1 mL up to 80 mL) and with channel sizes of 2 µm or 6 µm to meet the needs of various applications. Common applications include sample preparation for mass spectrometry (MS)-based proteomics or industrial scale production of protein hydrolysates.

Prerequisites for industrial scale usage are efficient enzymatic digestion, easy scale up, and long column life. Efficient enzymatic digestion was proven on 1 mL scale, where a trypsin IMER with 6 µm pore size retained 59% activity of the same amount of dissolved trypsin in solution (Y. Mao, et al., 2017). Data shown in Table 1 demonstrate excellent scalability across different size monoliths. Variation in rates of hydrolysis was within 5% between 1 and 8 mL monoliths. Similar consistency is expected for larger volumes.

Table 1 Enzymatic activity of two CIM trypsin IMERs based on 8 and 1 mL bed volume and 6 µm monolith channel sizes

CIM-trypsin IMERs volume	Permeability in deionized water (m^2)	Activity U (µmol/min) per mg trypsin based on substrate BAEE
8 mL	2.63×10^{-11}	12.8
1 mL	1.91×10^{-11}	12.2

METHOD

Table 2 Process parameters for CIM trypsin IMERs s

Column:	**CIMmultus™ Trypsin/Aldehyde-8 Advanced Composite Column (Pores 6 µm)**
Column conditioning:	10 CV ultra-pure water (16.8 MΩ cm at 20 °C) 10 CV Tris-HCl buffer, pH 8.7 2 CV substrate solution Flowrate 16 mL/min
Hydrolysis:	Substrate solution (80 mL 20 mg/mL whey protein isolate solution), recirculated at 32 mL/min
Washing:	10 CV water/1 M NaCl (pH 10.5), linear gradient 0-100% 1 M NaCl. 10 CV 100% 1 M NaCl (pH 10.5) 10 CV ultra-pure water 20 CV 19 mM $CaCl_2$ in 5% ethanol/water (v/v) (pH 3)
Storage	4 °C

Note: all applied solutions, including substrate solution, were filtered through 0.45 µm cellulose filter before use.

As shown by Yuhong Mao and Kulozik (2018), trypsin IMER functionality depends on environmental conditions. Flow rate, pH, temperature, ionic strength, etc., play important roles in the efficiency of the hydrolysis process, and composition of the resulting hydrolysates. The example below was performed with 80 mL of 20 mg/mL whey protein isolate **(WPI) in water, which was recirculated at 32 mL/min and pH 8.7. The degree of hydrolysis (DH) was recorded by pH-stat method. The DH is shown in Figure 1, as well as the evolution of the reaction velocity**.

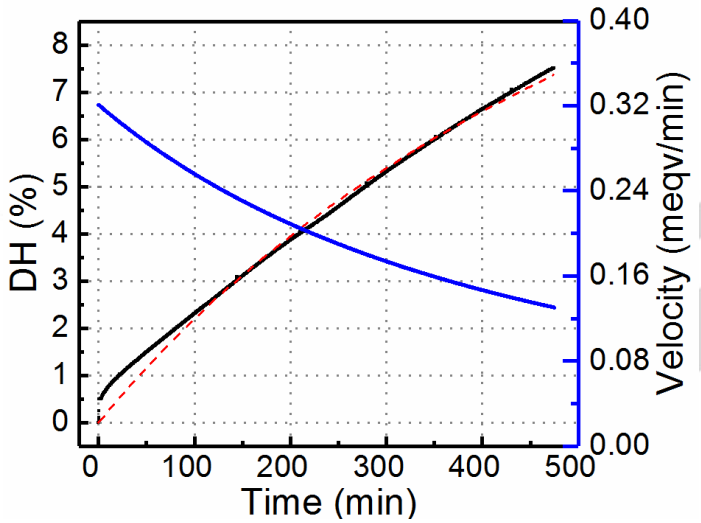

Figure 1 Evolutions of DH and reaction velocity during the hydrolysis of WPI.

Hydrolysis rate decreased only 12% between the first and 30th cycles (Figure 2A). Pressure drop at a flow rate of 6 column volumes (CV)/min (360 CV/h) was 0.045 MPa and increased to only 0.15MPa during 30th cycle.

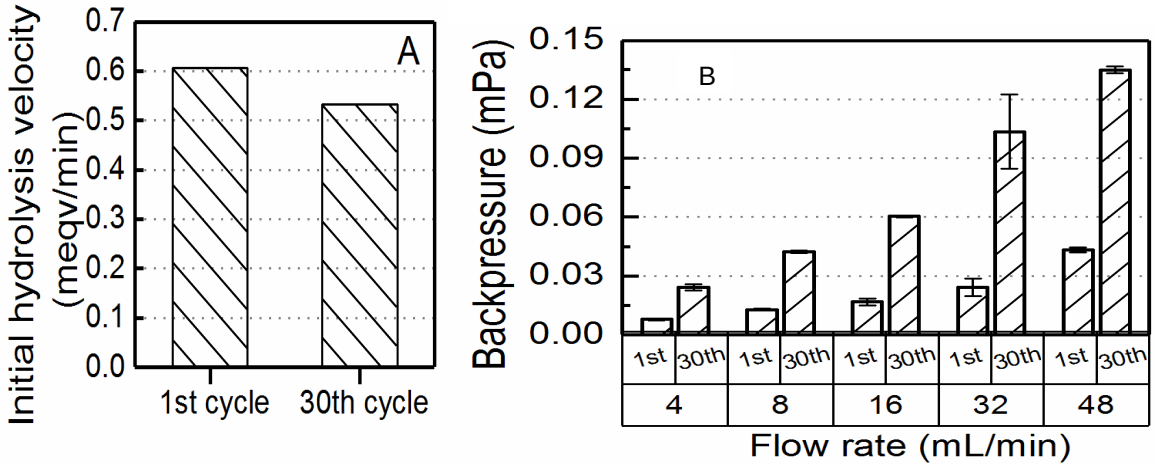

Figure 2: The comparison of initial hydrolysis velocity (A) and pressure-drop (B) at 1st and 30th cycle, respectively.

REFERENCES

- Mao, Y., Cernigoj, U., Zalokar, V., Strancar, A., & Kulozik, U. (2017). Production of beta-Lactoglobulin hydrolysates by monolith based immobilized trypsin reactors. *Electrophoresis, 38*(22-23), 2947-2956.
- Mao, Y., & Kulozik, U. (2018). Selective hydrolysis of whey proteins using a flow-through monolithic reactor with large pore size and immobilised trypsin. *International Dairy Journal*.
- Naldi, M., Černigoj, U., Štrancar, A., & Bartolini, M. (2017). Towards automation in protein digestion: Development of a monolithic trypsin immobilized reactor for highly efficient on-line digestion and analysis. *Talanta, 167*, 143-157.

Used products

Catalogue No.:	Product description
411.1208-6	CIMmultus™ Trypsin/Aldehyde-8 Advanced Composite Column (Pores 6 µm)

Related products

Catalogue No.:	Product description
311.1208-2	CIMmultus™ Trypsin/Aldehyde-1 Advanced Composite Column (Pores 2 µm)
110.1208-1.3	CIMac™ Trypsin/Aldehyde-0.1 Analytical column (Pores 1.3 µm)

For any additional information or further enquiries please contact us:

Tel.: +386 59 699 500

help@biaseparations.com
sales@biaseparations.com

www.biaseparations.com