VRIJE UNIVERSITEIT BRUSSEL

The faculty of Engineering of the Vrije Universiteit Brussel invites you to attend the public defense leading to the degree of

DOCTOR OF ENGINEERING SCIENCES

of **Ali Amini**

The public defense will take place on **Thursday 6th March 2025 at 4pm** in room **D.2.01** (Building D, VUB Main Campus)

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ON THE POTENTIAL OF MICROFLUIDIC LIQUID-CHROMATOGRAPHY SYSTEMS: PROTOTYPING AND PERFORMANCE ASSESSMENT OF MICROFLUIDIC CHIPS AND A ROBOTIC INTERFACE ENABLING MASS-SPECTROMETRY DETECTION

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Abstract of the PhD research

Microfluidics has emerged as a pivotal technology across diverse fields, offering a multitude of advantages, including minimal sample and reagent volume requirements, the possibilities to integrate multiple functionalities, and process parallelization. High-resolution 3D printing (3DP) technology provides opportunities to create microdevices for liquid chromatographic (LC) separations allowing integrating unique channel structures and microstructures. As such, 3DP will provide new possibilities to establish a novel generation of devices for multi-dimensional LC. Digital light processing (DLP) 3D printing was explored for prototyping microfluidic chips for high-pressure liquid-chromatography separations. Effects of printing parameters on microchannel geometry, 100-500 µm i.d. squared and circular channel, and surface roughness were assessed. Moreover, pressure resistance and solvent compatibility were investigated. Applying thermal post-curing and encasing the chip in an aluminum holder as a micro-to-macro-interface with the HPLC instrument increases the chip pressure resistance up to 650 bar. Moreover, a novel approach for the *in-situ* UV synthesis of polymer monolithic support structures is demonstrated, as a first step to a fully functional 3D printed micro device enabling high-pressure liquid-phase analysis and proof-of-concept of a reversed-phase (RP) chromatographic gradient separation of intact proteins is demonstrated using an aqueous-organic mobile-phase with isopropanol as organic modifier.

In spatial three-dimensional (3D-)LC, components undergo separation within a 3D space featuring orthogonal retention mechanisms, promising unprecedented resolving power. Parallel 2D and 3D analysis ensures high sample throughput. A robotic-microfluidic interface was developed that facilitates the coupling of spatial 3D-LC with mass-spectrometry imaging detection. The prototype microfluidic device includes a 3D microfluidic flow distributor with 16-parallel outlets in a 4 × 4 configuration, integrated with sixteen monolithic capillary columns. Positioned on a motorized XYZ stage, the microdevice deposits effluents in a series of prints on a target plate for subsequent mass-spectrometry imaging analysis. Furthermore, the deposited sample volume can be automatically adjusted from 60 nL to 2.5 µL by varying the flow rate and deposition time interval. Moreover, cross-contamination between deposited effluents was investigated and minimized by optimizing the movement of the robotic-microfluidic interface. As a proof-of-concept, intact proteins were analyzed by parallel RP chromatographic gradient separation and deposited effluents were characterized by matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS). RP-MALDI-MS spectra of deposited effluents among all the 16 parallel columns show similar retention times for intact proteins, confirming the practicality of this detection methodology for parallel channels and spatial 3D-LC.