Design and Application of an Immobilized-Enzyme Microfluidic Reactor for Rapid Online Digestion of Various Natural Macromolecules

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Enzymatic degradation of macromolecules (e.g. proteins or biodegradable polymers) can provide insight into their chemical structure by studying the constituting fragments. Herein, long digestion times remain an important bottleneck for high-throughput analysis. Alternatively, macromolecules can be digested using an immobilized-enzyme reactor (IMER), enabling shorter digestion times in the order of minutes to seconds due to a much higher enzyme-to-substrate ratio and reduction of diffusion distances. I will present our progress on the design and application of a cyclic-olefin-copolymer microfluidic reactor, containing enzymes immobilized on a polymer monolithic material via a 2-vinyl-4,4-dimethylazlactone linker. The potential of the IMER is demonstrated for the analysis of complex protein mixtures in dried blood spots (DBS). Compared to a conventional in-solution digestion workflow, a comparable number of protein identifications could be achieved by use of the IMER while reducing the digestion time from 16 hours to 5.6 minutes and omitting the sample pretreatment steps (denaturation, reduction, and alkylation). Present data suggest that protein digestion times well below one minute are feasible. Depending on the application, other enzymes specific for biopolymers (e.g. cellulose and lignocellulosic materials) or synthetic polymers (e.g. polyesters) can be immobilized. Incorporation of the immobilized-enzyme reactor in a modular microfluidic platform with low dead-volume connections, allows for in-line implementation of the reactor in an LC\texttimes{}LC workflow.