New miniaturized tools for the analysis of biomolecules in biological fluids

Protein analysis is mainly carried out using the "bottom-up" approach which is based on enzymatic digestion of proteins and analysis of resulting peptides by liquid chromatography coupled with tandem mass spectrometry. Usually, enzymatic digestion is carried out in solution. However, this procedure is long. The use of immobilised enzyme reactors (IMER) and their hyphenation with LC-MS/MS increases the reliability and sensitivity of the overall method. The study conducted during this thesis had two distinct objectives. The first was to evaluate the potential of IMERs for glycosylation characterisation. The complementarity of IMERs of pepsin and trypsin, developed in classical format by grafting the proteases on a Sepharose support, allowed to identify the N-glycans on the 4 glycosylation sites a pregnancy hormone contained in 2 drugs. The second objective was the miniaturization of these tools. To do this, monoliths obtained by sol-gel approach in the presence of organosilanes or by radical polymerization of organic monomers were synthesized in situ in capillaries of 100 µm internal diameter. The better repeatability of synthesis of organic supports led to their selection for functionalization by trypsin or pepsin. In parallel, a miniaturised set-up for the analysis of digests was carried out. This will allow the subsequent inclusion of IMER in the overall analytical device.