# Preparation and Characterization of a Trypsin Enzyme Reactor designed for Proteomic Assays

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**Introduction:** Trypsin has been immobilized in different supports to achieve a maximum of efficiency, increasing the number of identified peptides and reducing the reaction time. The immobilization has several advantages, such as – biomolecule stability and bioreactor reuse. Moreover its association with the benefits of magnetic supports encourage the use of this approach in proteomic analyses. **Objectives:** Immobilization and characterization of trypsin on the surface of magnetic beads. **Experimental:** Trypsin was immobilized on amine-terminated magnetic particles. For monitorig the bioreactor activity, a LC-UV method was validated to quantify the formed product *p*-nitroaniline using benzoyl-Arg p-nitroanilide (BAPNA) as substrate. The bioassay was optimized varying the reaction time and buffers. For the bioreactor characterization, a kinetic study with BAPNA (0.620 – 40.9 µM) was carried out. The developed bioassay was applied to digest 5.00 µg of bovine serum albumin (BSA) at different time (1, 2, 4 e 8 hours) and the samples obtained were analyzed in a LC-MS/MS system. **Results and Discussion:** The results obtained indicated a higher production of PNA using $NH_4HCO_3$ (100 mM, pH 8) as buffer and 8 min as reaction time. The kinetic result ($K_m$ = 1679,5 ± 75,7 µM) demonstrates the immobilized enzyme showed affinity for the substrate, suggesting that the active sites are available after the immobilization. In the proteolysis assays, the results obtained with 8 hours of digestion were similar (number of identified peptides), to those observedin the standard BSA digested, decreasing the proteolysis time from 16 (usual digestion protocol) to 8 hours. **Conclusions:**The prepared bioreactor was efficient, reducing the proteolysis time and maintained the activity. Besides the same bioreactor was used throughout the study, demonstrating the capability of reuse. The results achieved up to now indicates that the prepared trypsin bioreactor is a potent tool for proteomic assays.

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