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A semi-continuous magnetic particle-based process for the controlled attachment of PEG (PEGylation) to proteins is described for the first time. Trypsin and 2 kDa mono-activated PEG were used to systematically develop the steps in the process. Proof of concept was shown in a microfluidics system to minimize reagent consumption. Two streams containing (i) 1.2 g/L trypsin and (ii) 4 g/L magnetic adsorbents derivatized with the reversible affinity ligand benzamidine were pumped into a pipe reactor. At the exit, a third solution of activated PEG (0-40 g/L) was introduced and the solutions immediately fed into a second reactor. Upon exiting, the mixture was combined in a third reactor with a fourth stream of free amine groups to stop the reaction (50 mM lysine). The mixture continued into a high-gradient magnetic separator where magnetic supports, with PEGylated trypsin still attached, were captured and washing and elution steps were subsequently carried out. Analysis of the conjugates (with SDS-PAGE & LC-MS) showed that the extent of PEGylation could be controlled by varying the reaction time or PEG concentration. Furthermore, the PEG-conjugates had higher enzyme activity compared to PEGylation of non-immobilized trypsin.

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