PEGylation of L-Asparaginase in Microfluid Systems

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The enzyme L-asparaginase is responsible for converting the amino acid L-asparagine into L-aspartic acid and ammonia. This enzyme has important applications, especially in the pharmaceutical industry, where it is employed as an antileukemic biopharmaceutical for the treatment of acute lymphoblastic leukemia (ALL). In order to improve the performance of biopharmaceuticals, in recent years, techniques have been developed to improve biopharmaceuticals by bioconjugation, within these techniques PEGylation stands out. This technique allows the reduction of immunogenicity, increased solubility and half-life time in plasma, and greater protection against proteolytic attack. Despite the advantages of PEGylated biopharmaceuticals, also called *biobetters*, some limitations associated with PEGylation reactions need to be addressed, such as: the reduced conversion of the bioconjugation reaction, a result of the hydrolysis of the reactive PEG, and the reduced selectivity, resulting in a heterogeneity of PEGylated species formed by parallel reactions between the reactive PEG and the functional amino acids of the proteins. Both the extent of conversion and the selectivity of the PEGylation reaction of protein biopharmaceuticals are highly sensitive to the experimental parameters of the process (such as: reaction time, PEG:protein molar ratio, stirring and pH).

Thus, this innovative study aims to optimize the bioconjugation reactions of L-asparaginase by applying passive continuous-flow microreactors in two geometries (*i.e* crossing-channels with a reduced and high number of sections), aiming at increasing the PEGylation yield and selectivity of the reaction (monoPEGylation). The reactive PEG to be used is methoxy-polyethylene glycol carboxymethyl *N*-hydroxysuccinimidyl ester (mPEG-NHS) of three molecular masses (10, 20 and 40 kDa). It is intended to compare the yield of reactions

obtained in microfluidics by passive micromixers with those obtained in batch processes by magnetic stirring in glass vials. The PEGylation reaction in microfluidics, was conducted in a crossing-channel geometry of the micromixer, in which the inlets will be fed with syringes, injecting the reactive PEG solution (mPEG-NHS) of different sizes and the solution of L-asparaginase in 100 mM phosphate buffer (pH = 7.5), in a constant flow rate (20-200 $\mu L.min^{-1}$), so that it results in a more specific residence time and provides a mixture of the reaction between the reactive PEG and the L-asparaginase in a laminar flow with chaotic advention.

The technology developed in this work by PEGylation in microreactors intends to be transposable to other biopharmaceuticals an alternative to conventional batch processes with low yield and selectivity.

Keywords: L-asparaginase, microreactors, PEGylation.

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