Extended Abstract

Immobilized Enzyme Reactors Based on Nucleoside Phosphorylases (NPs) and Nucleoside 2'-Deoxyribosyltransferases (NDTs) for the In-Flow Synthesis of Nucleoside Analogues [†]

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Enzymes are increasingly used as biocatalysts for the production of Active Pharmaceutical Ingredients and fine chemicals [1,2]. A well-known strategy to increase enzyme stability and to allow the reuse of biocatalysts for multiple cycles is immobilization. Moreover, when flow systems are employed for biocatalyzed reactions, the process can benefit from accurate control of the reaction parameters, improved mass transfer, and reduction of substrate/product inhibition effects [3].

Immobilized enzyme reactors (IMERs) can be successfully applied in drug discovery for the rapid preparation of chemical libraries. In addition, single or multi-enzyme IMERs can be connected to different separation and detection systems.

In this work, two analytical IMERs were developed as prototypes for biosynthetic purposes, and their performances in the in-flow synthesis of nucleoside analogues of pharmaceutical interest were evaluated. Two classes of enzymes were tested: nucleoside phosphorylases (NPs) and nucleoside 2'-deoxyribosyltransferases (NDTs).

The NP-based bioreactor was prepared by co-immobilizing uridine phosphorylase from *Clostridium perfringens* (CpUP) and a purine nucleoside phosphorylase from *Aeromonas hydrophila* (AhPNP) on an aminopropyl silica column [4], while the second IMER was obtained by covalent immobilization of nucleoside 2'-deoxyribosyltransferase from *Lactobacillus reuteri* (LrNDT) on an epoxy silica column. As the chromatographic support, a monolithic material (Chromolith® columns) was selected due to its low operative back-pressure and fast mass transfer.

The in-flow synthesis of 5-iodo-2'-deoxyuridine, 5-fluoro-2'-deoxyuridine, and 2',3'-dideoxyinosine was then performed by exploiting both CpUP/AhPNP- and LrNDT-IMERs. Reaction monitoring and conversions were assessed by a reverse phase liquid chromatography system coupled to UV detection. LrNDT-IMER allowed to achieve higher conversions in shorter times for the investigated reactions.

References

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