19660 | Functionalized Carbon Xerogels as a Novel Platform for the Immobilization of L-

Asparaginase

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## Abstract

In the pharmaceutical sector, the enzyme L-asparaginase (ASNase, EC 3.5.1.1) is used to treat acute lymphoblastic leukemia (ALL), the most common type of cancer in children. In the food industry, this enzyme is used to reduce carcinogenic acrylamide on starchy-rich foods. Taking into consideration the ASNase important applications, it is of great interest to improve its enzymatic properties through its immobilization. Immobilization not only allows easier recovery and reuse of enzymes, but also contributes to the improvement of their stability.

In this work, the ASNase immobilization was studied by physical adsorption onto functionalized carbon xerogels (CX), a very promising material due to its high surface areas and adsorption capacities, as well as the possibility to precisely customize its porosity. Initially, the ASNase immobilization was tested onto CXs with different functionalizations. After selecting the best material, a central composite design with three factors (contact time, pH and enzyme concentration) combined with response surface methodology was applied to optimize the immobilization process in order to attain the highest relative recovered activity (RRA) of the immobilized ASNase. The results obtained revealed that ASNase concentration is the factor that most influences the predicted response.

The best results were obtained using the CX-OX-600, 81 min of contact time, pH 6.2 and 0.36 mg/mL of ASNase, reaching RRA values of 103% and an immobilization yield (IY) of 100%. Finally, under optimal conditions, the immobilized ASNase showed an exceptional operational stability, retaining 97% of its initial activity after 6 reaction cycles. Nevertheless, temperatures of 60°C led to denaturation of immobilized enzyme. The kinetic parameters indicated a 1.25-fold increase in the immobilized ASNase affinity for the substrate.

All these results confirm the CXs potential as a support material for the ASNase immobilization by physical adsorption.