OPTIMIZATION OF THE IMMOBILIZATION PROCESS OF TRYPSIN IN CALCIUM ALGINATE BEADS

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Introduction. Ca-alginate beads represent one of the most widely used carriers for the immobilization of enzymes. In this sense, the immobilized enzymes have several advantages over the enzymes in free solution since through this method facilitates the recovery and reuse of enzymes. However, it is necessary to take into account the concentration of calcium chloride ($CaCl_2$) and sodium alginate (AlgNa) to avoid diffusion of nutrients and product through the porous matrix, this is one of the most important disadvantages presented (1).

The aim of this study was to evaluate the effect of the concentration of $CaCl_2$ and AlgNa on the trypsin enzymatic activity.

Methods. The effect of $CaCl_2$(M) and AlgNa(%) concentration on the immobilized trypsin activity (AU/ml) (2) and immobilization rate (%) (3) were evaluated using a central composite rotatable design (Table 1) in order to optimize the immobilization conditions. The beads obtained from the optimal region were observed under a scanning electron microscope also were determined relative activity after 10 cycles of use (3).

Table 1: Experimental design: process variables and their levels.

Factors	Low level	Middle level	High level
AlgNa (% w/v)	2.0	2.5	3
$CaCl_2$ (M)	0.5	0.93	1.36

Results. The optimization was performed using the methodology of overlapping surfaces, setting a criterion find immobilization conditions for the best values of enzymatic activities and immobilization rate. The optimization results indicated that the best theoretical conditions for immobilization of trypsin are: $CaCl_2$ concentration: 1.28M; AlgNa concentration: 2.92%, resulting in an enzyme activity of 120.87 AU/ml with an immobilization rate of 95%. Experimentally, under these conditions, the enzyme activity was: 122±0.3 mAU/mL, indicating a good fit of the proposed model. The obtained spheres showed under optimal conditions a porous lattice structure (Fig. 1).

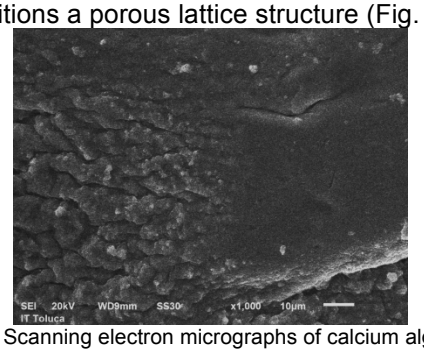

Fig. 1 Scanning electron micrographs of calcium alginate beads the area obtained at the optimum point (Cross Section).

To study the stability of the immobilized enzyme under the optimized conditions, enzymatic activity was measured daily and the activity of the immobilized biocatalyst under optimized conditions no showed significant difference after 4 uses, after this decreased by only 30% after 8 uses.

Conclusions. The results of this study demonstrated that the optimal conditions for the immobilization of trypsin were 2.92% alginate and 1.28M $CaCl_2$. The trypsin immobilized maintained its initial activity after 8 uses.

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