THE USE OF FERROMAGNETIC MATRICES FOR ENZYME IMMOBILIZATION

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The interest to investigate ferromagnetic matrices for enzyme immobilization began as an alternative procedure to overcome the limitations on the use of Dacron-hydrazide in powder. These particles (Fe_3O_4; magnetite) have been synthesized by thermal co-precipitation of ferric and ferrous chloride at high pH together with the polymer. These composites combine the advantages of the magnetism with the chemical groups of the polymer through which the enzyme molecule will covalently bind.

Firstly, amyloglucosidase was covalently immobilized on ferromagnetic Dacron particles (Carneiro Leão et al., 1991) and, afterwards, trypsin from the intestine of Tilapia Niloticus (*Oreochromis niloticus*), usually discarded (Amaral et al., 2006), and sulfatase from the liver of the muscle *Aplysia cervina* were also fixed on them.

Magnetic composites based on other polymers (chitosan, levan and a semi-interpenetrated network of polysiloxane and polyvinyl alcohol; POS-PVA) have been also synthesized and used as matrices for trypsin, α–L-rhamnosidase and β-galactosidase immobilization.

These magnetic water insoluble enzymatic derivatives combine the advantages of immobilized enzymes, such as, reuse, immediate removal from the reaction mixture, higher thermostability, products obtainment without enzyme contamination etc.; with those provided by the magnetism: easy removal from the reaction mixture by applying a magnetic field, applications in magnetically stabilized fluidized beds etc.

Immobilized trypsin on ferromagnetic Dacron retained 25.6 mg/g matrix and 18.5 mU/mg protein (29% of the free enzyme) and presented lower values of K_m and optimum pH compared with the free enzyme (Amaral et al., 2006). This preparation showed to be stable during two months stored at 10^o C in buffer and could be reused. Trypsin was also covalently fixed on ferromagnetic levan, via polysaccharide, but also it was adsorbed on the particles. However, this adsorbed enzyme leached out from the matrix during the washing procedures and showed lesser thermal stable.

Immobilized sulfatase on ferromagnetic Dacron and POS-PVA particles presented 3.17 U/mg protein and 1.85 U/mg protein, respectively, corresponding to 36.26% and 21.23% of the specific activity of the free enzyme. These preparations were reused eleven and seven times, respectively, without reduction of enzymatic activity. Both preparations showed similar apparent K_m, optimal pH and temperature, and higher thermal stable compared with the values estimated for the free enzyme.

α–L-Rhamnosidase covalently immobilized on magnetic particles of Dacron, POS-PVA and chitosan retained 36.6%; 40.4% and 4%, respectively, of the specific activity of the free enzyme. No enzymatic activity reduction was observed after these magnetic enzyme derivatives being reused ten times.

β-Galactosidase covalently immobilized on ferromagnetic POS-PVA was capable to hydrolyze all the lactose presented *in natura* milk after about two hours of incubation.

Recently, Maciel el al. (2008) by using X-ray diffraction and infrared spectroscopy demonstrated the presence of magnetite in the magnetic particles synthesized with levan.
Based on the above results one can conclude that the co-precipitation of ferrous and ferric chloride in the presence of polymers yields magnetic particles that can be used as matrices for enzyme immobilization. This attractive procedure is simple, low cost and very versatile. Investigations on the use of these magnetic matrices in continuous reactor and as nanoparticles for controlled release drug delivery are in progress.

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