# Hierarchically porous polyHIPE carriers for the immobilization of lactase

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Porous polymer monoliths are increasingly used in a variety of separation processes, including stationary phases for various chromatographic techniques, high-throughput bioreactors, thermoplastic microfluidic devices, and as solid supports for enzyme immobilization and catalytic reactions. Among the monomers used for the preparation of such monoliths, glycidyl methacrylate (GMA) and methyl methacrylate (MMA) are the most widely used. GMA is particularly attractive due to its reactive structure, which combines a polymerizable methacrylate group with an epoxy functionality suitable for post-polymerization modifications.

Several templating methods have been developed for the preparation of porous, cross-linked poly(GMA) monoliths. One very effective method is the polymerization of the continuous phase of a high internal phase emulsion (HIPE), which results in materials with highly interconnected, open-cell porous structures. HIPEs are emulsions consisting of two immiscible liquids, where the internal (dispersed) phase has a volume fraction of more than 74%, exceeding the maximum packing density of uniform spheres. The resulting polyHIPE materials typically have a structure characterized by large voids formed by the droplets of the internal phase and smaller interconnected pores (windows) between them [1].

Poly(GMA) with reactive epoxy groups and adjustable porosity offer an excellent platform for the immobilization of enzymes. Various immobilization mechanisms have been explored, including direct covalent binding, surface activation with crosslinkers such as glutaraldehyde, and non-covalent interactions such as hydrogen bonding and electrostatic forces. Porous structures are particularly advantageous as they provide a larger surface area and facilitate the stabilization of enzymes in the architecture of the material [2].

β-Galactosidase, a versatile enzyme with important applications in the food, pharmaceutical and biotechnology industries, is mainly used for lactose hydrolysis in milk production, supporting the development of lactose-free products. It also plays a role in the synthesis of prebiotic oligosaccharides, bioactive compounds and specialty sugars. Its broad specificity and operational stability have led to extensive research on its immobilization on various supports [3].

In this work, β-galactosidase was immobilized on hierarchically porous polyGMA supports prepared by the emulsion-templating method (polyHIPE). Two immobilization strategies were compared: direct covalent binding and surface activation by glutaraldehyde. Immobilization of β-galactosidase on highly porous poly(GMA) by glutaraldehyde activation resulted in higher enzyme loading (0,43 mg/100 mg polymer) and a significantly improved catalytic performance with optimal activity at pH 6.5 and 35°C compared to direct binding without activation. In flow reactors, complete lactose conversion was achieved at 0.5 $mL \cdot min^{-1}$, while higher flow rates reduced efficiency.

**Keywords:** polyHIPE, glycidyl methacrylate, immobilized enzymes, glycoside hydrolase enzyme

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