Crosslinked mercerized cellulose membranes for the affinity chromatography of papain inhibitors

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Abstract

Using a methodology described by the authors in previous papers, macroporous cellulose membranes possessing large pore sizes (0.4–1 μm) and high porosity (about 55%) were prepared from filter paper by crosslinking or mercerization followed by crosslinking. Epoxy and aniline groups were attached to activate the membrane, and a biomolecule ligand (bovine serum albumin, trypsin or papain) was coupled via diazotization. A detailed comparison between the mercerized and non-mercerized membranes showed that the former was more efficient for biomolecule immobilization. The uniformity of the ligand distribution was investigated using triazine dye and bovine serum albumin as test ligands. The activities of the membranes containing trypsin or papain as ligand for the hydrolysis of N-benzoyl-l-arginine p-nitroanilide were also determined. Using papain-containing affinity membranes, papain inhibitors were separated from potato tubers. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

In recent years, the membrane affinity chromatography of biomolecules had a fast development, because their pharmaceutical potential required reliable and efficient methods of separation and purification [1–5]. Numerous materials, such as nylon, polysulfone, glycidyl methacrylate, chitosan, cellulose and cellulose derivatives [6–11], were used to prepare matrices for membrane affinity chromatography. The synthetic polymeric materials are suitable, from a mechanical point of view, for membrane preparation, but many of them are less suitable for ligand immobilization, because their lower compatibility increases the probability for denaturation of the biomolecules.

In contrast, the natural macromolecular materials are compatible with the usual ligands, but often difficult to process as membranes.

Cellulose is highly compatible with biomolecules, but being insoluble in almost any solvent, it is difficult to process as a membrane [12]. However, acetate cellulose and nitrate cellulose are soluble in aqueous solutions, and membranes could therefore be prepared through the phase-inversion method; in addition, ion exchange groups or affinity ligands could be coupled to the crosslinked membranes [13]. Composite macroporous cellulose membranes could also be obtained by grafting an acrylic polymer to a cellulose backbone; they have been used for the purification of immunoglobulins and the removal of endotoxins [14].

In a previous paper, macroporous cellulose membranes were prepared from high quality filter paper...