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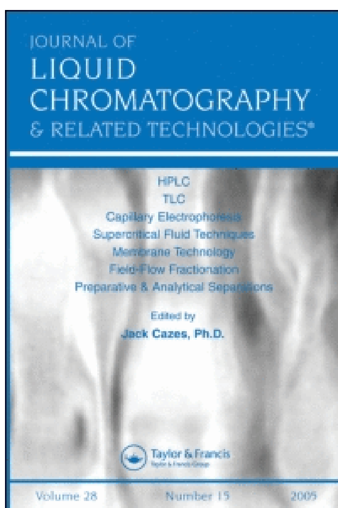

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High-Performance Membrane Chromatography. A Novel Method of Protein Separation

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HIGH-PERFORMANCE MEMBRANE CHROMATOGRAPHY. A NOVEL METHOD OF PROTEIN SEPARATION

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ABSTRACT

Basing on the fact that only short layers of a chromatographic column contribute to the separation in the interaction chromatography, 1 mm thick membranes from macroporous methacrylate polymer provided with functional groups were synthetized and used for protein separation. The chromatograms show that the separation is fully comparable with that experienced on a filled column but the advantage of a membrane is up to two orders of magnitude lower pressure during the process and very high loading reaching up to 40 g/m^2. This recommends the high performance membrane chromatography also for large scale preparative separations.

INTRODUCTION

The history of high-performance liquid chromatography (HPLC) of proteins is only a little longer than one decade. (1). Three chromatographic modes are the most important: steric exclusion, ion-exchange (IEC), and hydrophobic interaction chromatography (HIC) (2).

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The last two represent the interaction type of chromatography, in which biopolymers interact in various ways with the sorbent located at the top of the chromatographic column and eluted when the composition of the mobile phase is gradually changed (gradient). The capacity factor k′ defined as the molar ratio of the separated compound in the stationary and the mobile phase changes dramatically with the solvent mixture composition (3). Up to a certain composition, k′ is so high that the macromolecule is contained in the stationary phase only and does not move along the column. However, on reaching a defined point, a small change of the solvent quality causes a rapid decrease in k′ to a value close to zero. The macromolecule dissolves in the mobile phase and passes through the column almost without any retention. In the gradient chromatography based on principle of selective desorption the thickness of the layer in which the most effective separation process takes place is rather short and depends on the adsorptivity of the protein, steepness of the gradient, linear flow rate, and on the ratio between pore volume of packing and free volume of the column (4).

Thus, the length of the column does play an important role and very short columns may be used.

The majority of chromatographic column packings are macroporous and possess porosity even in the dry state (aerogels). Their morphology is characterized by a globular structure: this means that the particles consist of mutually connected spherical entities, globules, sized some tenths of micrometer. The interstitial spaces between them are pores (5). Knowing that only a thin layer of the support at the column top is actually saturated with the separated protein mixture, one can easily imagine that the layer, in fact flat bed column, can be used alone. Combining of the idea of the

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short column layer and globular morphology results in a flat board (membrane), consisting of a polymer, whose internal structure is identical with that of a macroporous sorbent.

EXPERIMENTAL

The membranes have been synthesized by the radical polymerization of glycidyl methacrylate and ethylene dimethacrylate in presence of porogenic solvents similar to that used in the production of beads (6), but not in suspension. The polymerizing mixture fills in the space between two heated plates. The membrane thickness can be adjusted by the distance between the plates. The inner surface of membranes has to be furnished with suitable functional groups usually used for HIC or IEC by simple modification of epoxy groups present in the poly[glycidyl methacrylate-co-ethylene dimethacrylate] described elsewhere (7). Subsequent hydrolysis by 0.5 mol/l sulfuric acid for 3 hours at 80°C destroys the residual epoxide groups, which may otherwise interfere by attaching the separated substances covalently and preventing 100 % recovery of separated species.

The separation of protein mixtures is carried out in a closed cylindrical vessel, 2 cm high and 2 cm in diameter, provided with a magnetic stirrer, directly connected to a detector. After the protein has been sorbed from its buffered solution, successive desorption is accomplished by pumping the second buffered solution, differing in the content of the chaotropic agent.

RESULT AND DISCUSSION

Because of our long-term experience in the formation of macroporous poly[glycidyl methacrylate-co-

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TABLE 1

Basic Characteristics of Membranes

Nr.	Polym. feed, %		S_g	L	r_g
	GMA	EDMA	m^2	m/s.Pa	nm
G-5	5	95	250	-	5[a]
G-25	25	75	139	8.0	10[b]
G-40	40	60	80	22.4	17[b]
G-50	50	50	60	40.0	23[b]
G-60	60	40	43	-	25[a]

S_g specific surface area; L permeability; r_p mean pore radius measured on beads of the same composition (a) or calculated from flux vs. pressure dependency.

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ethylene dimethacrylate] (GMA-EDMA) beads for HPLC the membranes too are made from the same copolymer. Their properties shown in a few examples in the Table 1 can be varied by modifying the GMA/EDMA ratio, the amount and type of the porogenic solvent, the polymerization conditions, etc.

The membranes possess properties usually reported for ultrafiltration membranes. The dependence of flux on pressure is linear and the slope is indirectly proportional to the fraction of the crosslinking agent in the monomeric mixture. A comparison between the mean pore size and permeability (Table 1) indicates that the pore size distribution of the membrane has to be broad, which is indeed required for the application. The broad pore size distribution covers both relatively large transportation pores (canals) and small separation ones. The former secure the high flow rate at a low pressure,

while in the big number of the small pores furnished with a large surface area, interactions with the compounds present in the solution take place.

Figure 1 compares the separation of a protein mixture on a microcolumn 140 mm long filled with the C4 alkyl modified GMA-EDMA microparticles and on a membrane 1 mm thick based on the same material. The separation efficiency is fully comparable, with the exception that for column chromatography pressure up to 2 MPa has to be used, while on the membrane the pressure does not exceed 0.1 MPa. Figure 2 shows the separation of another model mixture which is as good as the separation described in the literature with a special C18 silica column 4,6 mm i.d. and 20 cm long[9]. The most serious advantage of the membrane over the column is again the much lower pressure used (0.1 MPa instead of 8 MPa) and very high loading. The resolution during the chromatographic process can be governed by varying the flow rate of the mobile phase and by modifying the solvent composition gradient. The separation is acceptable even when quite steep gradient is used.

The chromatograms document clearly that the membrane separation developed in this study is very efficient; the technique can be therefore called high performance membrane chromatography (HPMC). Compared with HPLC, the advantage of the suggested method consists in a much lower pressure used for reaching the same separation rate, irrelevant heavy duty hardware, and particularly, in much higher loading, which achieved up to 40 g of ovalbumin per 1 m^2 of the membrane 1 mm thick. This makes HPMC well suited also for preparative separations, in which also interactions others than shown (ion exchange, bioaffinity, metal chelate affinity, immunoaffinity etc.) can be engaged.

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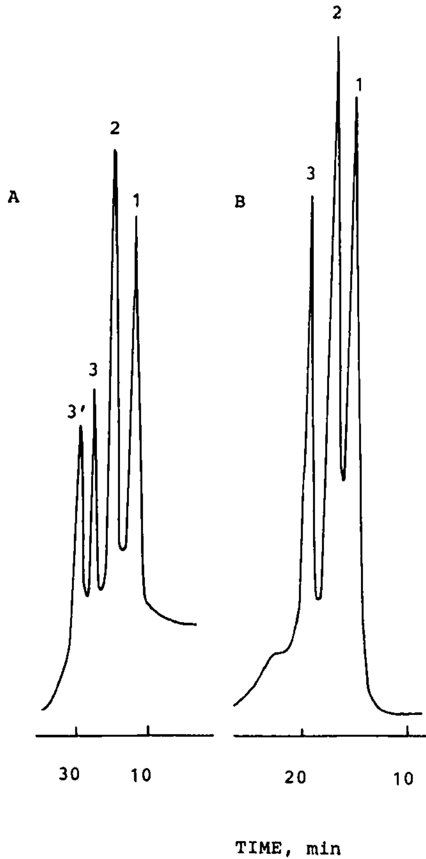

Figure 1. Separation of protein mixtures. Mobile phase gradient from 2 mol/l ammonium sulfate in 0.02 mol/l phosphate buffer pH 6.8 to buffer only. A - membrane 1 mm thick, elution rate 0.5 ml/min, pressure 0.1 MPa, gradient length 30 min, loading 0.1 mg of each protein; 1 - ribonuclease, 2 - ovalbumin, 3,3 chymotrypsin; B - capillary column 0.5 mm i.d. 140 mm long, elution rate 10 µl/m in, pressure 5 MPa, gradient length 20 min, loading 0.25 µg; 1 - ovalbumin, 2 - lysozym, 3 - chymotrypsin.

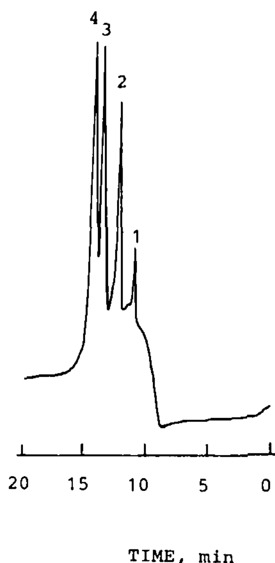

Figure 2. Separation of myoglobin (1), ovalbumin (2), lysozyme (3), and chymotrypsinogen (4) using G-50-C8 membrane. Conditions see Fig. 1, total loading 5 mg of proteins

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