Notes

Wedge-compressed membrane filters: their formation and application to small-scale filtration chromatography

Nitrocellulose membrane filters (UFS), either intact or impregnated with non-ionic detergents or proteins, were shown recently to be a convenient medium for rapid electrophoretic and chromatographic operations, deproteinization, and also for special determinations of very small amounts of proteins[1–8]. A regular microporous structure of the membranes[9] and the narrow distribution of pore sizes were important conditions for successful experiments with this material.

In the present investigation we wanted to probe the idea of forming a continuous pore-size gradient by pressing the membrane structure into a wedge between two plane blocks, under given conditions, and then fractionating molecules of different sizes by passing them from the larger pores towards the smaller ones (Fig. 1). It was assumed that during this sieving–chromatographic process the high-molecular weight substances would move more slowly than those having a lower molecular weight, the retardation being due mostly to the higher frequency of collisions with the membrane structure. The larger particles would be finally intercepted and immobilized in zones where the mean pore size begins to be smaller than the effective diffusion volumes of the given particles. The smallest particles could pass even through the line h_2 of maximum compression. Thus a separation of different particle size populations could possibly be achieved under simple experimental conditions and using very small amounts of material (*cf.* refs. 1–8). However, first of all it was necessary to find suitable materials and conditions for the realization of this idea.

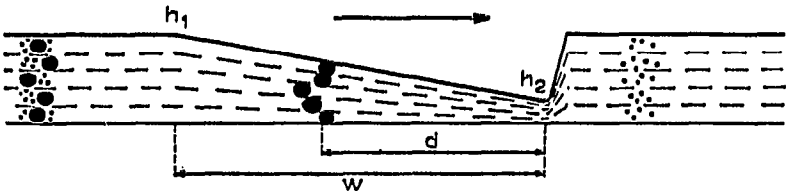

Fig. 1. Scheme of the separation of particles on a wedge-compressed membrane (longitudinal section). h_1 and h_2 = Lines of minimal and maximal compression of the membrane structure (*cf.* Fig. 3); d = part of the wedge unpenetrable for the greater particles; w = wedge length. Dashed lines indicate schematically the deformation of the pore sizes. Direction of flow indicated by the arrow.

The proposed microtechnique is in a certain sense the reverse of the principle of gel filtration on dextran gels[10,11] and differs also from methods using discontinuous pore-size gradients, *e.g.* stacked layers of gels of growing concentrations[12,13] or cascades of membranes having different permeability[14,15].

Nitrocellulose membranes VUFS* (produced by VCHZ Synthesia, Uhříněves,

* New name: Synpore 8, distributed by Chemapol, Prague.

Czechoslovakia), 0.05 mm thick, pore size 0.1–0.3 μm, in strips usually 3–4 cm long, were impregnated with 2 % Tween 60 as was described previously[1], to minimize the unwanted adsorption of particles[1,5,6]. The membranes were stored in the buffer to be used for chromatography (0.1 *M* TRIS–1 *M* NaCl, pH 7.9 adjusted by HCl).

The arrangement of the pressing device was relatively simple (Fig. 2) but sufficient to ensure reproducible conditions for the production of a standard series of wedge-compressed membranes. Best results were achieved when the blocks A and B, used to press the membranes, were made of hard steel: lathe knives "Poldi Radeco" 2.5 × 2.5 × 16 cm. (Softer steel was not suitable since it was easily and irreversibly deformed during wedge-pressing. Glass blocks even 2.0 cm thick were broken at the pressures necessary here.) The two operational sides of the steel blocks were first carefully plane-ground and then fine-polished to brightness on a mirror glass, using appropriate carborundum and sapphire polishing powders suspended in mineral oil. Special care must be paid to the pressing edge of the upper block A; no flaws discernable at 40 × magnification should be present. The edge must not be strictly sharp but somewhat curved (*cf.* Fig. 1), otherwise the membrane would be cut through during pressing.

The wet membrane with sufficient excess buffer was laid with its glossy side down on the block B (*cf.* Fig. 2), the pressing block A was placed above, supported at one side by two suitable steel supports (blades of 0.08 mm thickness were used). Then a steel or brass string of 3–4 mm diameter was laid on the upper block A at a given distance *a* from the edge and the whole system was compressed for 5–10 sec in a hydraulic press at laboratory temperature, using suitable controlled pressures in the region of 15–100 atm. The constancy of the parameters *P*, *a*, *b* and *c* was very important for the reproducibility of the experiments. The calculation of the angles and of the actual pressures acting on the membrane was not necessary here.

The membrane was then transferred with forceps from the press into the buffer and could be stored there for 1–2 days without any observable macroscopic change of the wedge-compressed area. This relative "irreversibility" of the deformation of the membrane for a fairly long time is very important and convenient from the practical aspect of the method. In most experiments, however, chromatography was usually begun within 1 h of pressing.

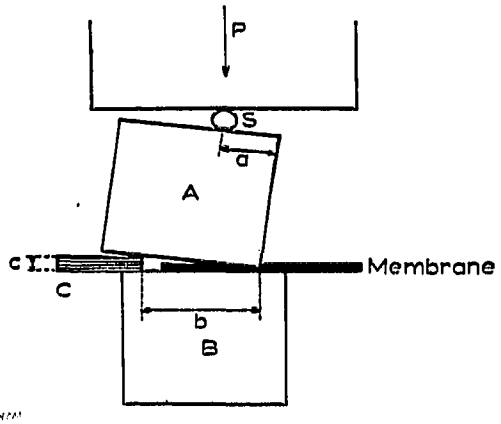

Fig. 2. Scheme of the pressing device. P = Head of the hydraulic press; S = steel string; A and B = polished pressing blocks of hard steel; C = flat steel supports; *a*, *b* and *c* = distances important for reproducible compression.

The pore size gradient was easy to observe, since the intact membrane was not transparent, whereas after compression a continuously increasing transparency could be seen along the membrane, reaching its maximum at the line h_2. When the membrane was dried slowly at laboratory temperature even the line h_1 (see Figs. 1 and 3) could be detected and marked very accurately, since the compressed area remained wet and dark for a longer time than the quickly drying uncompressed area.

Horizontal chromatography was performed as described before[6,7]. To ensure a more regular suction of the buffer through the membrane a junction consisting of a wet PUFS* membrane (pore size 4 μm) was placed between the paper wicks and the chromatographic strip. For the detection of proteins, the still wet membranes were dipped into solutions of Ponceau S or amido black 10B in 3–5 % trichloracetic acid; this method was most suitable[16].

It was also found convenient to use an ordinary indelible pencil to make the necessary marks (the position of the start, of line h_1 etc.) on the wet membrane, because this pigment adhered very firmly to nitrocellulose during the necessary operations.

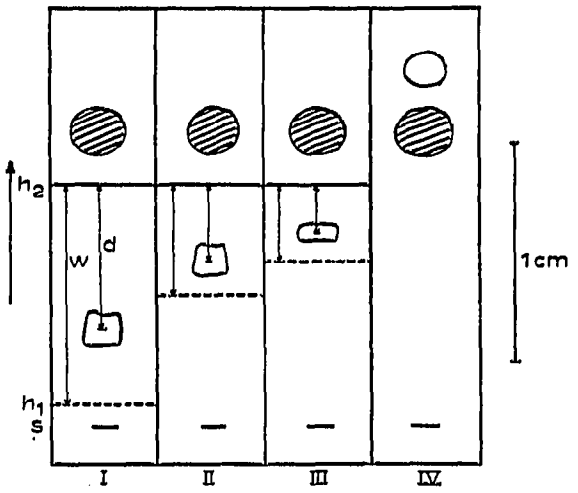

Fig. 3. Sieving-chromatography on wedge-compressed nitrocellulose membranes VUFS impregnated with Tween 60. Light spots: a high molecular protein fraction of modified serum; dark spots: phenolsulphophthalein. s = Start; h_1 = line of minimal compression (*cf.* Fig. 1); h_2 = line of maximal compression; w and d, *cf.* Fig. 1. Membranes No. I, II and III were compressed at a pressure of 25 atm, with a = 8 mm and b = 15 mm (*cf.* Fig. 2); c was 0.08 mm for No. I; 0.16 mm for No. II; 0.24 mm for No. III; membrane No. IV was not compressed. Buffer: 0.1 *M* TRIS–HCl + 1 *M* NaCl, pH 7.9; separation for 4.5 h; about 10^{-4} ml of 1 % samples were applied.

The separation of phenolsulphophthalein and the high-molecular protein fraction (mol. wt. of the order of 10^6), separated by gel filtration on pearl-condensed agar[17] from a blood volume expander "Resorba" made of modified bovine serum, was chosen as a model in order to investigate whether the proposed technique really operates. The results of several experiments are shown schematically in Fig. 3. The wedge lengths w corresponding to the given angles of compression were in very good agreement with the expected calculated values. This means that the eventual deformation of the steel blocks can be neglected here.

The ratios d/w or $1-d/w$ remained constant (within the experimental error) on

* Now Synpore 1.

all three membranes (I, II and III) and were independent of the distance between the start and the line h_1. It would seem that these ratios possibly have a similar meaning to R_F values in chromatography. It may be also concluded from our results that the particles did not escape the sieving process by streaming on the surface of the membrane. The sieving effect of the wedge-compressed membrane structure becomes most evident when comparing strips No. I, II and III with the uncompressed strip No. IV.

It may be summarized that the proposed microtechnique seems to offer the possibility of separating, in a relatively short time, very small amounts (10^{-4} to 10^{-5} ml samples) of substances differing in molecular size. The manipulation and detection is simple and the membranes store well for documentation. For further details on the method, its applications and limitations, see ref. 18.

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