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The use of PVC membrane filters as specific chromatographic carriers for rapid ultra-micro-analysis of proteins

Membrane chromatography and electrophoresis on cellulose nitrate[1-3] and acetate[4] membranes have proved to be very convenient techniques, especially for the simple and rapid analysis of very small amounts of biological material. In the course of further investigations on other types of membrane filters for similar purposes, we have recently studied polyvinylchloride (PVC) filters (Sartorius) which do not seem to have been used yet in this field. The results we achieved with these membranes indicated that the behaviour of the PVC carriers towards proteins is more like that of cellulose nitrate than of cellulose acetate. However, important differences between the behaviour of proteins on PVC and nitrocellulose membranes were observed, in particular the firmer binding of the proteins, which opens many new and promising experimental possibilities. Some special details of the pretreatment necessary when working with PVC membranes are briefly given below.

Materials and methods

PVC membrane filters (Sartorius, Göttingen, G.F.R.) SM 12801, 12804, 12806 and 12807, in strips 3–10 mm wide and 10–30 mm long, were used. They were first soaked in 40–50% aqueous ethanol, and then washed thoroughly with the developing solution, *e.g.*, water; 0.9% and 10% NaCl; 0.1 *M* acetate buffer, pH 3.7 and pH 6; 0.1 *M* phosphate buffer, pH 7.5; 0.1 *M* veronal acetate buffer, pH 8.6, 0.1 *M* borate buffer, pH 9.06. To prevent desiccation of the "slower" membranes (12804, 12806, 12807) during manipulation, 10% glycerine or ethyleneglycol was added to those solutions. 0.1–1 μl of human serum albumin, whole serum, dog haemoglobin, horse myoglobin, ovalbumin, trypsin, as 0.01–2% solutions, were placed on the wet membrane by means of a thin calibrated capillary[1] or a wick[5] of filter paper or cellulose acetate membrane (Millipore). In quantitative estimations it was convenient to let the drop of sample soak into the starting edge of the strip from the surface of the plexi-glass. Ascending one-dimensional development was used during chromatography. The starting edge of the strip was placed in contact, perpendicularly, with a filter paper wick wetted with buffer, while the other end was pressed gently between a dry filter paper wick and a glass slide in order to maintain a regular flow of buffer. In short runs (1–5 min) and especially on the "faster" SM 12801 strips no closed humid chamber was necessary. Indelible pen or crystal violet were suitable for marking the start, 5–10% solutions of either potassium bichromate or copper sulphate or chloride were used to visualise the rate of flow of the buffer Proteins were stained on the wet membranes with 0.5% Amido Black 10 B or 0.01% nigrosine in 5% trichloroacetic acid and the background destained with water[1-5].

Results

It was found that all the proteins tested were firmly adsorbed to the PVC membranes even in alkaline developing buffers and during chromatography formed a very homogeneous layer with very sharp contours. The area covered by the adsorbed protein under defined conditions was directly proportional to the absolute amount

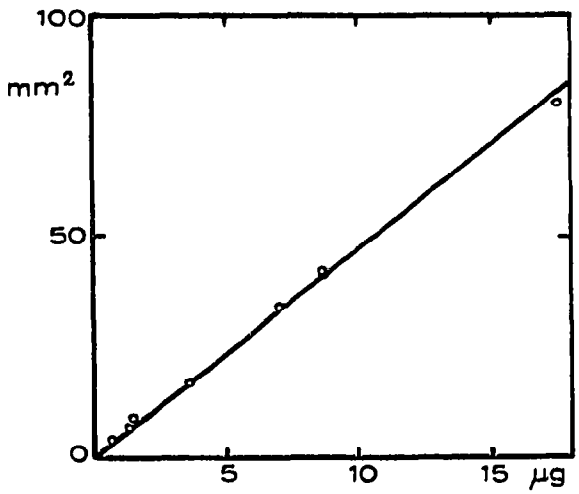

Fig. 1. Calibration curve of dog haemoglobin (fresh stroma-free haemolysate) PVC membranes Sartorius SM 12801 were used, the given amounts of haemoglobin applied in volumes 1 µl–3 µl, and chromatographed in 0.9% NaCl for 1–2 min

of the given protein (Fig. 1). This fact extends remarkably the range for the rapid quantitative estimation of proteins by membrane chromatography[1] to the region of neutral and alkaline pH values and to low molecular weight proteins (on nitrocellulose there was some restriction to acid pH values and higher molecular weights[3]).

The adsorption of the proteins could be prevented by previous impregnation of the membranes with either the same protein, or polyethyleneglycol (Polywachs 20,000, Hüls, G.F.R.), or neutral detergents such as 2% aqueous Tween 20 (Atlas, U.S.A.). The latter is able to substitute and thus elute adsorbed proteins from PVC strips similarly to what was observed on nitrocellulose[2,3]. Impregnated PVC membranes are then suitable as carriers for the microelectrophoresis of proteins under the usual conditions[2,3,5]. Certain changes in the adsorption of the proteins tested were observed in 0.1 *M* NaOH and 8 *M* urea; however, no marked changes were found in 0.1 *M* HCl and 10% NaCl.

Further details and results concerning the use of intact or impregnated PVC membranes in biochemical analysis and the binding mechanism of substances to PVC will be reported on later.

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