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Membrane chromatography of different molecular weight polyethylene glycols on polyvinylchloride ultrafilters

In a previous communication[1] we described the group separation of high- and low-molecular-weight polyethylene glycols (PEG) by membrane chromatography on cellulose nitrate. The behaviour of the PEG was generally similar to that of proteins and amino acids on the same carriers[2]. Quite recently, we have reported[3,4] that polyvinylchloride (PVC) membrane filters were suitable for use as chromatographic carriers and that they adsorbed proteins more firmly and over a much broader range of pH and molecular weights than nitrocellulose[5]. Furthermore, we noted that PEG 20 000 was also firmly adsorbed to PVC in a similar way.

In the present paper, we have investigated whether a quantitative microestimation of PEG could be achieved by membrane chromatography on PVC filters in a similar manner to that observed with proteins[3,4], and if so, whether there is any noticeable relationship between the molecular weights of different PEG fractions and the areas of the corresponding PEG layers. The last question was intended as a simple model for analoguous experiments with proteins.

Materials and methods

0.25% aqueous solutions of polyethylene glycols of average molecular weights 400, 600, 1500, 4000, 6000 and 20 000 (Hüls, Marl, G.F.R.) were used and applied to the starting edge of the chromatographic strips in volumes of 0.25–2 μl as described earlier[3,4]. Strips (3–4 mm × 10–20 mm) of PVC Sartorius membrane (Göttingen, G.F.R) SM 12801 were soaked with 40% ethanol, washed with distilled water, and were then used for one-dimensional ascending chromatography[3,4] with water as the developing agent. Chromatography lasted 1–3 min. The PEG was detected with Dragendorff reagent (see ref. 6). The stained areas were measured with a transparent millimeter scale and plotted against μg of PEG applied.

Results and discussion

Polyethylene glycols of molecular weight 1500, 4000, 6000 and 20 000 were adsorbed and spread as homogeneous and sharply outlined spots; however, the developed layer of PEG 1500 was markedly less stained with Dragendorff reagent. Samples of PEG 400 and 600 were not adsorbed and migrated freely with the front in one round, well defined spot in the same way as on nitrocellulose[1]. The calibration curves (Fig. 1) were linear within a 3% deviation about the mean but different for various PEG samples of different molecular weights; as was observed similarly with proteins on PVC membranes[3,4]. These results have shown that a simple and rapid microestimation of given fractions of PEG with molecular weights above 1500 can be achieved by membrane chromatography on PVC filters, and that a simple group separation of high- and low-molecular PEG fractions is possible on PVC as well as on nitrocellulose.

However, it was most interesting to note that the logarithms of the areas formed by equal quantities (in μg) of different PEG samples when plotted against

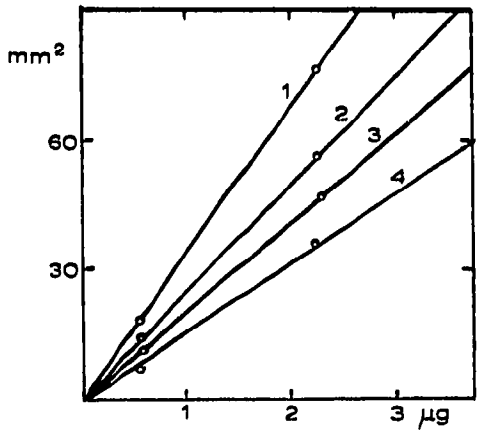

Fig. 1. Dependence of area on the amounts of different PEG samples. 1 = PEG 1500; 2 = PEG 4000; 3 = PEG 6000; 4 = PEG 20 000. Development in water on PVC membranes (Sartorius SM 12801) for 3 min.

the logarithms of the corresponding molecular weights, showed a nearly linear relationship (Fig. 2). This curve which obviously expresses an exponential function of a general formula $A = k/M^n$ can be used as a calibration curve for the estimation of the average molecular weights of PEG fractions in a given region. This fact can be interpreted by the formation of a very regular immobilised film of defined thickness for each PEG fraction on the surface of the microporous network of the particular PVC membrane filter. We have assumed that, in general, similar forces and mechanisms and principles of binding are probably involved here as was proposed for nitrocellulose membranes[6]. There also seems to be a certain distant analogy to the study of monomolecular films of proteins spread on the surfaces of liquid substrates[7].

We also attempted to construct an "ideal" calibration curve based on the following hypothetical and very simplified assumption: We considered that four groups of particles of a standard material having a standard density, are present as cubic particles of a relative volume (or molecular weight) ratio 20 000 n: 6000 n: 4000 n: 1500 n (*i.e.*, similar to the ratios of the PEG fractions tested) and are spread

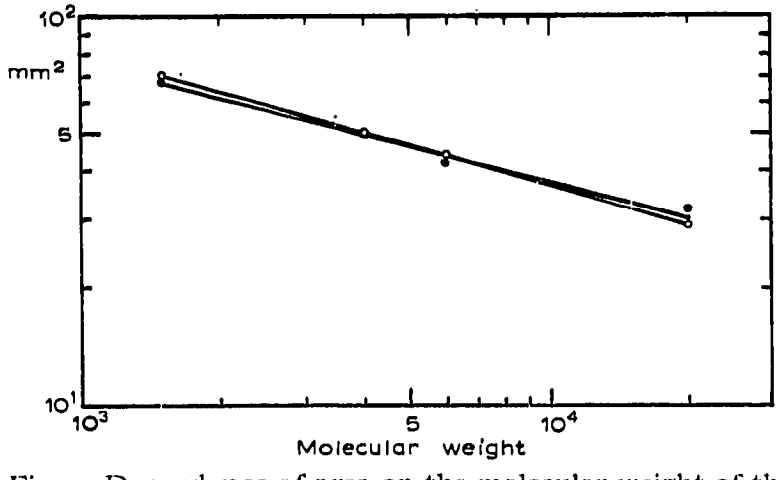

Fig. 2. Dependence of area on the molecular weight of the PEG. ● The calibration curve is valid for amounts of 2.0 µg PEG as derived from Fig. 1. ○ The hypothetical calibration curve (see text).

separately on a surface in monolayers with particles packed tightly side by side. The base areas x^2n of the cubes corresponding to the individual volumes x^3n were then calculated and related to one standard total volume, *e.g.* 20 000 n, in all cases. The ratio of the total areas covered by the hypothetical monolayers of an equal weight of standard particles differing only in their molecular size was 737 n: 1100 n: 1260 n: 1746 n, respectively. When n was taken as equal to 0.04 and the logarithms of the total areas were plotted *versus* the logarithms of the individual volumes a straight line was obtained (Fig. 2) which was very similar to that constructed from experimental data. This good analogy between hypothesis and experiment seems to verify the previous assumption and throws more light on the behaviour of the PEG fractions, and perhaps also of other substances, during membrane chromatography on PVC filters.

Further investigation dealing with a more detailed interpretation of our findings is in progress now. From preliminary results[8], it can be expected that the simple chromatographic principle used here for the experiments with PEG could also be applied to the determination of the molecular weights of proteins. Among the various techniques used for the estimation of the molecular weight of high-molecular substances, the proposed microchromatography on PVC membranes seems to be one of the most rapid, inexpensive and easy to perform.

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