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MEMBRANE CHROMATOGRAPHY OF DYES ON NITROCELLULOSE FILTERS

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SUMMARY

Various types of nitrocellulose membrane filters were tested as carrier materials for rapid small scale chromatography on strips 3–4 cm long. Model mixtures of 7–8 synthetic dyes were fractionated into their individual components in single chromatographic runs by using the developing systems: ethanol–chloroform (1:1); ethanol–chloroform–acetic acid (20:20:1); or ethanol–ammonia (10:1). The best results were achieved on membranes having mean pore sizes round 0.2–0.4 μm and especially on "Synpor 8 and 9" and "Millipore HA" membranes. Some basic general characteristics of membrane chromatography and its differences from PC and TLC are pointed out and discussed.

Nitrocellulose membrane filters were recently shown to be a convenient material for small scale chromatography and electrophoresis of proteins, as well as for some other substances (see ref. 1). The membranes were used either intact or impregnated with detergents or proteins and separations were done solely in aqueous developing solutions. Under those conditions it was possible to achieve some specific group separations of different types of substances during chromatography[2,3].

The present experiments were undertaken to investigate the applicability of nitrocellulose membranes to the chromatographic resolution of complex mixtures of low molecular weight substances into their individual constituents and also by using appropriate organic solvents. Mixtures of some randomly chosen dyes served as a model system. In addition, we wanted to obtain information as to whether "membrane chromatography" in general (using thin "selfcarrying" standard sheets of chemically homogeneous material with a fine foam-like microstructure and a standardized narrow distribution of pore sizes[4]) could be an alternative to paper chromatography (PC) and thin layer chromatography (TLC[5,6]) (which needs a mechanical support, spreading apparatus and often adhesives for the powdered adsorbents).

At the same time, the experiments served to test nitrocellulose as an adsorbent or carrier, the latter use has not yet drawn wider attention in chromatography.

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EXPERIMENTAL

Material and methods

Nitrocellulose membranes Synpor (Chemapol, Prague) type 1-10, Sartorius (Membranfiltergesellschaft, Göttingen) type 11001-11011 and Millipore (Millipore s.a., Paris) type VM, VC, HA and RA were used in strips, usually 0.5–1 cm wide and 3–4 cm long. Strips of the same size of acetylcellulose, Oxoid (Oxoid, London), Celotate (Millipore, s.a., Paris), Whatman No. 1 filter paper and thin-layer sheets of silica gel "Siligram" (Kavalier, Votice, ČSSR) and Eastman-Kodak "6060" (London), served as reference adsorbents for a rough comparison of the chromatography of the dyes in the given solvents.

Standard mixtures of 0.1–0.5 % ethanolic solutions of the following dyes were most convenient for our purposes: eosin, orange G, *p*-aminoazobenzene, methyl red, fuchsin, sudan yellow, rhodamine B, methylene blue; with solvents containing ammonia, fuchsin was omitted.

Three solvent systems: 96 % ethanol–chloroform (1:1), 96 % ethanol–chloroform–acetic acid (20:20:1) and 96 % ethanol–25 % ammonia (10:1) were found to be most effective, among a series of other developing systems tested, in separating the given mixture of dyes.

Ascending chromatography was done in 50–100 ml Erlenmayer flasks. The strips were hung on a wire, passing tightly through the cork stopper, and when necessary they could be equilibrated in the vapours of the solvent. The membranes were then immersed in the developing solution placed on the bottom of the flask, by carefully lowering the wire. Samples (volumes of the order of 0.1 μl) were applied by means of a thin capillary or a wick of a thin, hard filter paper[7]. The width of the start streak did not exceed 0.2 mm. The start was usually 0.5 cm above the surface of the solvent. Distances of 1.0 and 1.5 cm led to practically the same results. After chromatography, the strips were laid on a filter paper and their longer sides were covered (about 1 mm from each side) by glass slides to prevent slight deformation in the membranes, which occurs when the strips are dried freely in air. It was often advantageous to treat the dry membranes after chromatography with paraffin oil to make them transparent.

RESULTS AND DISCUSSION

Nitrocellulose membranes proved to be a very good medium for microchromatography of the dyes examined in all three developing systems. Fig. 1 illustrates the formation of distinct spots of the individual dyes in a single run within a very short distance and a short period of time. In some cases, circular or "semicircular[5]" development led to the formation of sharper zones especially in the vicinity of the start. Two-dimensional microchromatography (Fig. 2) in ethanol–chloroform–acetic acid (20:20:1) in the first direction, followed by ethanol–ammonia (10:1) in the second direction, permitted a still better separation of the individual spots.

Equilibration of the strips in the vapours of the solvent for 1–60 min had no practical effect when the system ethanol–ammonia was used. In the other two systems, containing chloroform, a somewhat sharper formation of the spots near to the start was observed after an equilibration for at least 15 minutes. A very sharp

resolution of the dyes was also achieved on membranes which had been impregnated either with Tween 20, Tween 60 or bovine serum prior to chromatography (*cf.* ref. 2). However, the positions and R_F values of some substances were changed (*e.g.* fuchsin and orange G interchanged their positions in the solvent ethanol–chloroform–acetic acid on strips impregnated with Tween 20). The same changed sequence of the two dyes was observed on intact Millipore membranes but not on the Sartorius ones.

Comparison of the results achieved on different membranes showed that the velocity of the advancing solvent front was generally greater on membranes having greater mean pore size, but the separation of the spots was worse.

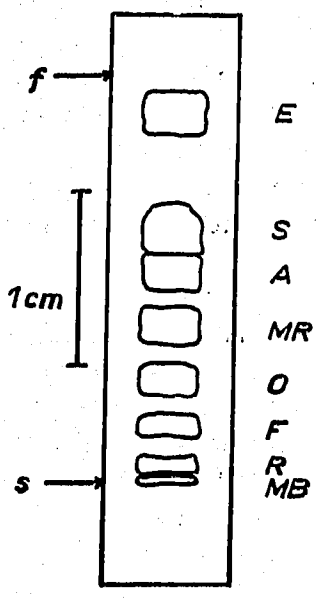

Fig. 1. Microchromatography of dyes on "Synpor 9" membranes. Solvent system: 96% ethanol–chloroform–acetic acid (20:20:1). Time 15 min. Start streak 0.2 mm wide. E = Eosin; O = orange G; A = *p*-aminoazobenzene; MR = methyl red; F = fuchsin; S = sudan yellow; R = rhodamine B; MB = methylene blue; s = start; f = front.

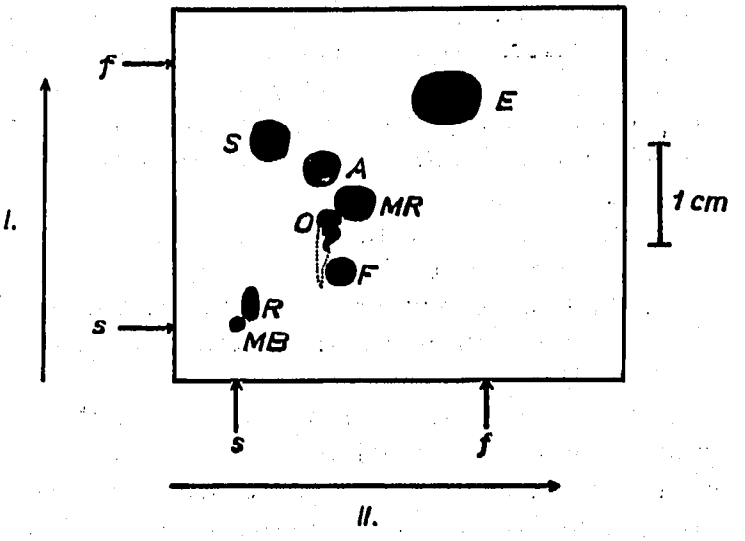

Fig. 2. Two-dimensional chromatography of dyes on "Synpor 8". Direction I: 96% ethanol–chloroform–acetic acid (20:20:1); direction II: 96% ethanol–25% ammonia (10:1). Running time 15 min in both directions, start spot diameter 0.6 mm. For nomenclature of dyes see Fig. 1.

An arbitrary classification of the quality of the chromatographic separation was made using five degrees of assessment; 1 = "very good" (well formed spots, no tailing); 2 = "good" (prolonged spots, slight tailing); 3 = "medium" (still longer spots, longer tailing); 4 = "poor" (partial resolution but no formation of individual spots, enormous tailing); 5 = "no resolution" (various difficulties, irregular development).

The results summarized in Fig. 3 indicate that "Synpor" membranes having mean pore sizes in the range of 0.2–0.4 μm were most suitable for chromatography of the given dyes under given conditions. A similar finding was made for "Millipore" membranes, whereas on "Sartorius" filters the quality of separation seemed to be less dependent on the mean pore sizes. Very good results were achieved with "Synpor 9" in all three solvent systems, together with "Millipore HA" in ethanol–chloroform–acetic acid (20:20:1) and "Sartorius 11002" in ethanol–ammonia (10:1). Comparative runs made in the same solvent systems on paper, the acetyl cellulose membranes

Celotate and Oxoid were generally unsuccessful. Thin layers of silica gel permitted a "medium" separation in the system ethanol–chloroform but the length of the strips was not sufficient to make a better separation possible. This does not mean, of course, that a suitable solvent system could not be found for this carrier medium. However, it was not the aim of this work to investigate in detail the chromatography of dyes on other carriers.

It seems to us that the present results with the dyes as well as former results obtained with proteins and other substances allow some generalizations concerning chromatography on membranes to be made (although the experiments up till now have mostly been done on nitrocellulose filters, and the possibility of separating various other types of substances on this material is still to be investigated). There seem to be several advantages in using membrane filters especially for rapid microscale chromatography. There are also some substantial differences between this type of chromatography, paper chromatography and thin-layer chromatography, which led us to propose the term "membrane chromatography". The following characteristics and practical advantages should be pointed out, to complete the facts already mentioned in the introduction:

Membrane filters have a very uniform microporous structure, the whole material of the strip is chemically homogeneous and its active surface is continuous. This is different from the rough fibrous structure of filter paper used in PC and also from the discontinuous physical and mechanical structure of thin-layers of powdered adsorbents used in TLC. The fine structure of the membranes enables the application of minute samples in very sharp start lines, a fact which has a great positive influence on the quality of the separation (*cf.* Figs. 1 and 2). No mechanical support is necessary as in TLC[6]. Cutting of strips to wanted sizes and handling during all the necessary operations is very convenient, as is their preservation for documentation. The strips can easily be made transparent, *e.g.* with paraffin oil. Membranes of various chemical

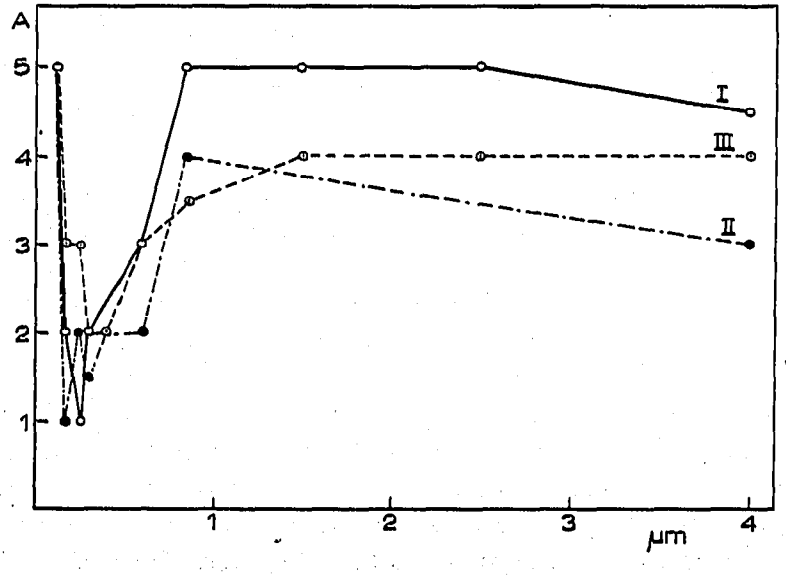

Fig. 3. Quality of the separation depending on mean pore sizes of the Synpor membranes. A = Arbitrary degrees for classifying the quality of separation: (1) very good, (2) good, (3) medium, (4) poor, (5) no separation (*cf.* text); μm = mean pore size. Solvent systems: (I) ethanol–chloroform (1:1); (II) ethanol–chloroform–acetic acid (20:20:1); (III) ethanol–ammonia (10:1).

nature are produced commercially and are available in standard qualities as filtration sheets with given porosity and pore sizes. According to the present experiments no special activation seems to be necessary nor was there a basic need for a long equilibration in the atmosphere of the chamber.

However, the following limitations of nitrocellulose membranes should also be mentioned. Some organic solvents *e.g.* acetone, pyridine, methanol, ethyl acetate, glacial acetic acid, which dissolve nitrocellulose[4] , cannot, of course, be used as developing systems or as solvents for the detecting agents. (However, they are suitable for dissolving those parts of the strips containing the wanted isolated fraction.) Concentrated sulphuric acid, a general detection agent in TLC, also attacks nitrocellulose. Developing systems containing a high proportion of lower alcohols may, after chromatography, cause a slight deformation ("curling") of the membranes during drying in air.

It may be concluded that membrane chromatography seems to offer the possibility of rapid microscale separations of various substances not only in aqueous solutions but also in some organic developing systems in a similar way as in PC and TLC.

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