Chromatography of streptothricins on carboxymethylcellulose

The streptothricins, a commonly occurring group of antibiotics, have been the subject of repeated attention on the part of investigators, owing to their high biological activity[1]. However, their isolation as individual compounds from their mixtures is as yet a rather complicated task. Although it has been shown in a number of studies that streptothricins can be fractionated by partition chromatography on cellulose[2–5], this method did not find extensive use as it is cumbersome and very inefficient.

In the course of studies of polymycin, a new antibiotic belonging to this group, we developed a convenient procedure for its separation based on ion exchange chromatography on carboxymethylcellulose (CMC). Later this was successfully applied to other streptothricin mixtures.

Fig. 1 gives the results of the analytical fractionation of five streptothricin antibiotics containing different numbers of components. To compare the data of several runs we recalculated the results as shown on the right-hand side of Fig. 1. This illustrates the close relation between the components of the various preparations.

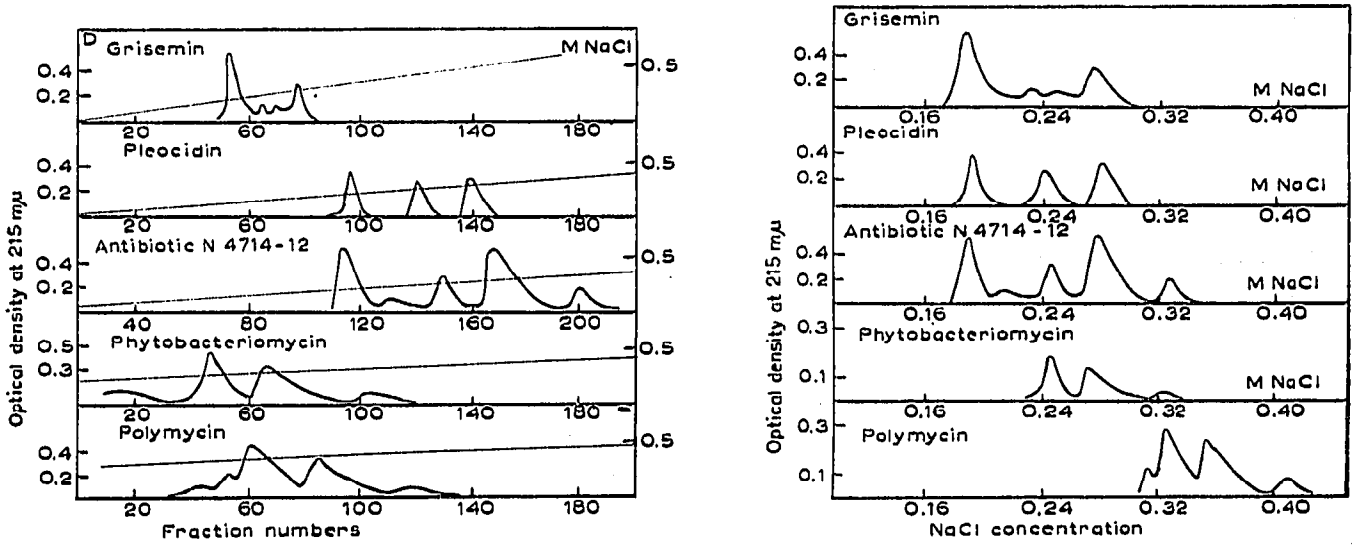

Fig. 1. Chromatography of five streptothricins on a CMC column. Column: 0.9 × 40 cm. Adsorbent: Na form of CMC, capacity 0.55 mequiv./g. Amount of substance: 20–35 mg hydrochloride in 0.5 ml H_2O. Rate: 15–20 ml/h. Fraction volume: 5.9 ml (grisemin); 4.8 ml (pleocidin); 5 ml (phytobacteriomycin); 4.6 ml (antibiotic No. 4714-12); 5.2 ml (polymycin). Eluent: NaCl solution with linear concentration gradient.

To show the reliability of the method a number of components were isolated from eluates with the aid of Amberlite IRC-50 in the Na form and were compared with the initial antibiotic preparations by paper chromatography (see Fig. 2). The right-hand side of Fig. 2 shows a comparison of the chromatographic behaviour of polymycin and antibiotic No. 4714-12, applied as a mixture, and of their constituents. It was found that the initial antibiotic preparations contain components with different R_F values. These values were found to cover mobilities of components of all chromatographically analysed streptothricins. Hence the polymycin + antibiotic No. 4714-12 mixture provides a convenient reference for the chromatographic analysis of new streptothricin preparations.

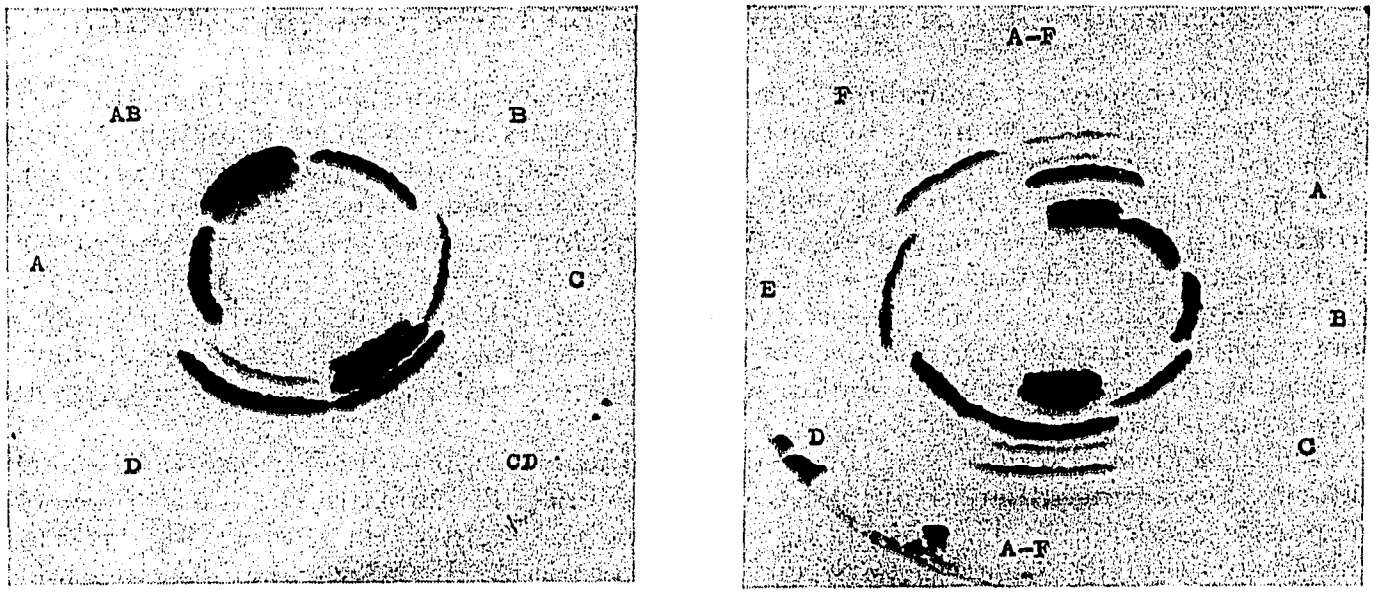

Fig. 2. Radial chromatograms of the isolated components and the initial streptothricins. Solvent: *n*-butanol–pyridine–acetic acid–water (15:10:3:12). Development: twice developed with the same solvent. Reagent: 0.25% ninhydrin in ethanol. Left-hand side: AB = initial polymycin; A, B = components of polymycin; CD = initial phytobacteriomycin; C, D = components of phytobacteriomycin. Right-hand side: A–F = mixture of polymycin and antibiotic No. 4714-12 applied as a single spot; A, B = components of polymycin; C, D, E, F = components of antibiotic No. 4714-12.

From the above results it can be seen that the proposed method has considerable advantages over partition chromatography on cellulose and, we believe, may find ready use for analytical and preparative purposes.

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Received November 11th, 1963

Preparative separation of vitamins K_1 and K_3 from vitamins $K_{2(30)}$ and $K_{2(35)}$ by column chromatography

The earlier chromatographic methods for the isolation of vitamin K compounds entailed the use of Permutit[1], silica gel[2], Decalso[3], $MgSO_4$ and $ZnCO_3$[4]. The K vitamins are destroyed during chromatography on some adsorbents, such as Decalso or alumina[5] and as a consequence, prolonged chromatography must be avoided. Differ-