A Note on "Carrier Displacement" Chromatography

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If a mixture of dissolved substances is displaced through an adsorption column by a substance with an adsorption affinity higher than for any of the substances to be separated, a "procession" of adjoining zones of characteristic concentrations is set up, each zone representing a pure component. The width of each zone measured as the volume it occupies in the effluent is proportional to the amount of substance in that zone. This procedure, which was first described by one of us [1], has since been applied to a number of separations for analytical and preparative purposes with amino acids, peptides, fatty acids, carbohydrates etc. Its chief advantage depends upon the possibility of avoiding the effects of »tailing» which makes chromatographic separations based upon adsorption extremely difficult for substances with curved isotherms, which normally characterize strong adsorption. For the same reason the method has also been used in separations on ionic exchange columns [2, 3] and in partition chromatography on buffered columns of high capacity, where the distribution isotherm is no longer linear [4]. The fact that the zones do not spread out after the stationary concentration has been reached, makes it possible to use very large columns and thus to effect difficult separations. A detailed theory shows that the plate efficiency of the method is very high [4]. One disadvantage of the method, however, has been that the zones are in close contact, and thus there is some difficulty in cutting the fractions properly in the effluent, particularly for narrow zones (small quantities). It has been suggested by one of the authors [5] that this difficulty might be overcome by interposing between the zones to be separated a number of substances of intermediate adsorption affinities, which would form part of the "procession" and afterwards could be removed by evaporation or extraction. It was also mentioned by Synge and Tiselius [6] that some phenomena observed in the successive elution of adsorption columns might be interpreted as resulting from this sort of displacement, and the analogy to the separation of closely related substances by distillation with "carriers" was pointed out.

We have recently applied such a procedure to separations of amino acids and peptides with results which seem very promising. As a "carrier system" we have used a mixture of homologous alcohols, all in aqueous solution. Thus in one experiment 0.2 mg methionine + 0.2 mg of leucyl-glycyl-glycine + 0.2 mg of phenylalanine were dissolved in a mixture of 8 ml 1 % isoamyl alcohol (boil. point 128°—132°) and 2.5 ml 1 % *n*-butyl alcohol, both in 0.1 *N* HCl. The mixture was displaced with a solution of 1 % *n*-amylalcohol in 0.1 *N* HCl through a column containing 7.8 ml of a mixture of equal parts of active charcoal (Carboraffin Supra) and Super-Cel. The resulting diagram, as obtained by successive interferometric observations in the effluent, shows three sharp boundaries at 33.9 ml, 49.2 ml and 68.0 ml separating the three alcohols. Subsequent investigation by paper chromatography showed that all methionine had collected at the first boundary, all leucyl-glycyl-glycine at the second and all the phenylalanine at the third and that there were zones of 12 ml resp. 14 ml of medium devoid of amino acids or peptides in between.

In displacement chromatography the substances appear in the order of their adsorption affinity, and thus this order should not depend on amounts or concentrations as long as the adsorption isotherms do not intersect. Consequently a given substance will always tend to collect

against one definite boundary, namely the one which separates zones of carrier substances of slightly higher and slightly lower affinity. As an illustration the following cases studied so far, using homogloous alcohols as carriers, may be quoted.

Valine: *tert.* butanol/water
Leucine: *sec.* butanol/*tert.* butanol
Methionine: *sec.* butanol/*tert.* butanol
Leucyl-glycyl-glycine: *iso*amyl/*n*-butylalcohol
Phenylalanine: *n*-amyl/*iso*-amylalcohol
Glycyl-tryptophan: benzylalcohol/*n*-amylalcohol

The procedure, for which we suggest the name »carrier displacement chromatography» has several advantages. The zones are sharp and of appreciable concentration, and the degree of separation can be predicted from the known behaviour of the carrier system. The spacing between the zones can be varied at will, by chosing suitable amounts of carriers and varying their proportions, provided that the adsorption column is large enough to give complete separation in the carrier system. Obviously it should be possible to apply the same principle to ionic exchange and displacement partition columns.

This type of separation can easily be demonstrated by a very simple arrangement. If the outlet of a small column (for example about 1 ml of a mixture of charcoal and Super-Cel 1 : 5) is allowed to touch the centre of a large circular filter paper between two glass plates, and an experiment similar to that described above is carried out (with correspondingly smaller volume), the filter paper will take up the effluent. After drying and spraying with ninhydrin, it will show a number of concentric rings corresponding to the amino acids and peptides present at the alcohol boundaries.

The work is continued with particular attention to peptide separations. A more detailed report will appear elsewhere.

The Use of Ethyl α-Thienylcyanoacetate in the Synthesis of Substituted α-Thienylacetic Acids

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The preparation of substituted thienylacetic acids has been a rather cumbersome problem. This can be illustrated by the synthesis of benzylthienylacetic acid, which has been obtained by the following steps [1]: Ethyl α-thienylglyoxylate was prepared from thiophene and ethyl oxalyl chloride. The free acid was prepared and converted to benzylthienylhydroxyacetic acid by means of benzylmagnesium chloride. This acid was reduced with stannous chloride yielding benzylthienylacetic acid.

The same acid has now been prepared from ethyl α-thienylcyanoacetate by benzylation with the aid of anhydrous potassium carbonate and benzyl chloride [2]. The ethyl benzylthienylcyanoacetate was then converted to benzylthienylacetic acid. The method is very useful for preparing other substituted thienylacetic acids, especially if the corresponding halogen compound cannot be converted to the Grignard compound.

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1. Tiselius, A. *Arkiv Kemi* **A 16** (1943) no. 18.
2. Partridge, S. M., and Westall, R. G. *Biochem. J.* **44** (1949) 418.
Partridge, S. M., and Brimley, R. C. *Biochem. J.* **44** (1949) 513.
Partridge, S. M. *Biochem. J.* **44** (1949) 521.
3. Spedding, F. H. *Faraday Society Discussion on Chromatography 1949.* In the press (See particularly Figs. 13—15).
4. Martin, A. J. P. *Biochemical Society Symposia.* No. 3, p. 4 (Cambridge University Press 1949).
5. Tiselius, A. *Kolloid-Z.* **105** (1943) 101.
6. Synge, R. L. M., and Tiselius, A. *Acta Chem. Scand.* **3** (1949) 231.

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