The Nature of Inorganic Chromatography on Cellulose Columns.

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A careful study of the movement of zones of ferric ions under different eluting conditions on cellulose columns suggests that the process is one of simple partition between two liquid phases.

INORGANIC chromatography on cellulose is often suggested to be a form of partition chromatography in which the cellulose acts as a support for the stationary aqueous phase while the organic phase flows through the column. No evidence has been produced to support this view, and the complexity of many chromatographic procedures has obscured the nature of the underlying process. An analysis of the latter can be made in two ways. First it might be possible to show that the movement of zones of inorganic ions on the columns in simple elution chromatograms obeys the theoretical relation deduced by Martin and Synge (*Biochem. J.*, 1941, **35**, 1358) between this movement and the bulk distribution coefficient of the individual ions between the two liquid phases. Secondly a general comparison could be made between the separations possible on cellulose columns and those obtained in bulk distribution experiments. The latter comparison is difficult, for whereas simple two-component two-phase systems, in which the phases are readily separable, are prefered in bulk extractions, phase separation is no problem in chromatograms and complex three-component two-phase systems are more often used (Wells, *Quart. Reviews*, 1953, **7**, 307). The first approach was therefore considered more profitable.

The theoretical relationship deduced by Martin and Synge (*loc. cit.*) is that the distribution coefficient is given by

$$D = \frac{[\text{M}] \text{ in mobile phase}}{[\text{M}] \text{ in non-mobile phase}} = \frac{V_a}{V_o} \frac{1}{(1/R_\text{F} - 1)} \quad . \quad . \quad . \quad . \quad (1)$$

where V_a is the volume of the stationary aqueous phase, V_o that of the mobile organic phase, and R_F is the ratio of the rate of movement of the concentration maximum of the zone of the substance M under study to that of the mobile phase, or

$$R_\text{F} = V/V_o \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

where V is the volume of the mobile phase which has flowed from the column when the peak of the zone reaches the end of the column, and V_o is often called the "dead" volume of mobile phase in the column. Relation (1) can be tested in two ways. The values of D and R_F can be compared for a number of cations, one solvent system being used, or for one cation, changes being made in the solvent system. We used the latter method taking ferric ion as our test case. As we had previously determined the distribution of ferric ions between solvents containing aqueous hydrochloric acid, methanol, and ethyl ether (to be published), we investigated the behaviour of ferric ions on cellulose columns in the presence of these solvent systems.

Equation (1) holds only if the partition isotherm is linear and if the process on the column is one of pure partition between two liquid phases. We examined both conditions.

The Effect of Iron Concentration on the Partition.—The first condition can be tested in three ways: the bulk distribution should obey Nernst's law, the elution peak from the column should be symmetrical, and the R_F value should be independent of the column length. In our solvent systems (see Table), Nernst's law was obeyed at iron concentrations less than 10^{-3}M but above this very considerable deviations appear. The elution peaks obtained in the column experiments were not exactly symmetrical despite the fact that we worked at concentrations lower than 10^{-3}M but the asymmetry was small. At higher concentrations tailing peaks occurred and in such concentrated solutions as are commonly employed in inorganic chromatography, elution analysis cannot be efficient because the partition isotherms are curved. The success of inorganic chromatography on cellulose is due to large differences between the partition coefficients of the individual metals and to the accidental application of gradient elution analysis (p. 1720). The R_F did not vary with column length in our experiments.

The Part played by Adsorption.—If adsorption affects chromatography on cellulose the cations must be adsorbed on to the cellulose from the stationary phase. Thus, ferric ions should not travel down a column at the solvent front ($R_F = 1{\cdot}0$) when an *adsorption* chromatogram is run with cellulose as the adsorbent and with the stationary phase from a partition chromatogram as the mobile eluting solvent. We have carried out a number of such experiments using different aqueous phases as eluting solvents. When the acid concentration was sufficiently high to prevent formation of ferric hydroxide iron was eluted very close to the solvent front and we conclude that negligible adsorption occurs.

Equilibrium of Phases on the Column.—Before R_F values could be measured it had to be shown that the solvents on the column were in equilibrium with one another. This difficulty is not often met in chromatography but we showed that it is necessary to run large volumes of solvent through a cellulose column before the mobile and the stationary phase are in equilibrium. We packed our columns in the stationary phase which had been equilibrated with the solvent to be used as the mobile phase, but even then equilibrium was not established until large volumes of solvent had passed. In several cases we followed the acidity of the effluent and found that when our three-component solvents (Table) were used, equilibrium was reached after some 50 ml. of solvent had passed. However, if a column 20 cm. $\times$ 1 cm. is packed in the aqueous layer formed in equilibrium with pure ether at an initial aqueous acidity (HCl) of 0—5M and the ether layer is then used as the mobile phase, the acidity of the effluent changes continuously until more than a litre of solvent has passed through the column. A gradient of acidity and a gradient of water content of both phases are established along the column; such gradients greatly adjust the nature of the processes taking place (Williams, *Analyst*, 1952, **77**, 905).

The uptake of acid by the stationary phase on a cellulose column and the changes in water content of the two phases from the equilibrium value established in bulk are such that the nature of the stationary phase is unknown when a chromatogram is run. We have therefore limited our examination of the movement of ions on such partition columns to that found in solvent systems in which we had previously shown the partition to be dependent upon the acidity of the organic layer and to be extremely insensitive to changes in the aqueous layer. Over considerable ranges of acidity there is a simple relation between the acidity of the organic layer and the distribution of the ferric ion which allows the latter to be calculated from the former (to be published). It cannot be assumed that

any similar simple relationship will hold for other solvent systems or for other cation distributions and it was for this reason that we restricted attention to the ferric ion.

The Volume of the Phases on the Column.—In order to determine R_F from equation (2) V_o must be found. The simplest method uses a frontal analysis with a coloured non-partitioning dye in the mobile phase. The break-through volume of the dye front is then the dead volume of the mobile phase. Using azobenzene we found the dead volume of our columns to be 0·3 ml./ml. of column volume.

The volume of the stationary phase, V_a, was obtained by measuring the amount of liquid taken up by the cellulose. The experiment was carried out with several of the solvents which we used as stationary solvents; V_a was 0·25 ± 0·05 ml./ml.

Rate of Flow of Solvent.—If the rate of flow of the solvent is below 5 ml./hr. there is no difficulty in obtaining R_F values independent of the rate of flow. At higher rates larger R_F values are obtained and equilibrium is not established on the column. Apparently most separations on cellulose columns described in the literature are not run near to equilibrium.

To keep the column in equilibrium, the substance to be chromatographed must be put on in the mobile phase. We applied the iron as 0·2 ml. of solution of ferric chloride in the organic layer. Few inorganic ions can be applied in this way so that the column equilibrium is usually destroyed on introducing the cation solution.

The R_F Values.—After the iron had been introduced elution with the organic phase was begun and the effluent collected on a fraction collector designed for us by Mr. C. A. Baker. The fractions were analysed for iron by the thiocyanate method. The R_F values determined at different values of D are given in the Table. The plot of $1/(1/R_F - 1)$ against D is a straight line and the slope of the line V_a/V_o is 0·75 which corresponds closely with the value determined directly (see above). We therefore consider that chromatography of ferric ions, at least, is a pure partition process.

Survey of Chromatography of Other Cations on Cellulose.—We have shown above that no direct correspondence can be expected between inorganic chomatography on cellulose columns, as generally performed, and bulk partition experiments. However, the following points are noteworthy.

(1) In three-component solvent systems D may well pass through a maximum and then a minimum with changing acidity. R_F will follow the same pattern, as we have shown for the ferric ion, but these changes will occur at different acidities for each cation.

(2) A rough guide to the order of R_F of different cations is given by the order of ease of formation of the complex extracted. This guide will be of value only at low acid concentration in the solvents, as it is entirely upset by the formation of anionic complexes in the aqueous phase.

(3) The extracting powers of solvents parallel their effect on R_F values. Among solvents which are all members of the same homologous series, the lowest members extract most efficiently and the R_F value of a given cation in these solvents also increases in this order (Burstall, Davies, Linstead, and Wells, *J.*, 1950, 516; Lederer, *Analyt. Chim. Acta*, 1951, **5**, 185).

(4) Our experiments with ferric ions on cellulose led us to suppose that many separations on such partition columns depended fortuitously on a gradient of the solvent along the column. Therefore we have made a number of experiments using a deliberately imposed

R_F values for the migration of ferric ions on cellulose columns under different conditions.

[The solvents in the different experiments were made by the addition of 5 ml. of aqueous acid of initial normality, N_a^I, to methanol (7·5 ml.) and ethyl ether (25 ml.). The lighter phase was used as the mobile phase and the heavier phase was equilibrated with the adsorbent.]

N_a^I	0·05	0·10	0·19	0·32	0·39	0·46
D_{Fe}	0·015	0·03	0·07	0·16	0·23	0·33
R_F	0·04	0·08	0·10	0·18	0·24	0·29
$1/(1/R_F - 1)$	0·04	0·08	0·11	0·21	0·31	0·40

solvent gradient in the elution of cations from cellulose. The gradient is controlled by an external mixing vessel as described by Alm, Williams, and Tiselius (*Acta Chem. Scand.*,

1952, **6**, 826). The method permits the separation of cations which differ greatly in partition coefficient in one operation, *e.g.* iron, cobalt, nickel, and copper can be eluted as chlorides, whereas many of these mixtures require frequent changes of solvent with stepwise elution (Burstall *et al.*, *loc. cit.*).

As the procedure we recommend is readily adapted to many separative problems in inorganic chromatography we will defer further description until a later publication. Since this work was finished Lederer (*Nature*, 1953, **172**, 635) has described the application of the same technique in paper-sheet chromatography, showing that it has considerable advantages over the usual procedures.

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