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Frontal chromatographic techniques in preparative chromatography

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ABSTRACT

This paper describes the use of frontal chromatography as a preparative method and its application to large-scale purifications, demonstrating that it can provide a useful alternative to the more familiar elution and displacement modes of operation of a chromatographic column.

INTRODUCTION

Although the operation of a chromatographic column in the frontal mode was first described by Tiselius [1] in 1943, the method does not appear to have been developed as a preparative technique. Tiselius [1] defined three distinct modes in which a chromatographic column may be operated, namely elution, displacement and frontal. Whilst elution chromatography has found widespread use in preparative chromatography and displacement chromatography has, more recently, been revived as a preparative technique by Horváth *et al.* [2] and by Verzele *et al.* [3], frontal chromatography appears to have been largely ignored. Guiochon and Katti [4] suggested that this may be attributable to the method not appearing well suited to the preparation of pure compounds. However, although the method is not universally applicable, in those cases where it can be applied, it provides a cost-effective alternative to either elution or displacement.

THEORY

Consider a chromatographic system in which the combination of stationary and mobile phases is such that the capacity factor, k', for a particular analyte is infinite. If a solution of the analyte in mobile phase is continuously loaded onto the column the solute will be adsorbed, occupying all adsorption sites sequentially and forming a narrow band which broadens until, when all the adsorption sites on the column are occupied, it begins to elute in a concentration which rapidly increases until it is equal to the loading concentration. The resulting chromatogram will appear as in Fig. 1.

Now consider a three-component mixture, A, B and C, where k' for each

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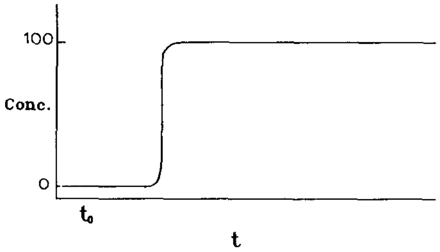
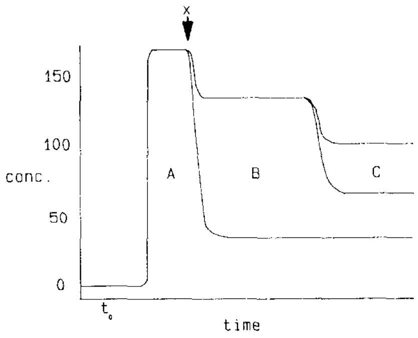


Fig. 1. Frontal chromatogram, single solute. Concentration (Conc.) is expressed as a percentage of the loading concentration. t = Time; t_0 = dead time.

component is infinite, but where the adsorption isotherms of the components are different so that their affinity for the stationary phase is C > B > A. In the ideal case, where the difference between the adsorption isotherms is large, if a solution of the mixture is continuously loaded onto the column, all three components will be adsorbed, but the most strongly adsorbed component C will displace the other components whilst B will displace A, resulting in the formation of three distinct bands. As loading is continued, these bands will broaden until, when all adsorption sites are occupied, component A will start to elute in a concentration equal to the sum of the loading concentration and the amount of A being displaced from the column by B and C. When all component A has been displaced from the column, its elution concentration falls towards its loading concentration and B starts to co-elute in a concentration which rapidly increases until it is equal to the sum of its loading concentration and the amount being displaced from the column by component C. When all component B has been displaced from the column, its elution concentration falls towards its loading concentration and component C starts to co-elute with A and B in a concentration which rapidly increases to its loading concentration *i.e.* the column eluate has the same composition as the solution being loaded. The resulting chromatogram will appear as in Fig. 2 whilst the on-column situation will be as diagramatically represented in Fig. 3. In practice, the difference between the



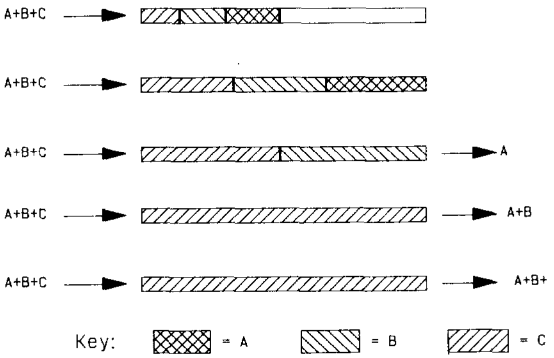


Fig. 2. Frontal chromatogram, three solutes (A, B and C). Concentration is expressed as a percentage of the loading concentration. For point X, see text.

Fig. 3. Diagramatic representation of the on-column situation during the development of a frontal chromatogram with three solutes (A, B and C).

adsorption isotherms is not always large and the analytes compete with each other for the available active sites on the stationary phase, resulting in the boundary between adjacently eluting analytes being less sharp, its sharpness depending upon the relative magnitudes of the adsorption isotherms of the analytes. The material adsorbed onto the column at the point where the composition of the feed solution and the eluate become equal will not be pure component C, but will be contaminated with component B and possibly with some component A, the degree of contamination being determined by the relative magnitudes of the adsorption isotherms. Slow mass transfer kinetics of the system would also lead to the boundaries between successively eluting components being less well defined.

Thus, there exist two situations during the development of a frontal chromatogram which may potentially be exploited in a preparative role:

(1) The least retained component is eluted in a pure form up to the point where the next component starts to elute (point X, Fig. 2)

(2) When most of the adsorption sites on the column are occupied by the most strongly retained component. In this case the column may be washed free of non-adsorbed compounds and the material on the column desorbed into a "stronger" solvent. The relative magnitude(s) of the adsorption isotherms of the most strongly retained component and of the component(s) preceding it will determine the degree of purity obtainable in this case.

Where the required component of a multi-component mixture is not either the most or the least retained component (as is usually the case), it is still possible to use frontal chromatography for its isolation, but a two-column process is required. The mixture is treated as if it consists of three components, the component of interest, all less strongly retained components and all more strongly retained components. The mixture is loaded onto a column until all the component of interest along with the less retained components have eluted, but elution of the more strongly retained components has not yet started. The eluate from the first column is then loaded to a second column until the less strongly retained components have eluted, but the component of interest has not. The component of interest can then be obtained by desorption into a "stronger" solvent. Once the optimum loading for each of the columns has been determined, provided that the composition of the feedstock remains constant and column performance does not deteriorate, the process may be represented thus:

Column 1

(1) Load an aliquot of feedstock solution.
(2) Wash column free of non-adsorbed compounds.
(3) Desorb the more strongly retained components into a "strong" solvent.
(4) Regenerate column with original mobile phase.

Column 2

(1) Load an aliquot of the partially purified feedstock solution.
(2) Wash column free of non-adsorbed compounds.
(3) Desorb the required component into a "strong" solvent.
(4) Regenerate column with the original mobile phase.

The purity obtainable for any given component will depend upon the sharpness of the boundaries between the eluting components of any mixture (*i.e.* the relative magnitude of the adsorption isotherms of the components) and the applicability of the method will thus be limited to those cases where there are large differences in the isotherms or where the purity requirements of the products are less stringent.

EXPERIMENTAL, RESULTS AND DISCUSSION

Instrumentation

A Prep 500 instrument (Waters Assoc., Milford, MA, U.S.A.) was modified by removing the radial compression module and fitting a 250 mm × 75 mm Annular Expansion column (Separations Technology, Wakefield, RI, U.S.A.) or a 500 mm × 50 mm axially compressed column (Amicon, Stonehouse, U.K.). The spare port on the flow splitter of the Prep 500 was connected to a Model 7520 injection valve (Rheodyne, Cotati, CA, U.S.A.) fitted with a 0.5-μl internal sample loop, this valve being connected to an analytical h.p.l.c. instrument comprising a Model 6000A pump and Model 440 detector (Waters Assoc.) and a Model PM8251 chart recorder (Phillips, Cambridge, U.K.), so that by operating the valve an analysis of the eluent could be made at any point during the preparative run (Fig. 4). Larger-scale work was carried out on a Model K'3000 instrument fitted with a 500 mm × 200 mm axially compressed column (Amicon).

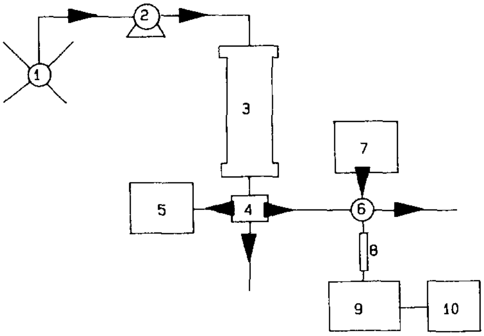


Fig. 4. Diagramatic representation of the preparative instrumentation. 1 = Five-way valve; 2 = preparative pump; 3 = preparative column; 4 = flow splitter; 5 = refractive index detector; 6 = injection valve; 7 = analytical pump; 8 = analytical column; 9 = UV detector; 10 = chart recorder.

Example 1

Deprotection of a protected synthetic pentapeptide using trifluoroacetic acid in the presence of anisole, gave a crude product containing 75% of the required pentapeptide and 25% of related impurities. Additionally, the product contained anisole and anisole derivatives and was obtained as the trifluoroacetate salt. The product was required as the free base with an assay of >96%. A solution of the crude product in water (4%, w/v) was prepared, the pH was adjusted to 5.9 (sodium carbonate) and the solution was extracted with dichloromethane to remove anisole related compounds. The resulting solution (Fig. 5a) was pumped at a flow-rate of 100 cm^3 min^{-1} onto the 250 mm × 75 mm Annular Expansion column packed with 500 g of 20–45 μm irregular C_8 bonded silica (Amicon), and the column eluate was monitored by high-performance liquid chromatography (HPLC) simply by operating the injection valve when required. Fig. 6 shows the analytical chromatograms collected during the course of a preparative run. At the point where the breakthrough of

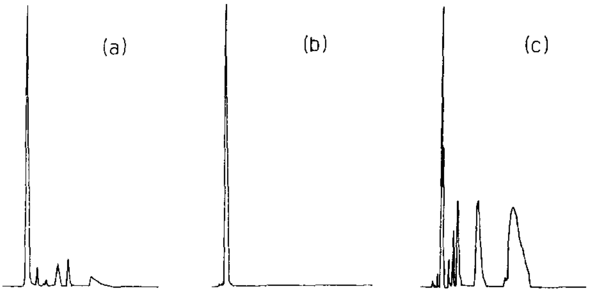


Fig. 5. Analyses of fractions, example 1. (a) Crude product, (b) purified product, (c) methanol solution. Analytical conditions: water–acetonitrile–phosphoric acid (400:100:5), 2.0 cm^3 min^{-1} on 250 mm × 4.6 mm Partisil 5 ODS 3. Detection, UV at 254 nm.

impurities commenced (Fig. 6f), loading was stopped and the column was washed sequentially with water (500 cm^3), methanol (2000 cm^3) and water (500 cm^3) at a flow-rate of 200 cm^3 min^{-1}. The analytical chromatogram Fig. 6h shows a decrease in concentration as the water wash elutes and Fig. 6i shows the methanol wash. The aqueous eluate was examined by HPLC (Fig. 5b), and found to contain product with a purity of >98% whilst the methanol solution (Fig. 5c), contained mainly impurities. Once the optimum load volume had been determined on the first run, subsequent runs were effected by loading the predetermined volume of crude product solution then using the above wash sequence before recommencing loading. This cycle was conveniently carried out by using a five-port valve fitted to the pump inlet to switch between sample, water, methanol and water. Fraction cut points were determined with the aid of the refractive index detector on the Prep 500 instrument. A total of 25 cycles was carried out. The chromatogram of the first preparative run is given in Fig. 7, this having been constructed from the analyses taken during the run. It should be noted that this is not the full frontal chromatogram of the separation, but only of that part which is required to obtain the required purification, the washes being added before complete development of the frontal chromatogram has occurred. An aliquot of the aqueous solution containing purified product was then loaded onto the column so that breakthrough did not occur, the column was washed free of salts with water and the

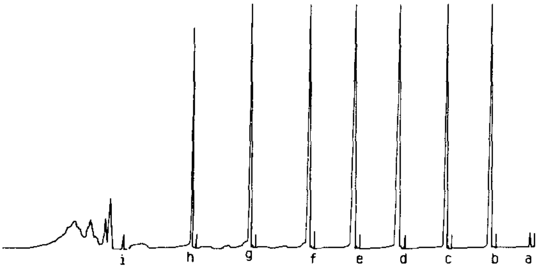


Fig. 6. Monitoring of the eluate from the purification step in example 1. The letters indicate the points at which injections were made during the preparative run. Analytical conditions: water–acetonitrile–phosphoric acid (400:100:5), 4.0 cm^3 min^{-1} on 50 mm × 4.6 mm Hypersil 3 ODS. Detection, UV at 254 nm (2.0 a.u.f.s.). Injection 0.5 μl of column eluate.

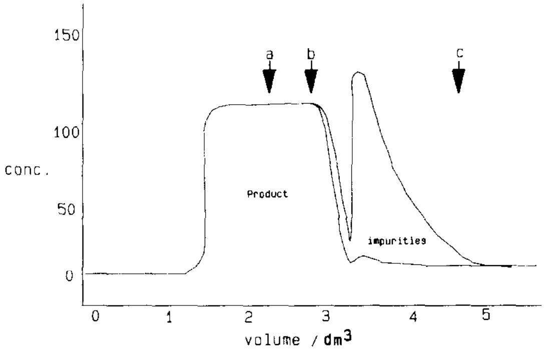


Fig. 7. Preparative chromatogram for the purification step in example 1, constructed from the analyses taken during the run. Point a: water wash started; point b: methanol wash started; point c: column regeneration wash started.

product was desorbed into methanol. After regeneration of the column with a water wash, the cycle was repeated. Treatment of the total batch required 140 cycles. The methanol eluate from these runs was combined, evaporated to dryness, the residue was dissolved in a minimum volume of methanol and the product precipitated with acetone. The precipitate was collected by filtration and dried *in vacuo* to give 3.14 kg of product which assayed at 98.5%.

In this case the required compound was the fastest running component of the crude product and, therefore provides an example of the simplest form of frontal chromatography in preparative applications, although a second step was required to effect the desalt. From analyses of the crude product and of the methanol solution from the first step the yield for the purification step was estimated at 97% whilst the yield for the desalt step was close to 100%. No deterioration of the column performance was found during this work. Each kilogram of purified product obtained used a total of 109.5 dm^3 of methanol (16 dm^3 for purification, 93.5 dm^3 for desalt) and required 21 h of instrument time (5.5 h for purification, 15.5 h for desalt). Projecting this to a large scale, assuming a high degree of automation, recovery of solvent and a column lifetime allowing the purification of 60 kg of product per kg of stationary phase, it was shown that, had it been possible to use recrystallisation to purify this material, a recovery of $>95\%$ would have been required to obtain an equivalent purification cost. A limited study of elution chromatography for this purification on the same size column used for this work showed that the purification would have required 33 h/kg (*cf.* 5.5 h/kg), considerably more solvent and a desalt step would still have been necessary. The processing cost would have been an order of magnitude higher and, although the cost differential is somewhat smaller when product recovery is included in the calculation, the lower throughput would require larger equipment and consequent capital investment in order to achieve an equivalent production rate.

Example 2

Fluorination of an anhydronucleoside with hydrofluoric acid resulted in a complex mixture in which the required product was present at a level of 20–25%. In addition to the unwanted products shown by HPLC (Fig. 8a) (the required material is

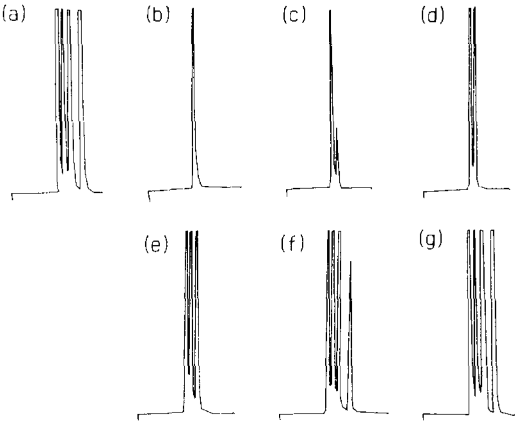


Fig. 8. Analyses of fractions from the small-scale frontal experiment, example 2. For explanation see text. Analytical conditions: water–tetrahydrofuran (80:20), 2.0 cm^3 min^{-1} on 250 mm × 4.6 mm Partisil 10 ODS. Detection, UV at 254 nm (2.0 a.u.f.s.). Injection 5.0 μl of column eluate.

the fourth major peak), late-running impurities were present which were not detected by UV or were not eluted in the mobile phase used for the analysis. Numerous attempts to isolate the product by crystallisation were unsuccessful. A solution of the reaction product in water (4%, w/v) was pumped onto a 250 mm × 4.6 mm Partisil 10 ODS column (Whatman, Maidstone, U.K.) at a flow-rate of 1.0 cm^3 min^{-1} and 1.0-cm^3 fractions were collected and analysed, Fig. 8b–g show the analyses of fractions 10, 15, 20, 25, 30 and 35, respectively, and demonstrated that frontal chromatography was likely to be successful preparatively. A solution of the mixture (627 g) in water (15 l) was divided into ten aliquots and an aliquot was pumped onto a 250 mm × 75 mm Annular Expansion column containing 500 g of 20–45-μm C_8 bonded silica (Amicon) at a flow-rate of 100 cm^3 min^{-1}. The column was then washed sequentially with water (500 cm^3), methanol (2000 cm^3) and water (500 cm^3) before the next aliquot was loaded. The aqueous solution containing the required product and the less strongly adsorbed impurities, was divided into twenty aliquots, an aliquot was loaded onto the column, and the column was subjected to the same wash sequence as above before loading the next aliquot. The methanol washes were collected and combined. On each of these runs a small fraction of the aqueous eluent (100 cm^3) immediately before the methanol began to elute, contained some impure product. These fractions were combined and rechromatographed in a single run, the methanol eluate from which was combined with the other methanol fractions and the solvent was evaporated to dryness to give 126 g of product which assayed at >95%. The chromatogram of the preparative separation for the second step of the purification is given in Fig. 9, this having been constructed from analyses of collected fractions. Sufficient data were not available to determine the individual elution profiles of the three impurities within the impurity fraction.

In this example, the required component was the slowest running on the analytical system used, which suggested that a single stage process might be used for its isolation, since the required purity of the product was not high (95%) and, once the

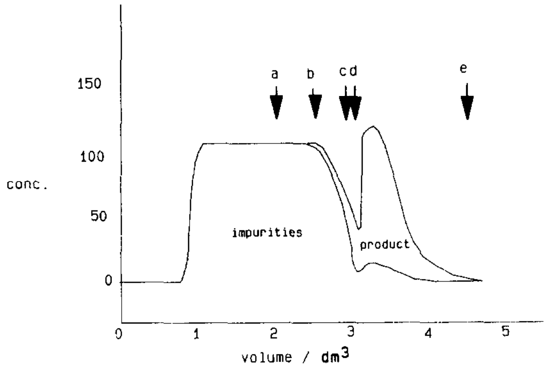


Fig. 9. Preparative chromatogram from the second purification step, example 2, constructed from the analyses of collected fractions. Point a: water wash started; point b: methanol wash started; point c: collect aqueous fraction; point d: collect methanol fraction; point e: column regeneration water wash started.

product had been isolated from the bulk of the impurities, its purity could, if necessary, be improved by recrystallisation. However, on commencement of the preparative work, it soon became apparent that other, slow-running, impurities were present which either did not elute on the analytical system or were not detectable by UV and it was therefore necessary to use a two stage purification. Evaporation of the methanol from the first step of the purification yielded a black tarry residue which contained only a trace of the required product. A small amount of product was lost in the aqueous liquors from the second purification step. The overall yield of product from the two stage purification was estimated to be 95%. This example demonstrates the use of frontal chromatography on a small column in order to predict the likelihood of success on a larger scale.

Example 3

Recrystallisation of a crude product from 10% brine gave good quality product plus mother liquors which, in addition to various impurities, contained approximately 2% (w/w) of the product which represented a loss of about 10% of the process yield. Evaporation of the liquors and second cropping recovered only a small proportion of the available material because of decomposition during the evaporation step. Frontal chromatography was therefore investigated as a recovery method. The mother liquors were passed down a 500 mm × 200 mm axially compressed column packed with 10 kg of 20–45-μm C_8-bonded silica at a flow-rate of 1.2 dm^3 min^{-1}, which effectively removed the bulk of the late-running impurities including highly coloured materials, with a minimum loss of the required product. Approximately 800 dm^3 of liquors could be treated before the breakthrough of impurities in a concentration high enough to affect the quality of the product after subsequent crystallisation occurred and the column required regeneration by washing with methanol (40 dm^3) and water (20 dm^3). Experiments were then conducted on either a 250 mm × 75 mm Annular Expansion column or on a 500 mm × 50 mm axially compressed column, both containing 500 g of 20–45-μm C_8-bonded silica, to determine optimum loading and flow-rate, each experiment being carried out by loading an aliquot of partially purified liquors then washing the column sequentially with water, methanol and water. The first water wash and the methanol wash were then examined by analytical HPLC to determine if product was being lost in the water and the purification efficiency. When the optimum conditions had been determined, the process was transferred to the 500 mm × 200 mm axially compressed column. A proportion of the water wash which followed the methanol wash was combined with the methanol wash, the methanol was stripped off and the purified product was obtained by adding strong brine to the residual aqueous solution and cooling to effect crystallisation.

Since large amounts of mother liquor were available in this instance, it was possible to carry out a more detailed examination of conditions and hence to optimise the process. Table I shows the results obtained during the optimisation experiments. The initial experiments on each column determined the optimum load volume, showing that too low a load gave a product of inferior quality whilst too large a load led to product being lost through breakthrough. Of the two columns used, the 500 mm × 50 mm gave either a higher recovery of product at the same purity or a higher purity for the same recovery when using an equivalent linear flow-rate, 200 $cm^3 min^{-1}$ on the 75 mm diameter column being equivalent to 100 $cm^3 min^{-1}$ on the 50 mm diameter column since the effective diameter of the former is reduced by the tapered rod used to produce the Annular Expansion effect. An investigation into loading flow-rate carried out on the 50 mm diameter column showed that, up to 125 $cm^3 min^{-1}$ there was no effect on the separation efficiency, but above this point efficiency

TABLE I

RESULTS OF OPTIMISATION EXPERIMENTS (EXAMPLE 3)

The 75 mm diameter column was an Annular Expansion type and the 50 mm column an axially compressed type. Assay is the externally standardised assay of the residue obtained from evaporation of the methanol eluate. Breakthrough indicates whether the aqueous eluate immediately preceding the methanol eluate contained product.

Column diameter (mm)	Load volume (dm^3)	Flow-rate ($cm^3 min^{-1}$)	Assay (%)	Breakthrough
75	2.50	200	56	No
75	2.75	200	64	No
75	3.00	200	73	No
75	3.50	200	82	Slight
75	4.00	200	85	Yes
50	3.50	100	69	No
50	4.00	100	77	No
50	4.25	100	88	No
50	4.50	100	91	Slight
50	4.75	100	92	Yes
50	4.25	50	89	No
50	4.25	125	90	No
50	4.25	150	86	Slight
50	4.25	175	83	Slight

diminished with increasing flow, albeit slightly, up to 170 cm^3 min^{-1}, the highest flow obtainable within the pressure constraints of the instrumentation. This decrease in efficiency at higher flows would be predicted on the basis of mass transfer kinetics. From experiments on the isolation of product from methanol solution, it was found that the final crystallisation gave product of acceptable quality provided that the material in the methanol assayed at >85%. The wash volumes determined by experiment were: water 100 cm^3; methanol 2000 cm^3; water 500 cm^3, and were the minimum required to obtain optimum recovery/purity whilst minimising solvent usage and thus maximising throughput.

Having optimised conditions on the small scale, the method was transferred to the 200 mm diameter column without difficulty. Several hundred litres of liquors were processed and the overall economics of the process were determined. Because of the high recoveries as compared to the existing recovery method, chromatographic recovery was found to be cost effective.

General discussion

A general stratagem for investigating the possibility of using frontal chromatography for any particular preparative separation is first to find a combination of stationary phase and mobile phase which gives infinite k' for the components of the mixture and where the components are all soluble in the mobile phase. Running a small-scale experiment as in example 2 will then rapidly show whether further work is likely to be justified, taking into consideration the purity requirements for the product to be isolated.

Example 1 stands out as one of the very rare cases where it is possible to obtain a high purity product directly by frontal chromatography. In the majority of cases, as Guiochon and Katti [4] state, the technique is not well suited to the production of pure materials. It can, however, as demonstrated in examples 2 and 3 be extremely useful in the rapid and cost effective isolation of materials from a crude mixture where either the purity requirements of the product are less stringent, or where subsequent further purification is to be used.

The use of preparative chromatography in production can often be justified economically since the higher yields obtained compensate for the higher processing costs involved, particularly with high value products, even in those cases where a simple alternative such as recrystallisation is feasible. However, a combination of techniques can often prove most cost effective. Taking the example of recrystallisation, product will be lost in the mother liquors. This material can be chromatographically purified in one of two ways, either by using chromatography directly to obtain pure material or by using chromatography to enhance the quality of the material to that of the crude product thus allowing good quality material to be obtained by recrystallisation. Since the purity requirements are less stringent in the second case, much higher throughputs can be achieved and an additional advantage is that all product will be produced via an identical last step, *i.e.* recrystallisation. In this way overall yields close to 100% can be obtained, thereby more than compensating for the cost of the chromatographic step, even with low to moderate value products. In this context, the technique of frontal chromatography shows considerable promise.

CONCLUSION

In the examples studied here, frontal chromatography has been shown to be a simple and cost-effective purification technique, but further work will be required to determine whether its use will be restricted to a few very specific examples or if it can be improved as a more general preparative method. In view of the promise shown thus far, it would seem worth the effort in studying the effect of such parameters as loading concentration and the nature of the stationary phase (shape and particle size), as well as its application in normal-phase and ion-exchange chromatography.

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