Journal of Chromatography, 523 (1990) 63–80
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 746

Utility of the displacement effect in the routine optimization of separations by preparative liquid chromatography

JOAN NEWBURGER
Squibb Institute for Medical Research, P.O. Box 4000, Princeton, NJ 08543-4000 (U.S.A.)
and
GEORGES GUIOCHON*
* *Department of Chemistry, University of Tennessee, Knoxville, TN 37996-1600 and Division of Analytical Chemistry, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6120 (U.S.A.)*
(First received February 7th, 1990; revised manuscript received June 8th, 1990)

ABSTRACT

The utility of the displacement effect, previously observed between the first- and the second-eluted components of a particular binary mixture ("sample self-displacement effect"), is demonstrated by a further series of experiments. The use of this phenomenon is shown to be an effective, general approach for the separation of a binary mixture. When practical, it considerably facilitates the optimization of experimental conditions for a high production rate of very pure fractions, while permitting the achievement of high recovery yields. Using a 750 × 21.4 mm I.D. silica column, separations of several two-component mixtures were achieved for loads as high as 6 g per injection. A good recovery of the first-eluting component with a purity higher than 99% is easily achieved. As the loading increases, tailing of the first component into the second band can reduce the production of the second component. Nevertheless, the recoveries for both components can be substantially greater than those achieved with conventional elution chromatography. Factors found to influence recovery yield are α, k' and the mobile phase flow-rate. Guidelines are presented that can be used routinely to induce the self-displacement separation mode.

INTRODUCTION

Displacement chromatography is an attractive separation method [1]. Sharp boundaries form between successive bands in an isotachic displacement train and provide for very strong concentration gradients of closely eluted compounds, *i.e.*, for high-concentration fractions. Large productions per run and good recovery yields make the method extremely attractive. Its use at the laboratory level for multi-batch separations is hampered, however, by the long development time and by the lengthy and tedious regeneration steps after each injection to remove the strongly retained displacing agent. In contrast, the ability to induce a displacement effect between two closely eluted components of a mixture without resorting to an external displacer is a very attractive feature of high-concentration elution chromatography. This induction is possible, as we have shown previously, in a particular case [2–4].

When the sample size of a binary mixture is increased, and if the concentration

0021-9673/90/$03.50

of the second component in the feed is of the same order as or larger than that of the first component, displacement by the second band of the first band takes place and can be very strong. The second component pushes the first one in front of it, a shock layer appears between the two components and the mixed band between the two zones of almost pure compounds can be narrow if the experimental conditions are properly adjusted [2,3]. As noted above, the addition of a displacer is not necessary to achieve this result and a considerable increase in production rate can be easily achieved. We have termed this phenomenon "sample self-displacement", to emphasize the concept of interaction between the sample components during their overloaded elution.

Previous work was done with a mixture of epimers, having a relative retention near unity [2,3]. Accordingly, the generality of the phenomenon, in spite of strongly supportive theoretical evidence [5], was not proved. This work demonstrates experimentally that the phenomenon is general and can be used to increase production for any two-component batch purified using preparative liquid chromatography. Further, we show that the method can be used routinely and that it is easy to find the experimental conditions under which the phenomenon of sample self-displacement takes place.

EXPERIMENTAL

Apparatus

Preparative experiments were carried out on a Waters Assoc. (Milford, MA, U.S.A.) Model 3000 preparative liquid chromatograph, equipped with a Waters Assoc. Model 480 variable-wavelength absorbance detector and a Kipp & Zonen (Delft, The Netherlands) Model BD40 strip-chart recorder.

Isomer ratios were determined on an analytical high-performance liquid chromatographic (HPLC) system consisting of a Perkin-Elmer (Norwalk, CT, U.S.A.) Series 4 liquid chromatograph, an Applied Biosystems (Foster City, CA, U.S.A.) Model 783a variable-wavelength absorbance detector, a Perkin-Elmer ISS-100 autosampler and a Perkin-Elmer Model 7700 data collection system.

Reagents

Solvents were of HPLC grade, purchased from J. T. Baker (Phillipsburg, NJ, U.S.A.). Diethyl phthalate, dimethyl phthalate, α-tetralone, β-tetralone and benzosuberone were of 99% + purity from Aldrich (Milwaukee, WI, U.S.A.).

Preparative chromatographic conditions

The preparative column consisted of three identical 250 × 21.4 mm I.D. Dynamax 8-μm silica columns (Rainin Instruments, Woburn, MA, U.S.A.) connected in series with the minimum length of 0.040-in. tubing. For the experiment on the separation of a mixture of dimethyl and diethyl phthalate, one column or two columns in series were also used.

Prior to use, each column was washed with the following series of solvents: 100 ml of tetrahydrofuran (THF)–hexane (30:70), 300 ml of THF–hexane ethyl acetate (10:75:15) and 1 l of ethyl acetate–hexane (15:85). This procedure was found to be necessary to remove any trace of silica-deactivating solvents (*e.g.*, water) present in

the shipping solvent. Failure to wash the columns resulted in a substantial reduction of the displacement effect.

For the β-tetralone–diethyl phthalate and the diethyl phthalate–dimethyl phthalate experiments, the mobile phase was ethyl acetate–hexane (4:96). The flow-rate was 40 ml/min (0.25 cm/s), except as noted in the flow-rate study. Peaks were monitored at 300 nm.

For the β-tetralone–benzosuberone self-displacement experiments, the mobile phase was ethyl acetate–hexane (2:98) for the loading study or ethyl acetate–hexane (2:98), (3:97) or (3.85:96.15) for the k' study. For the elution chromatographic experiment, the mobile phase was ethyl acetate–hexane (0.5:99.5). The flow-rate was 50 ml/min and peaks were monitored at 310 nm.

Procedures

Test compounds were weighed out according to the desired ratio (25:75) and loadings, added to volumetric flasks and dissolved in mobile phase at concentrations of 0.3–3 g per 5 ml, 4-5 g per 7 ml or 6 g per 9 ml. If necessary, ethyl acetate was added to improve the solubility. Sample mixtures were injected onto the column by filling a 5-, 7- or 9-ml injection loop (Rheodyne, Cotati, CA, U.S.A.).

For each injection, fractionation began as soon as a slope change was noted. Fraction volumes were monitored indirectly by counting chart paper units. Most of the fraction volumes were 10 and 16 ml in the α-tetralone and β-tetralone experiments, respectively. Multiple volumes were collected for the first one or two and the last two or three fractions.

The fractions were analyzed to determine component ratios using a Waters Assoc. Nova-Pak 4-μm silica column (150 mm × 3.9 I.D.). In all studies, a mobile phase of ethyl acetate–hexane (2:98) was used and the peaks were monitored at 254 nm. Owing to the difference in molar absorptivities for the components of each mixture, it was not possible to obtain component ratios in the fractions by taking a simple ratio of integration area counts. Instead, masses for each compound per injection were determined by comparison with known standards. A standard of each component was made up fresh and injected several times for every fraction set analysis. The absorbance was linear for most of the concentration range, although for very small and very large amounts the fit was poorer.

Calculations

Recovery yields were calculated for each chromatogram by counting the number of chart paper units under the curve. This procedure was reproducible to within a few percent. The criteria for 95% and 99% pure materials required that all fractions of less than 94% and 98.5% purity, respectively, be discarded. The chromatogram areas of the impure, mixed fractions were discounted in determining the total pure material recovered.

The yields determined by this method are only approximate as the molar absorptivities for each component of the particular fractions are different. Yields from the different sets of mixtures should not be compared; however, reasonable conclusions regarding relative behavior can be drawn from injections of the same mixture. The reported "total yield combined" is the sum of the recoveries at the required purity for the first and the second components.

RESULTS AND DISCUSSION

Successful extension of the overload phenomenon, sample self-displacement, from the original laboratory application to a practical preparative tool has been predicted only in modeling studies [5–9]. A non-linear phenomenon was inferred from some published data [2,3,10,11], but actual band profile data were still lacking for confirmation at the beginning of this study. Therefore, at the outset of this study, chromatographic conditions were designed to mimic as closely as possible those used in the prior epimer study. Nevertheless, potential sample compounds were chosen at random from the Aldrich catalog to prove the generality of the overload technique. We have systematically investigated the effect of sample size and several other experimental parameters on the batch production of pure material from multi-gram injections of three selected mixtures. Detailed investigations of band profiles under overloaded conditions have been published recently [12–14]. In contrast to this work, however, they do not focus on the influence of the experimental conditions on the recovery yield and production rate.

Effect of sample size on the recovery of β-tetralone

In a previous study [3], the retention factor for the first component of the epimer pair, k', was 7 and the relative retention at infinite dilution, α, was 1.04. Of the compounds chosen from the Aldrich catalogue, a mixture of diethyl phthalate and β-tetralone came closest to matching those parameters, having k' and α equal to 6 and 1.1, respectively (see Fig. 1).

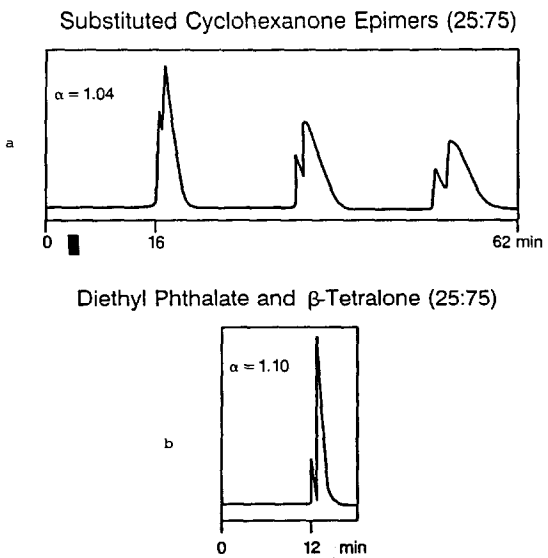


Fig. 1. Initial development of self-displacement chromatographic conditions. (a) Chromatogram of a 60-mg mixture of methyl *cis*- and *trans*-1-ethyl-2-oxo-4-(2-propenyl)cyclohexaneacetate. Three passes on one 250 × 21.4 mm I.D. column with recycling. Mobile phase, ethyl acetate–hexane (2.5:97.5); flow-rate, 40 ml/min (velocity, 0.25 cm/s). Relative retention calculated for the first pass. (b) Similar chromatogram obtained for a 60-mg mixture of diethyl phthalate and β-tetralone on a 250 × 21.4 mm I.D. silica column. Mobile phase, ethyl acetate–hexane (4:96); flow-rate, 40 ml/min.

The analytical separation of a 15-mg mixture of diethyl phthalate and β-tetralone (1:1) is shown in Fig. 2. The relative retention is 1.1 and near baseline resolution is achieved. Based on this chromatogram, practical experience with elution chromatography would suggest that a sample load of 50 mg might be reasonable if the separation is to be carried out under conditions of linear chromatography, with touching bands. However, these chromatographic conditions were developed specifically to induce the displacement effect.

Samples of 0.3–5 g were injected under the same experimental conditions as in Figs. 1 and 2. The chromatograms are shown in Figs. 3 and 4. In contrast to results expected in the linear elution mode, the compounds are resolved to the baseline when 300 mg are injected. The resolution is still excellent when the load is more than tripled to 1 g. When the injection size is increased to 3 g, very sharp fronts are recorded for the two components and differentiation between the two bands can be observed. Visually, however, the resolution is poor. The recovery yield and production are also given in Fig. 4 for the three sample sizes. Surprisingly, with the 3-g injection, there is only a narrow mixed zone behind the front of the second component band. The composition profile of the mixed zone is reported Fig. 5 for the three chromatograms shown in Fig. 4. The curves in Fig. 5 give the relative concentration of diethyl phthalate in the β-tetralone fraction collected after the sharp front (see the next section). The mixed zone is weakly polluted by a slight tail of the first component, but its purity exceeds 95%. If it is not essential that the purity of the collected fractions of the two purified components exceeds 95%, the recovery is nearly total. If the purity of the material recovered must exceed 99%, the yield is still high (67%).

With larger samples the yield decreases, but for a 5-g sample it is still 74% when combining fractions that are 95% pure. In practice, for the maximum production of purified material of the two individual components, each having a purity of 95 or 99%, sample sizes of 3 or 5 g, respectively, would be optimum.

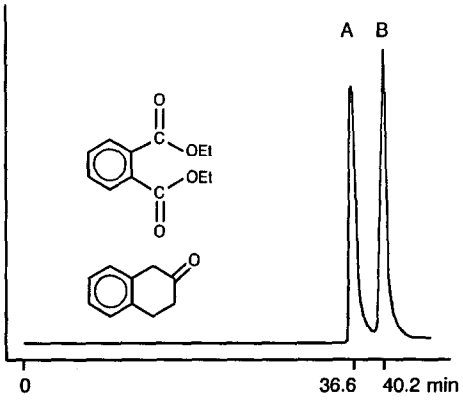


Fig. 2. Analytical separation of a 25:75 mixture of diethyl phthalate (A) and β-tetralone (B). Chromatograms for different sample sizes. Column, 750 × 21.4 mm I.D., packed with 10-μm silica particles. Mobile phase, ethyl acetate–hexane (4:96); flow-rate, 40 ml/min; sample size, 15 mg (1:1 mixture).

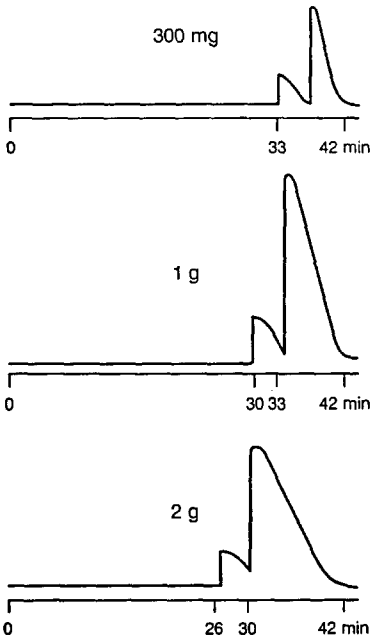


Fig. 3. Effect of sample size on the elution profile of a 25:75 mixture of diethyl phthalate and β-tetralone. Experimental conditions as in Fig. 2, except for sample sizes: 0.3, 1 and 2 g. Complete or near-complete resolution is achieved for sample sizes below 2 g.

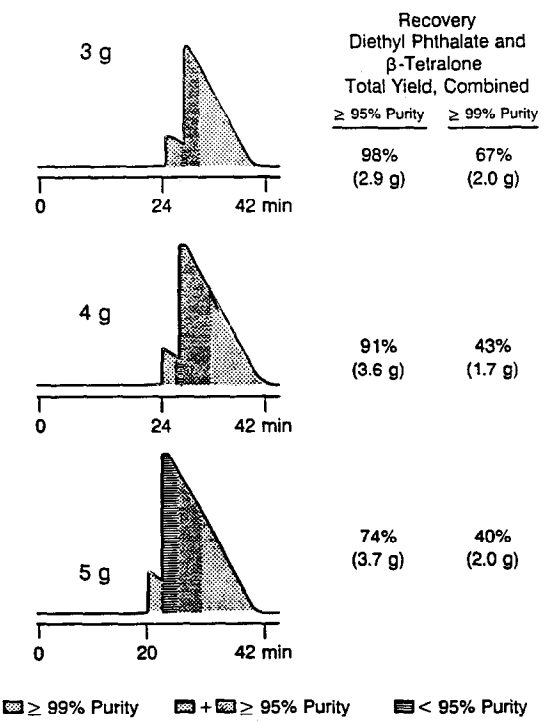


Fig. 4. Effect of sample size on the elution profile and the recovery yields for a 25:75 mixture of diethyl phthalate and β-tetralone. Experimental conditions as in Fig. 2, except for sample sizes: 3, 4 and 5 g.

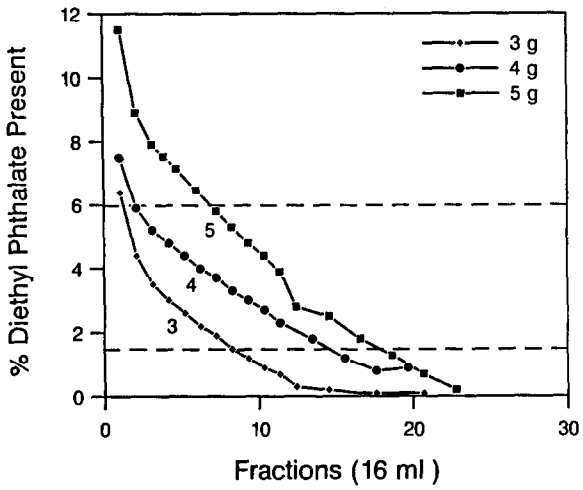


Fig. 5. Effect of sample size on the purity of fractions of β-tetralone collected during the elution of samples of increasing size of a 25:75 mixture of diethyl phthalate and β-tetralone. Plot of the diethyl phthalate concentration in collected fractions *versus* the fraction number. The collection of the first 16-ml fraction starts 16 ml after the front of β-tetralone appears in the chromatogram (Fig. 4).

Effect of sample size on the purity of β-tetralone

Although it would be valuable to draw the elution profile of diethyl phthalate, this is not possible because of differences in the molar absorptivities of the two compounds. Information about the elution behavior of the first component can still be obtained by plotting the percentage of diethyl phthalate present in the β-tetralone fractions. The fractions collected before the nearly vertical front of β-tetralone all consisted of diethyl phthalate in greater than 99.9% purity. The composition of the 16-ml fractions collected during the elution of the mixed zone, behind the steep front of the second component (see Fig. 3), is shown in Fig. 5. The concentration decay of diethyl phthalate behind the steep front of the β-tetralone band takes place as predicted by theory [5,6]. This explains the decrease in recovery of the two compounds when the sample size increases.

To obtain material that is 95% pure, only one or two fractions must be discarded from the 3-g injection, two or three fractions must be eliminated from the 4-g injection and six fractions must be removed from the 5-g injection. Further, it is evident from the plot that the overall purity of the combined material designated as 95% pure will be greater for the 3-g injection than for the 4- and 5-g injections.

The purity of the individual fractions of diethyl phthalate collected before the elution of the front of the β-tetralone band (not shown) exceeds 99% in all three instances. The loss in recovery yield is due solely to the tailing of the diethyl phthalate peak into the β-tetralone band and not to possible fronting of the β-tetralone band into the diethyl phthalate. Such fronting of the second component has never been observed [2,3].

Confirmation of the displacement effect

The observations made in the previous two sections are in excellent agreement

with our previous experimental results [2,3] and with the predictions of the theory of non-linear chromatography [5,6]. They seem to indicate the occurrence of a displacement effect. Proof of the strong degree of interaction between the two bands of this binary mixture is shown in Fig. 6. The top chromatogram shows the elution of the 3-g sample of the 1:3 binary mixture discussed above. The bottom part shows the superimposition of two chromatograms, corresponding to 0.75 g of pure diethyl phthalate and 2.25 g of pure β-tetralone. Both chromatograms exhibit triangular profiles with steep, almost vertical fronts, which are typical of an overloaded column. Because the sample size for β-tetralone is three times larger than that for diethyl phthalate, the retention time of the front of the former compound has become shorter than that of the latter. Based on consideration of these individual chromatograms, a reasonable degree of separation for the mixture, permitting the production of important amounts of purified fractions with a high recovery yield, would be ruled out by most chemists.

However, the retention times of the fronts on the two pure compound chromatograms are substantially different from those of the two fronts in the chromatogram of the mixture. In the previous section, we have shown that (i) the first front corresponds to the beginning of the elution of a pure band of the lesser retained component, (ii) the concentration of the first component in the eluate drops off abruptly at

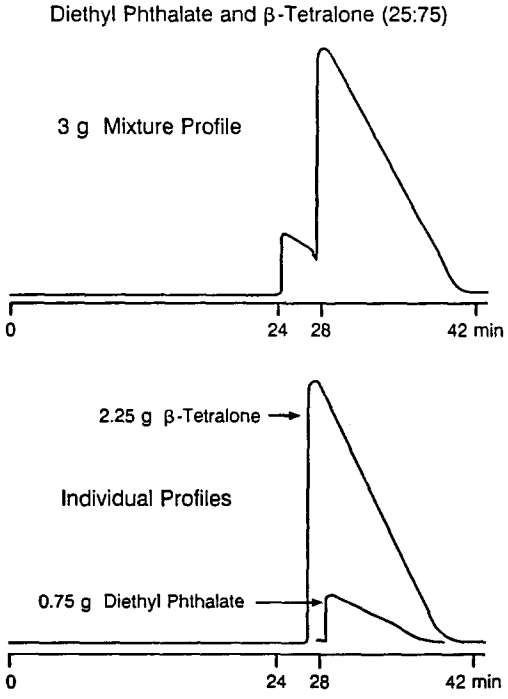


Fig. 6. Confirmation of the self-displacement effect. Comparison between the chromatogram of a 3-g sample of a 25:75 mixture of diethyl phthalate and β-tetralone with the chromatograms obtained from individual injections of the same relative amounts of the two pure compounds. Experimental conditions as in Fig. 2.

the second front, when the second component suddenly surges out of the column, and (iii) a thin mixed zone is eluted just after the second front. The 3-g line in Fig. 5 shows the tail of the elution profile of diethyl phthalate behind the β-tetralone band front. This tail lasts for only about 4 min and decays rapidly, from 6.5% in the first fraction to less than 1% in the tenth.

Clearly, the behavior of the two compounds during the elution of the mixture is not independent, in spite of their lack of structural similarity. Because it is more strongly absorbed, β-tetralone displaces diethyl phthalate, forcing the elution of the diethyl phthalate as an earlier band and permitting the collection of purified products. As we have shown previously [3], for this displacement effect to take place, it is important that the concentration of the second component in the feed is substantial; preferably, it should be comparable to or larger than the concentration of the first component [14]. Experimental results involving the detailed comparison between individual elution profiles obtained experimentally and calculated from the equilibrium isotherms, using the semi-ideal model [5,6,9], have demonstrated the reality of this effect and the importance of the relative composition of the feed on the magnitude of the displacement effect [12,13].

Effect of sample size on the recovery yield of α-tetralone

A second example of the displacement of the first component by the second is given by the separation of a mixture of benzosuberone (first-eluted component) and α-tetralone. The relative retention of the two compounds at very low concentration is 1.3, and higher loadings can be used. Fig. 7 shows two chromatograms, obtained on the injection of 4- and 6-g feed samples. The profile of the benzosuberone tail behind the front of the α-tetralone band is discussed in the next section. The resolution between the two bands is much better than that for the chromatograms in Fig. 4, owing to the better selectivity of the stationary phase. Accordingly, the recovery yields and the production of pure fractions are higher. The difference is greater for 99% than for 95% pure fractions. Absolute comparisons between the chromatograms obtained for this mixture and the previous mixture cannot be made because the molar absorptivities of the four compounds in question are different.

It is interesting to compare the performance of this separation with that achieved under more conventional conditions, with larger values of k' but using a shorter column, 1/3 in length (see Fig. 8). The production per run achieved under the present conditions, which promote the sample self-displacement effect, is much greater than those corresponding to slightly overloaded, but still quasi-linear elution. This result confirms that the production rate is increased by actions which promote the importance of the displacement effect. It is in agreement with independent theoretical work [9] showing that there is an optimum column length, corresponding to a low value of the column efficiency, and that the optimum retention factor, k', is small [9,15].

Effect of sample size on the purity of α-tetralone

The composition of the fractions collected during the elution of the mixed zone of the chromatograms shown in Fig. 7 is plotted in Fig. 9 *versus* the fraction number. The purity of the benzosuberone fractions eluted before the α-tetralone front always exceeds 99%. This is a general result. The second component front is nearly vertical

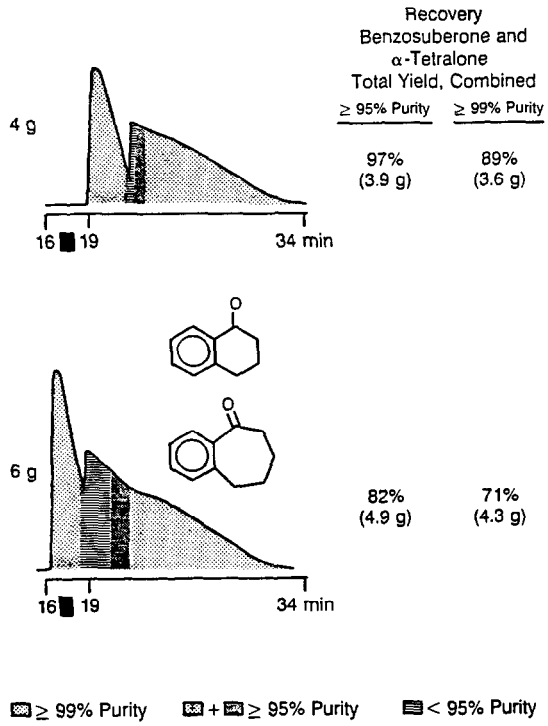


Fig. 7. Effect of sample size on the elution profile of a 25:75 mixture of benzosuberone and α-tetralone and on the recovery yield and production of pure material. Chromatograms for 4- and 6-g injections. Mobile phase, ethyl acetate–hexane (2:98); flow-rate, 50 ml/min (velocity, 0.32 cm/s); column length, 75 cm.

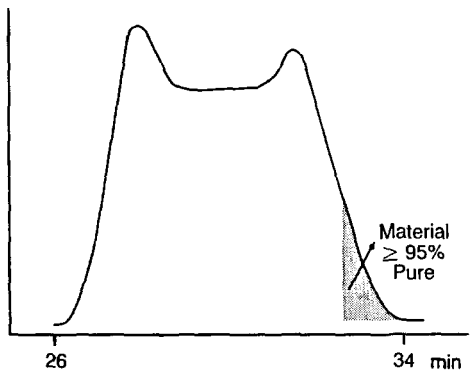


Fig. 8. Elution profile, recovery yield and production of pure material for a small sample (250 mg) of a 25:75 mixture of benzosuberone and α-tetralone, eluted with a weak mobile phase, under quasi-linear conditions, in the absence of self-displacement effect. Mobile phase, ethyl acetate–hexane (0.5:99.5); flow-rate, 50 ml/min (velocity, 0.32 cm/s); column length, 25 cm.

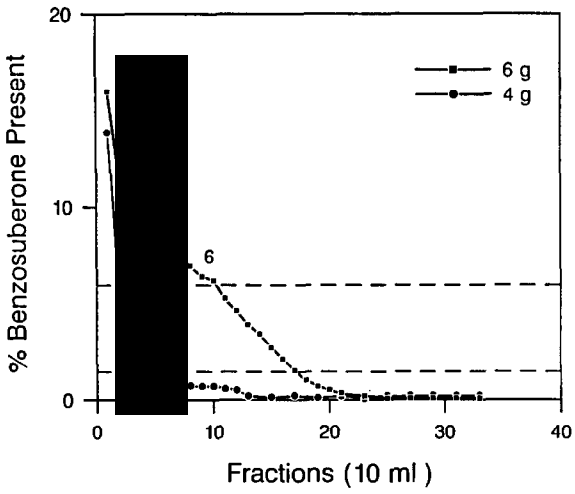


Fig. 9. Effect of sample size on the elution profile of a 25:75 mixture of benzosuberone and α-tetralone. Plot of the benzosuberone concentration in collected fractions *versus* the fraction rank..The collection of the first 10-ml fraction starts 10 ml after the front of α-tetralone appears in the chromatogram (Fig. 7).

and the fraction purity of the first component eluted before that front is usually much more than 99%.

Tailing of the first component (benzosuberone) into the second component band accounts for nearly all the lost production and the recovery yields fall below 100%. With a 4-g sample size, only one fraction has to be rejected to prepare 95% pure β-tetralone and five fractions if 99% compound purity is required. For a 6-g sample the number of fractions to be discarded is ten in order to prepare 95% pure α-tetralone and seventeen for 99% pure material. Using either purity criterion, the 6-g injection would result in a better production rate if absolute recovery is not an issue.

Effect of column length on the separation of diethyl and dimethyl phthalates

A third separation was developed with two homologous phthalate esters. The relative retention of the two components of this mixture is extremely high, 1.7, and the separation is very easy (see Fig. 10). With a 4-g sample, the resolution between the two bands greatly exceeds unity on the three-column series used in this experiment and also in all the previous experiments. The separation is total and any loss in recovery would not arise from overlapping chromatographic bands.

On the two-column series, the retention time decreased by one third, as expected. Although the resolution is lower, it still exceeds unity and the recovery yields are again total (Fig. 10). Comparing the top two chromatograms in Fig. 10, we note that the elution times of the two fronts are reduced by slightly more than one third. As the column is shorter, the extent of dilution is less and the bands are taller. The migration velocity of a front increases with its concentration [6,7]. From the second chromatogram, it does not seem that a significant further reduction of the column length will produce a marked improvement in the production rate. On the contrary, we expect that the two bands will interfere strongly, thereby leading to a drop in the recovery yield.

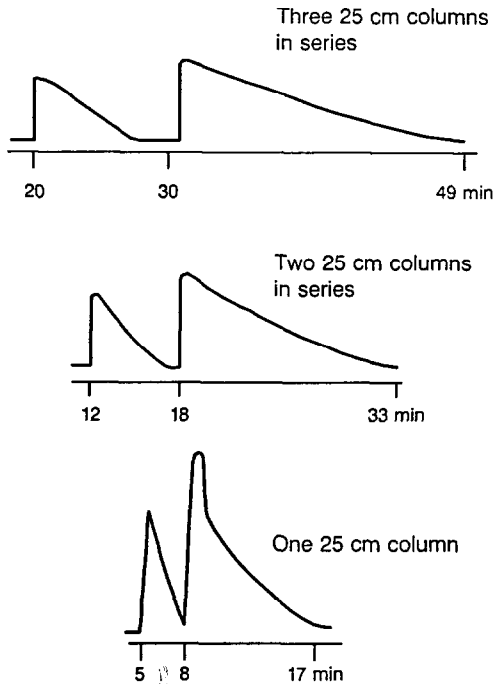


Fig. 10. Effect of sample size on the elution profile of a 25:75 mixture of dimethyl and diethyl phthalates. Chromatograms for a 4-g sample size on columns of different lengths. Experimental conditions as in Fig. 2.

The third chromatogram in Fig. 10 shows that the opposite of this expectation is true. Reducing the column length to one third of the original value leads to a chromatogram that exhibits touching bands. The resolution is nearly total and the separation is still complete. Pure fractions (99%) of both components can be collected with a recovery exceeding 99%. As the flow velocity was kept constant while the column length was reduced from 75 to 25 cm, the production rate has been effectively tripled, which is a noteworthy improvement.

The atypical elution profile of the two components on the third chromatogram must be considered carefully. The first component band is much narrower than in the previous two chromatograms and it is much taller. Again, the retention times of the two fronts have decreased more than proportionally to the decrease in the column length because the velocity of the fronts increases with their concentration. The second component band is now much taller than the first and exhibits a front "tower". All these non-classical features of the elution bands are evidence for the interaction that took place in the column during the progressive separation between the two bands and from which the profiles, just resolved, have not had time to recover completely [6].

Finally, a note of caution is appropriate when comparing the chromatograms in Figs. 4, 7 and 10. The compounds are different and so are the relative responses of the UV detector for the two components. Because of a variable degree of saturation of the detector during elution, the relative response is not even constant. The chroma-

tograms shown are absorbance profiles of the eluate during the elution of the sample and are not true chromatograms, *i.e.*, not true concentration profiles.

Effect of a change in k' on yield and purity

Fig. 11 shows a series of chromatograms corresponding to a 6-g feed sample of the benzosuberone–α-tetralone mixture already discussed (Figs. 7 and 8). The composition of the mobile phase has been changed by increasing the concentration of the strong solvent, ethyl acetate, from 2 to 3.85% (v/v), thereby decreasing the retention of the two components. The run time decreases from 33 to 21 min and the retention time of the first front from 16 to 11.5 min. (Note that the attenuation was different for the third chromatogram, which explains the shorter band height). The relative retention of the two components does not change significantly: α is equal to 1.21, 1.23 and 1.24 for ethyl acetate concentrations of 2, 3 and 3.85%, respectively. Thus, any change observed in the production rate cannot be due to a variation of the relative retention {theory predicts that the production rate with a given column is proportional to $[(1 - \alpha)/\alpha]^2$, *i.e.*, it predicts a change of 5% in the present instance, which is a negligible effect [15,16]}.

A significant increase in the recovery yield is observed, from 10% for 95% pure fractions to 13% for 99% pure fractions. Accordingly, the production rate increases

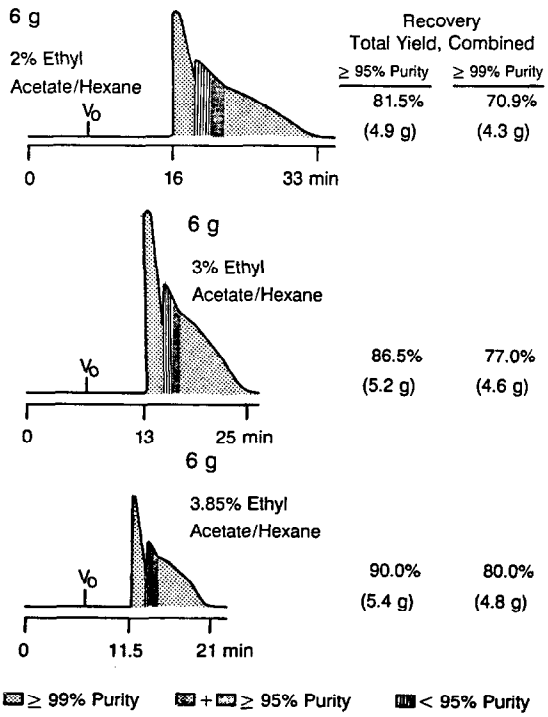


Fig. 11. Effect of mobile phase composition on the elution profile of a 6-g sample of a 25:75 mixture of benzosuberone and α-tetralone. Chromatograms, recovery and production for different concentrations of ethyl acetate in *n*-hexane (2%, 3% and 3.85%). Flow-rate, 50 ml/min.

considerably, as it is proportional both to the recovery yield and to the reverse of the run time. Assuming what is probable, *viz.*, that the change in the composition of the mobile phase does not modify the column saturation capacity, the enhancement of the displacement effect is due to the lesser dilution of the sample, in relation to faster elution. In contrast to the development of separations in the non-overload elution mode, the data suggest that the mobile phase composition should be adjusted so that the capacity factor for the first component, k', is low. For the third chromatogram in Fig. 11, k' for the first-eluted component is only 1.5. This conclusion is in agreement with theoretical results [7–9].

The composition of the fractions collected systematically after the elution of the second component front is plotted in Fig. 12 for the three chromatograms given in Fig. 11. The reason for the increase in recovery yield with decreasing retention is obvious. The number of fractions to be discarded to recover 95% pure products decreases from 10 (2% strong solvent) to 4 (3%) and to 3 (3.85%). The volume of the fractions to be discarded decreases much faster than the volume of eluate occupied by the band system at the column exit (see Fig. 11). Similarly, the number of fractions eliminated in order to prepare 99% pure products decreases from 17 (2%) to 8 (3%) and to 6 (3.85%). Comparable conclusions would be derived by considering the area under the curves in Fig. 12 (total amount of benzosuberone discarded) and the area of the α-tetralone band which has to be discarded.

In order to investigate in greater detail the influence of retention on production, another experiment was performed. The concentration of the strong solvent was decreased to 0.5% ethyl acetate and the column length was reduced to 25 cm to keep the run time comparable to the other experiments (34 min). These conditions correspond to what many consider to be the optimum for preparative elution chromatography. A 250-mg sample was injected (Fig. 8). The band profile is consistent with

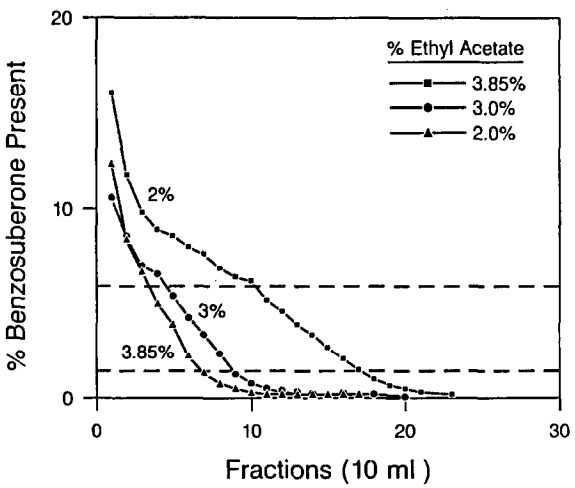


Fig. 12. Effect of mobile phase composition on the elution profile of a 6-g sample of a 25:75 mixture of benzosuberone and α-tetralone. Plot of the benzosuberone concentration in collected fractions *versus* the fraction number. The collection of the first 10-ml fraction starts 10 ml after the front of α-tetralone appears on the chromatogram (Fig. 11).

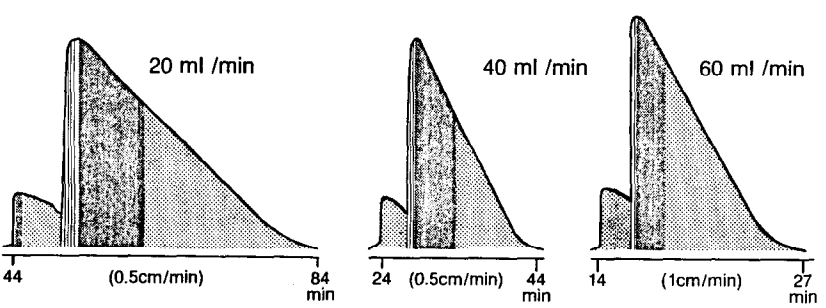
conventional linear elution chromatography. Unlike the chromatograms obtained under experimental conditions permitting the displacement effect to take place, a sharp boundary between the two component bands does not exist. Only a small amount of pure material can be recovered from this 250-mg injection, compared with almost total recovery for a 4-g injection under conditions promoting a strong displacement effect (see Fig. 7).

Clearly, in this example the self-displacement mode is superior for production.

Effect of mobile phase flow-rate on production

In two recent papers, it was predicted through modeling that the optimum value of the mobile phase velocity for maximum production rate is relatively very high, much higher than the optimum velocity for maximum column efficiency [8,9]. We investigated this phenomenon experimentally by repeating the separation of the mixture of diethyl phthalate and β-tetralone at three different flow-rates, 20, 40 and 60 ml/min. Because the flow resistance of the long column used (75 cm) reached the maximum operating pressure of the chromatograph, it was not practical to use higher values of the flow-rate. The results are reported in Figs. 13 and 14.

In Fig. 13, we see that the elution times of the main features of the chromatogram decrease approximately in proportion to the reverse of the flow-rate, as expected. As the sample (4 g) is large, the influence of the column efficiency on the elution profile is moderate and can be neglected as a first approximation. The recovery for 95% pure products increases slightly with increasing flow-rate, from 86% to 90%. The differences are of questionable significance. The recovery for the preparation of 99% pure fractions seems to remain approximately constant (49, 44 and 49%). Plotting the composition of the intermediate fractions *versus* their rank (Fig. 14) again shows only slight differences. The number of fractions to be discarded in order to prepare 99% pure material is nearly the same in all three instances, *i.e.*, eleven or twelve. These numbers reflect minor fluctuations of experimental conditions



Recovery Total Yield, Combined			
≥ 95% Purity	86% (3.4 g)	91% (3.6 g)	90% (3.6 g)
≥ 99% Purity	49% (2.0 g)	44% (1.7 g)	49% (2.0 g)

≥ 99% Purity + ≥ 95% Purity < 95% Purity

Fig. 13. Effect of mobile phase flow-rate on the elution profile of a 4-g sample of a 25:75 mixture of diethyl phthalate and β-tetralone. Chromatograms, recovery and production obtained at different flow-rates. Mobile phase as in Fig. 2.

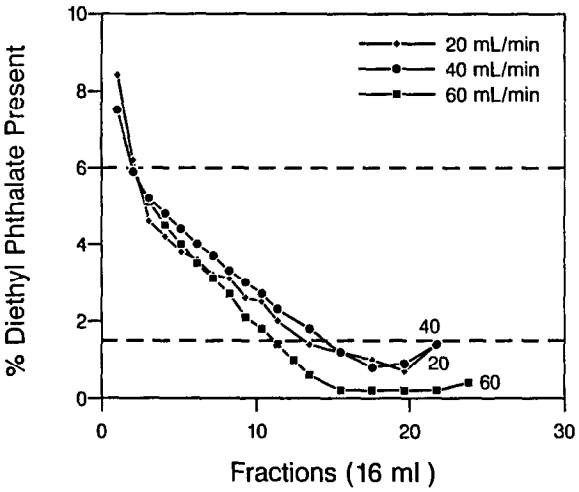


Fig. 14. Effect of mobile phase flow-rate on the elution profile of a 4-g sample of a 25:75 mixture of diethyl phthalate and β-tetralone. Plot of the diethyl phthalate concentration in collected fractions of β-tetralone *versus* the fraction number. The collection of the first 16-ml fraction starts 16 ml after the front of β-tetralone appears in the chromatogram (Fig. 13).

rather than a meaningful distinction. It is seen in Fig. 14, however, that the purity of the usable fractions with 60 ml/min flow-rate is systematically better than in the other two instances.

As the recovery yield and the production per run remain essentially independent of the flow-rate in the range investigated, we may conclude that the production rate increases as the flow-rate increases (*i.e.*, the amount produced per unit volume of mobile phase used is constant) and that the trend may be extrapolated to some extent, as suggested by theory. Obviously, the pressure capacity of the pump and the column tubing used may set limits much lower than the maximum production rate.

Guidelines for method development

The production amounts and rates presented in these studies have major ramifications for the practical purification laboratory. Fortunately, it is easy to induce the self-displacement effect. As one conclusion of this work, we summarize in the following set of guidelines the systematic optimization procedure that has been developed and is being applied routinely in the laboratory to a variety of problems involving compounds that cannot be discussed here. First, however, we should point out that these rules are valid for small production scales, when a small batch of a pure or very pure chemical has to be prepared rapidly, on an intermittent basis. Optimization of preparative processes for the large-scale production of commercial compounds would require and could support more quantitative and systematic investigations.

(1) Using an analytical-scale column, approximate conditions permitting the resolution of the components of interest are generated. A relative retention exceeding 1.1 is highly desirable but not essential. It is not necessary to use exactly matched column sets for scale-up, but it is helpful to select a packing material from the same manufacturer, having the same average particle size as the material in the preparative

column whose use is intended. We have found the optimum particle size to be about 10 μm or smaller. As a general rule, it is better to elute the minor component first, unless the relative retention under conditions where the major component is eluted first is extremely high. Strategies have been developed where the displacement effect can be used to advantage only if the concentration of the second component in the feed is comparable to or larger than that of the first component [17].

(2) A 25-cm long preparative column is equilibrated with the mobile phase of the selected composition. The column should be packed with particles of average size 10 μm or smaller and the flow-rate should be approximately one column volume every 2 min.

(3) Using a sample size of 10–40 mg (larger if the column diameter exceeds 20 mm), the mobile phase composition is adjusted for a capacity factor between 3 and 7 for the first component, if possible. Larger values of k' are acceptable but the recovery and/or the production will be lower. If a choice must be made between conditions giving maximum relative retention or an adequate value of k', the optimization of the former parameter is more important.

(4) A series of three 25-cm preparative columns are equilibrated with the mobile phase selected. Alternatively, one column is used in the recycle mode.

(5) the maximum load depends on the relative retention and the relative composition of the feed mixture. In most instances it will exceed several grams on a standard 21.5 mm I.D. column (conventional 1 in. O.D.) when α is greater than 1.1.

(6) The conditions obtained by following the procedure just described are not truly optimized, but they should provide good recoveries and excellent production rates. It may prove useful to experiment further with the flow-rate and the mobile phase composition to reduce the retention while keeping a high value of the relative retention.

CONCLUSIONS

The results of this study confirm the generality of the sample self-displacement effect. It takes place in all the preparative separations we have tried, provided that the sample size is large enough and that the bands of the components of the mixture interact during the separation. The displacement effect is stronger at high concentrations (large sample size, moderate retention factors) and high relative retention of the two components involved. Using the guidelines we have established, it is easy to develop separation methods where this effect is enhanced and used to advantage. High recovery yields can be achieved in spite of a poor-looking chromatogram and production rates are obtained which far exceed those possible under the more classical conditions corresponding to nearly linear behavior. The use of a high flow-rate does not reduce the yield significantly and further enhances the production rate.

The advantages of separations carried out under conditions that maximize the self-displacement effect are important and obvious in the case of the first component. For this reason, the compound of greatest interest in a feed should be eluted as early as possible. When the production of purified fractions of the second component is desired, the choice is less clear. This production must be balanced against losses in yield. Purity and, consequently, recovery are diminished by the tailing of the first component into the band of the second. In most instances, it may be better to reduce

the sample size, compared with the size preferred for purification of the first component. However, significantly large yields can sometimes be obtained and production rates of the second component may be substantially greater overall in comparison with those arising from conventional "touching bands" methodology. Even when complete resolution between two high concentration bands is achieved (Fig. 10), the displacement effect has been used with advantage, effectively compressing the first component band and increasing the resolution [9].

As a general rule, the production rate is much increased by operating the column under non-linear conditions.

ACKNOWLEDGEMENTS

We thank Mr. Norman Cole and Dr. Philip Funke (Squibb Institute for Medical Research, Princeton, NJ) for their support of this project. This work was supported in part by grant CHE-8901382 of the National Science Foundation and by the cooperative agreement between the University of Tennessee and the Oak Ridge National Laboratory.

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