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SOME ASPECTS OF PREPARATIVE-SCALE LIQUID CHROMATOGRAPHY

R. P. W. SCOTT and P. KUCERA
Chemical Research Department, Hoffmann-La Roche, Inc., Nutley, N.J. 07110 (U.S.A.)
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SUMMARY

The various factors that affect the loading capacity of a column are considered and it is shown that large-scale separations can only be achieved at the individual or combined expense of speed, scope and solvent consumption. An equation that predicts the maximum sample volume that can be placed on a column without impairing resolution is derived and experimentally verified. It is shown that, when the sample volume is progressively increased, the fronts of all peaks remain at constant retention while the retention of the rears of the peaks progressively increases. Conversely, under circumstances of mass overload the rears and fronts of all peaks tend to move toward positions of less retention, the maximum effect occurring with the major component in the mixture.

INTRODUCTION

The term preparative-scale chromatography can be applied to a wide range of sample loads from a few milligrams to tens, or perhaps even hundreds, of grams of material being separated at one time. A few milligrams of a pure substance may be required for structure elucidation by appropriate spectroscopic techniques or the isolation of 5–50 g of a pure intermediate may be needed for subsequent synthetic work. High levels of sample load will often require a range of special columns to be available together with suitable chromatographic apparatus specially designed to take such columns; further, appropriate operating conditions for such columns must also be known.

Considering the four attributes that can be obtained from a chromatographic system, namely, resolution, speed, scope and load, it is fairly obvious that to obtain any two of these attributes in excess, the other two will have to be sacrificed. Thus, in preparative-scale chromatography, load must be obtained at the expense of speed and scope and, in some instances, with reduced resolution.

For any chromatographic system there is a limiting charge that can be placed on the column before the resolution is impaired and it is obviously important to determine those parameters of the column system that will control the magnitude of the limiting charge. Loss of resolution from column overload can arise from two causes,

either excessive sample feed volume or excessive sample mass, the former usually resulting from poor solubility of the sample in the mobile phase.

During chromatographic development of a mixture, the individual solutes become separated and it is the capacity of the column to carry the individual solutes that largely limit the initial charge. Thus, the maximum sample load is limited by the level of the major component or components in the mixture together with the properties of the particular column and mobile phase employed. In this paper the general properties of a column that limit the sample size are discussed together with the effect of column overload by both excessive sample feed volume and excessive sample mass.

FACTORS THAT AFFECT THE CHARGE THAT CAN BE PLACED ON A LIQUID CHROMATOGRAPHIC COLUMN

For any column operating under given conditions it has been shown[1] that both the maximum sample feed volume and the maximum solute mass that can be placed on the column without impairing the column efficiency are directly proportional to plate volume and the square root of the efficiency. Thus

$$M = A\sqrt{n}\,(v_m + Ka_s)$$

where

M = limiting mass of sample
n = column efficiency
v_m = volume of mobile phase/plate
a_s = surface area of adsorbent per plate
K = distribution coefficient of the solute defined in appropriate terms
A = constant

Now

$$V_r = n\,(v_m + Ka_s)$$

where V_r is the retention volume of the solute.
Thus

$$M = \frac{AV_r}{\sqrt{n}}$$

If the peak is well retained, it can be considered that $v_m \ll Ka_s$ and further

$$V_r \rightarrow nKa_s \rightarrow VKdA_s \rightarrow \pi r^2 lKdA_s$$

where

V = volume of the column
r = radius of the column
l = length of the column
d = packing density of the adsorbent in g/ml
A_s = surface area of the adsorbent in m^2/g

Hence

$$M = \frac{A\pi r^2 dlKA_s}{\sqrt{n}}$$

Assuming n is a fixed value $(n)_c$ that just provides the necessary resolution between the solutes of interest then

$$(n)_c = \frac{l}{(h)_c}$$

where $(h)_c$ is the height equivalent to the theoretical plate at a constant linear mobile-phase velocity.

Further, for a significantly retained peak, where the band dispersion is largely determined by the resistance to mass transfer factors, it follows from the HETP equation that at a given flow-rate

$$h \approx \alpha\, d_p^2$$

where

α = constant
d_p = particle diameter of the support

It follows that

$$M = A\pi r^2 lKdA_s \left(\frac{\alpha d_p^2}{l}\right)_c^{\frac{1}{2}} \quad (1)$$

Eqn. 1 gives the various column parameters that condition the maximum load that can be placed on a column. The effect of these parameters will now be considered individually.

Column radius (r)

The loading capacity of a column will increase with the square of the radius or directly with the column cross-sectional area. Increasing the cross-sectional area of the column will also increase the quantity of stationary phase/plate and consequently the plate volume. Increasing r will not increase the retention time of the solute providing the column flow-rate is also proportionally increased to maintain the same mobile-phase linear velocity. It follows that, by increasing the column radius, more mobile phase is utilized for the greater loading capacities obtained. However, it should be pointed out that the solvent economy remains the same as the consumption of solvent per unit mass of solute separated will be independent of the column radius if the same mobile phase linear velocity is employed.

Distribution coefficient (K)

Increasing the distribution coefficient K will result in an increase in retention volume of the solute and thus the column loading capacity. K can only be increased for a given column by changing the nature or composition of the mobile phase, which

will result in both an increase in retention time and a greater volume of mobile phase being used. As the maximum sample load and the solute retention volume both increase linearly with the value of K, however, the solvent economy, in terms of the volume of solvent required to separate unit mass of solute, will remain constant.

Packing density (d)

The effect of packing density is theoretically important but, in practice, is not a significant variable as all columns that are reasonable well packed have packing densities approximately similar and close to their maximum.

Surface area per gram of adsorbent (A_s)

The loading capacity of a silica gel will increase with the surface area of the adsorbent for those silica gels currently in use for liquid chromatography[2]. However, high-surface-area adsorbents used, under isocratic conditions of development, will result in a narrow solute polarity range in which to operate; the polarity of the mobile phase being, of necessity, close to that of the solutes being separated. Thus, employing adsorbents of high surface area to increase the loading capacity of the column requires that both the speed and the scope of the chromatographic system are sacrificed.

Column length (l) and particle diameter (d_p)

It is seen from eqn. 1 that l and d_p are related, insomuch that the ratio d_p^2/l must remain constant to maintain the necessary minimum efficiency to achieve the required resolution. It follows that the effect of both l and d_p on the column loading capacity must be considered together but it should be emphasized that this is true only when loading effects of preparative-scale columns are being considered. From eqn. 1 it is seen that the loading capacity of the column will increase as the square root of the column length, but (d_p) has to be increased appropriately together with l if the efficiency is to be kept to the same minimum requirement for resolution. It follows that the column will become longer but be packed with coarser particles. Using eqn. 1 the fractional increase in load with column length and particle diameter is shown in Table I for a 25 cm × 4.6 mm I.D. column packed initially with 10-μm particle diameter silica gel.

It is seen from Table I that the loading capacity of the column can be doubled by increasing the column length by a factor of four and the particle diameter of the adsorbent by a factor of two. Increasing the loading capacity in this way also increases the solute retention volume and thus sacrifices both speed of separation and solvent

TABLE I

RELATIONSHIP BETWEEN COLUMN LENGTH, PARTICLE DIAMETER, RELATIVE PERMEABILITY AND RELATIVE LOADING CAPACITIES FOR COLUMNS HAVING A CONSTANT CROSS-SECTIONAL AREA AND THE SAME EFFICIENCIES

Column length (cm)	*Particle diameter (μm)*	*Relative loading capacity*	*Relative permeability*
25	10	1.0	1.0
50	14	1.4	2.0
75	17	1.7	3.0
100	20	2.0	4.0

economy as in this case the consumption of solvent per unit mass of solute separated would be doubled. However, in the example given, the column permeability is also increased by a factor of 4 and, as the column length is increased by the same ratio, no increase in column inlet pressure is required. When working with large-diameter preparative columns, it is highly desirable to maintain high column permeability in order to make less stringent the specifications of wall thickness to provide adequate mechanical strength.

It should be emphasized that the above conditions apply to columns that carry the maximum load but are not overloaded in the sense that the normal band width is not exceeded by more than 5%[1]. If the 25-cm-long column is initially overloaded then the advantages derived from increasing the column length may well be in excess of the relative loading capacities given in Table I.

COLUMN OVERLOAD

In practice it is usually not possible to construct a specific column for each preparative-scale sample presented for separation. Normally a limited number of columns are available for preparative work and the conditions of separation have to be adjusted for each sample to meet the limitations of the columns that can be used. The mobile phase is usually chosen to provide a suitably high separation ratio between the solute peaks of interest and then the column is overloaded, until the peak of the solute to be isolated is just separated from its closest neighbor. A column can be overloaded in three acceptable ways, *viz.*

(a) When samples that are relatively insoluble in the mobile phase are being separated, the column may be overloaded with respect to sample feed volume.

(b) For samples very soluble in the mobile phase, column overload can arise from excess of sample mass.

(c) Addition of sample in a solvent less polar than the mobile phase and which does not permit development to take place. Thus, when the sample is placed on the column, the solutes pile up on the front of the column and development only occurs when the more polar mobile phase commences to flow through the column. The net effect of this procedure is mass overload. A further possible method would be the addition of the sample in a solvent more polar than the mobile phase to overcome gross insolubility problems. This method is not recommended, however, as the more polar sample solvent would deactivate the silica gel and render the chromatographic properties of the column unstable.

The effect of overload due to sample feed volume will first be quantitatively examined.

(a) Column overload due to sample volume

Consider the situation explicitly depicted in Fig. 1. To determine the band spreading effects resulting from significant sample volumes the principle of the summation of variances should be employed. However, when the sample volume becomes excessive, the band spreading that results from column overload becomes equivalent, to the first approximation, to the sample volume itself. Thus, referring to Fig. 1 the peak separation in milliliters of mobile phase will be equivalent to the volume of sample plus the sum of half the base width of the respective peaks. Bearing in mind that half

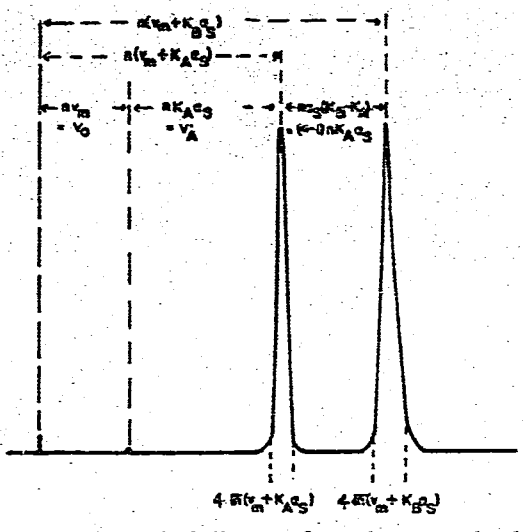

Fig. 1. Theoretical diagram for volume overload.

the peak width at the base is approximately twice the standard deviation of the peak, then from the plate theory[3], assuming that both peaks have the same efficiency, n.

$$(\alpha - 1)nK_A a_s = V_L + 2\sqrt{n}\,(v_m + K_A a_s) + 2\sqrt{n}\,(v_m + K_B a_s)$$

where

n = column efficiency
K_A and K_B = distribution coefficients of solutes A and B, respectively
α = separation ratio of solute B to solute A
V_L = sample overload volume

Rearranging

$$V_L = (\alpha - 1)nK_A a_s - 2\sqrt{n}\,[(v_m + K_A a_s) + (v_m + K_B a_s)]$$

Noting that

$$nK_A a_s = V'_A,\quad nK_B a_s = V'_B,\quad V'_A/V_0 = k'_A,\quad V'_B/V_0 = k'_B$$

and

$$k'_B = \alpha k'_A$$

where

$$V_0 = nv_m = \text{column dead volume}$$

$$V_L = V_0\left[(\alpha - 1)\,k'_A - \frac{2}{\sqrt{n}}(2 + k'_A + \alpha k'_A)\right] \qquad (2)$$

Eqn. 2 allows the calculation of the maximum volume of sample that can be placed on the column to maintain the separation of solutes A and B (B being eluted later than A) in terms of the column dead volume, the column efficiency, the separation ratio of solute B to solute A and the respective k' values of solutes A and B.

Taking a practical example of a preparative 4 ft. × 1 in. I.D. column having a dead volume of 300 ml and an efficiency of 2000 theoretical plates, the maximum sample volume was calculated using eqn. 2 for solutes of different α values and k'_A values. The results are shown in Fig. 2. It can be seen from Fig. 2 that the maximum overload charge varies widely from just over 200 ml for an α value between the solutes of 1.2 and a k'_A value of 2 to well over 2 l for solutes having an α value of 2.0 and the first solute having a k'_A value of 6. For solute pairs having high separation ratios chromatographed on large columns having dead volumes of 300 ml or more the volume of sample can be exceedingly high before the resolution of the solutes is impaired. The validity of eqn. 2 was examined experimentally.

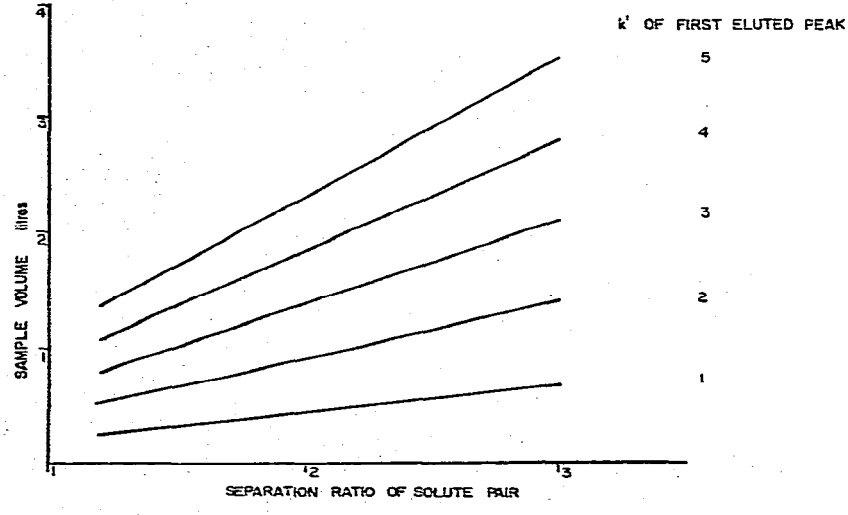

Fig. 2. Graph of maximum sample volume against separation ratio of solute pair for solutes eluted at different k' values.

Experimental. The apparatus used was a Waters Assoc. Model M-6000 pump which was connected directly to a Valco Model ACV-6-UHP-C-20 sample loop valve. The column was 25 cm × 4.6 mm I.D. and packed with Partisil 10 silica gel by the balanced density slurry packing technique described by Majors[4]. The column exit was connected to a LDC duomonitor, Model 1222 UV detector and operated at 254 nm. In order to demonstrate the effect of sample feed volume overload, the mobile phase chosen was heptane and the solutes employed were benzene, naphthalene and anthracene. The mass of sample employed was kept constant at 176 μg of benzene, 9 μg of naphthalene and 0.3 μg of anthracene, but sample volumes of 1 μl, 1 ml, 2 ml and 3 ml were injected and the chromatograms obtained are shown on the left-hand side of Fig. 3. The dead volume of the column determined from the retention volume

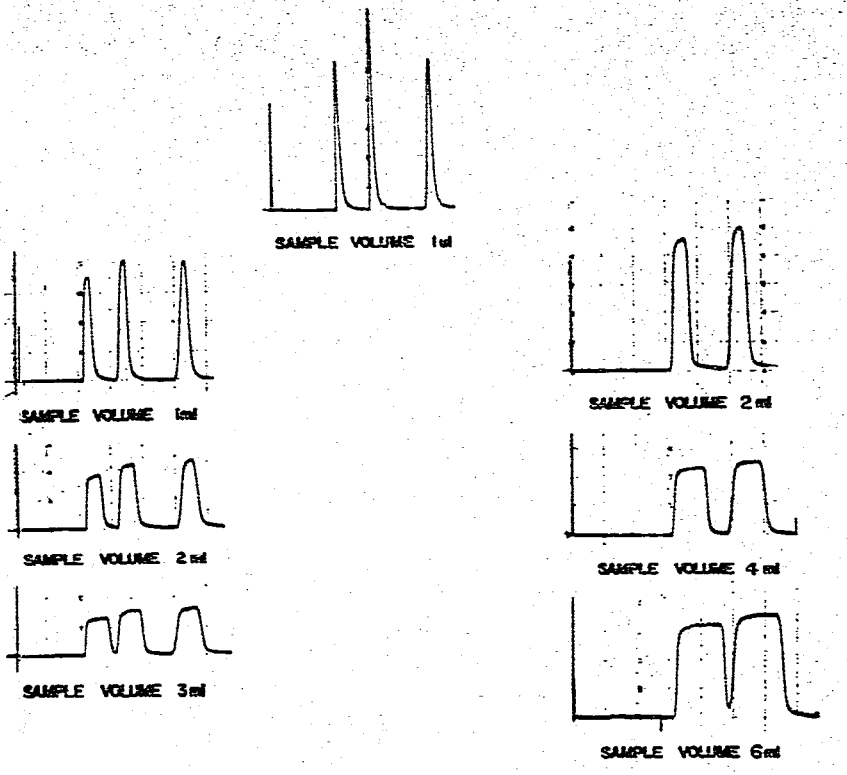

Fig. 3. Chromatograms demonstrating volume overload. Column, 25 cm × 4.6 mm I.D.; flow-rate, 1 ml/min; solutes, benzene, naphthalene and anthracene.

of an unretained solute was shown to be 3.48 ml and the retention volumes of benzene, naphthalene and anthracene were found to be 4.11, 8.11 and 15.0 ml, respectively. The efficiencies and k' value for each solute are shown in Table II together with the maximum permissible feed volume calculated from eqn. 2.

It is seen from Fig. 3 and Table II that the maximum permissible feed volume for benzene is accurately predicted by eqn. 2. In the same manner 2, 4 and 6 ml of solutions containing a total of 9.0 and 0.3 μg of naphthalene and anthracene, respectively, were injected onto the column and the chromatograms obtained are shown on

TABLE II

CALCULATED MAXIMUM FEED VOLUME FOR BENZENE AND NAPHTHALENE WHEN CHROMATOGRAPHED IN THE PRESENCE OF ANTHRACENE TOGETHER WITH THEIR RESPECTIVE k' VALUES AND EFFICIENCIES

Parameter	*Benzene*		*Naphthalene*		*Anthracene*
k'	1.18		2.33		4.31
n	1850		4480		5470
α	<	1.97	><	1.85	>
V_L	3.1 ml		6.1 ml		—

the right-hand side of Fig. 3. It is seen that again the maximum volume of charge for naphthalene that can be used while maintaining resolution between naphthalene and anthracene is accurately predicted by eqn. 2.

The sample feed volume overload experiments were then repeated over the range of 1–3 ml and 1–6 ml for the benzene–naphthalene–anthracene mixtures and naphthalene–anthracene mixtures, respectively. However, in the former case the sample feed volume was increased in 0.5-ml increments and in the latter in 1.0-ml increments. In Figs. 4 and 5 the retention of the front and back of each peak measured at the peak base is plotted against the volume of charge.

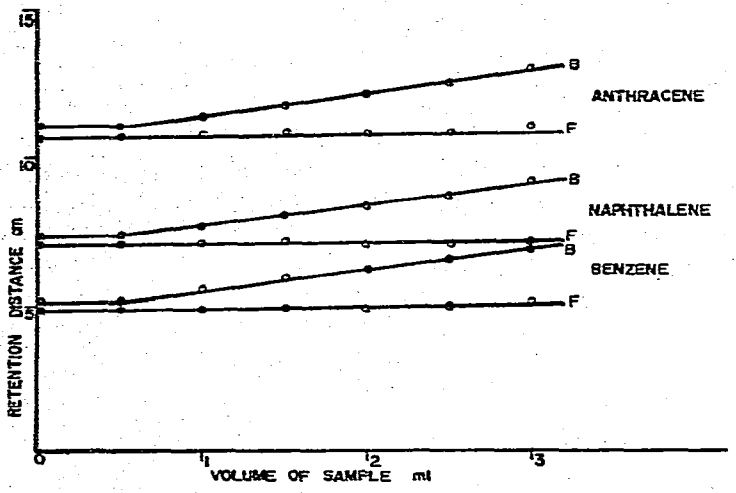

Fig. 4. Graph of retention distance of benzene, naphthalene and anthracene against volume of sample injected. B = Back of peak; F = front of peak.

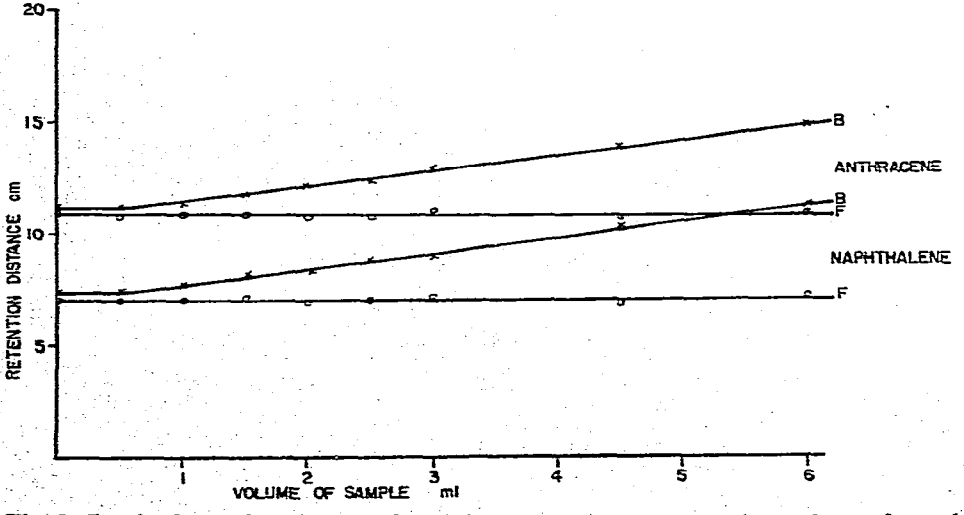

Fig. 5. Graph of retention distance of naphthalene and anthracene against volume of sample injected. B = Back of peak; F = front of peak.

Discussion. It is seen from Figs. 4 and 5 that the peak front for each solute has a constant retention distance irrespective of the volume of the charge. The retention of the back of the peak, however, only remains constant up to a charge level of 0.5 ml for the three-component mixture and 1 ml for the two-component mixture. Subsequent to this, the peak width increases linearly with the volume of the charge. The spread of the peaks is also the same for each solute and is not dependent on the nature of the solute or its k' value. It should be noted that, at the predicted feed volume of 3 ml, the back of the benzene peak has just reached the front of the naphthalene peak. In a similar manner, at a charge of 6 ml, the back of the naphthalene peak has just reached the front of the anthracene peak. The spread of the peaks toward greater retention times is characteristic of volume overload and, as will be seen later, is in contrast with the effect of mass overload, where the peaks spread toward reduced retention times as the mass of charge is increased.

Column overload resulting from increased feed volume of sample distorts the normal elution process of development toward frontal analysis and, in fact, when elution development is carried out with progressively increasing sample volume, the distorted elution development culminates in frontal analysis. This transition from elution development to frontal analysis is clearly shown in Fig. 6, where progressively larger volumes of charge were placed on the column up to a maximum of 16 ml. Frontal analysis curves can demonstrate the need to take certain precautions when employing injection procedures using a valve and sample loop. Owing to the parabolic velocity profile of the sample and mobile phase in the sample tube as the sample is pumped onto

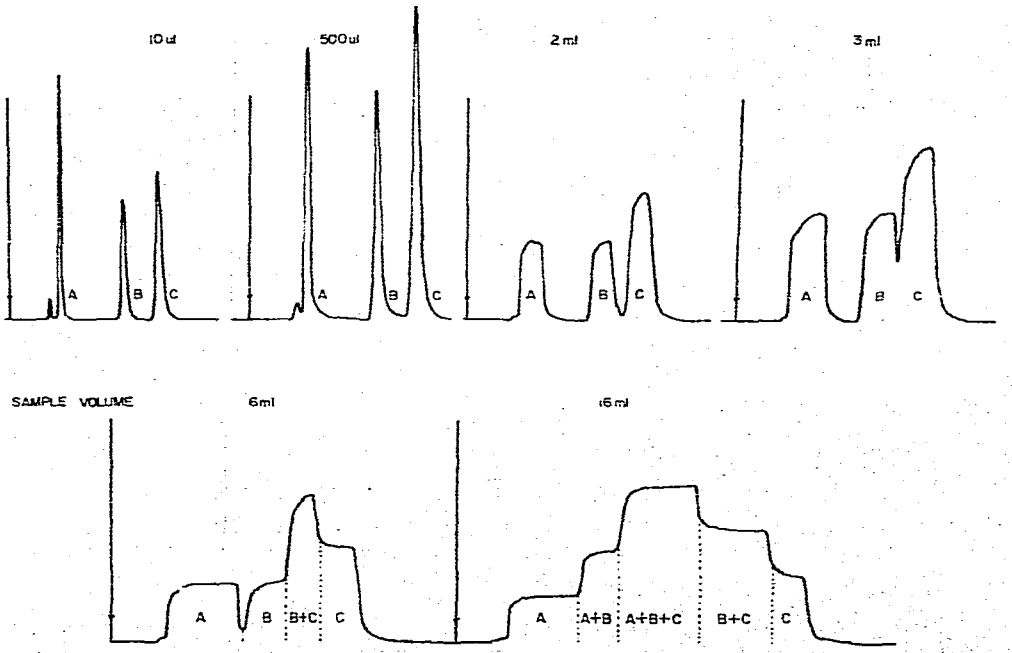

Fig. 6. Transition from sample volume overload to frontal analysis development. Column, 25 cm × 4.6 mm I.D., packed with Partisil 10; mobile phase, 5% (v/v) diethyl ether in *n*-heptane; flow-rate, 1 ml/min; sample volume, as indicated in the figure; solutes, (A) anisole, (B) benzyl acetate, and (C) acetophenone.

the column, dispersion of the sample load occurs resulting in the concentration profile of the sample taking the form of a tailing Poisson curve as opposed to a rectangular slug. Thus, if the sample loop is left in line with the mobile phase flow subsequent to the sample being injected, the peaks will exhibit serious tailing and this is demonstrated in the lower frontal analysis curves shown in Fig. 7. It is seen that the descending steps of the curves, which correspond to the tails of the normal elution curves, are very diffuse compared with the ascending steps, which correspond to the fronts of the normal elution curve. If the sample is placed on the column by allowing the mobile phase to flow through the sample loop for a given time, which is determined from the flow-rate and volume of sample selected for injection, and then the valve rotated to allow the mobile phase to pass directly to the column, the dispersion effect of the sample tube is eliminated. The improved effect of this method of sampling is shown in the upper frontal analysis curve in Fig. 7. It is seen that the ascending steps of the curve are very similar to the descending steps of the curve and the diffuse character of the descending steps in the lower frontal analysis curve has been virtually eliminated.

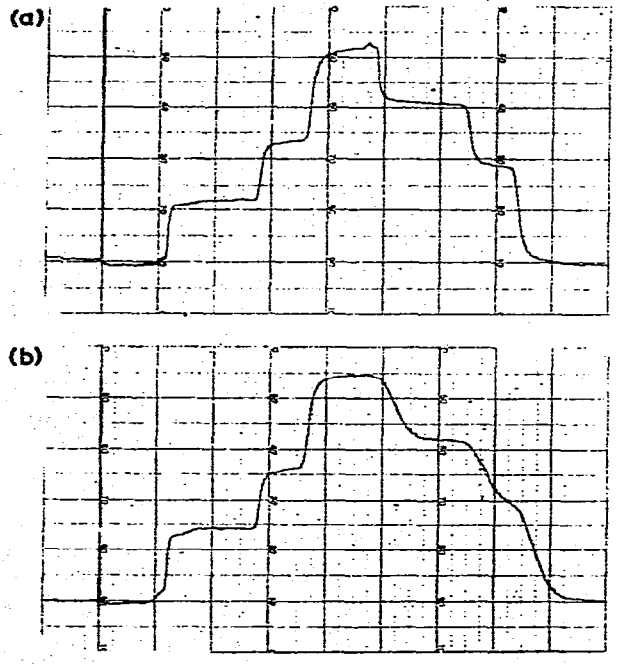

Fig. 7. Frontal analysis curves demonstrating dispersion effects resulting from the incorrect use of sample loop. (a) Sample loop turned off-line immediately on completion of injection. (b) Sample loop kept in-line during the entire chromatographic development. Column, 25 cm × 4.6 mm I.D., packed with Partisil 10; flow-rate, 1 ml/min; sample volume, 16 ml; solutes, benzene, naphthalene, and anthracene.

(b) Column overload due to sample mass

The effect of excessive sample mass on the development process that takes place in the column is extremely complicated, and for this reason it is difficult to treat mass overload quantitatively. A large mass of sample will modify the development

process in basically three ways. First, there will be a dispersive effect resulting from the limited capacity of the stationary phase. The sample will spread along the column, carried by the mobile phase, until it contacts sufficient adsorbent to permit it to be held on the stationary phase surface under equilibrium conditions. This will result in band spreading similar in form to sample volume overload and, if it were the sole effect of mass overload, could be treated quantitatively in a similar manner. The peaks so formed would be square topped and of similar shape to those shown in Fig. 3. However, superimposed on this band spreading process is the second effect of mass overload that results from the deactivation of the adsorbent and the increased effective polarity of the mobile phase. If the charge is massive, the sample occupies a significant portion of the column immediately after injection resulting from the first effect described above. The adsorbent thus becomes partially deactivated all the while the overloaded solute is in the column, causing all solutes contained in the mixture to be eluted more rapidly at reduced retention times. This effect is further aggravated by the higher polarity of the mobile phase resulting from the high concentration of the overloaded solute in mobile phase which will also cause other solutes to be eluted more rapidly. Thus, as a result, the retention times of all the solutes are reduced. Finally, the high concentrations of solute on the adsorbent surface that result from mass overload will result in non-linear adsorption isotherms and the eluted peaks will exhibit pronounced tails.

Experimental. The same chromatographic system used for volume overload study was also employed to investigate mass overload. It was considered interesting to demonstrate mass overload on two mobile phase–solute systems differing in polarity.

The first mobile phase system used was pure *n*-heptane and in this case the volume of sample was kept constant at 200 μl for the solutes benzene, naphthalene and anthracene. A chromatogram of a reference sample which contained 180 μg of benzene, 9 μg of naphthalene and 0.3 μg of anthracene is shown in Fig. 8. The mass of benzene was then increased to 8.1 mg and then to 16.9 mg. These chromatograms were obtained operating the UV duomonitor at sensitivity settings of × 0.02–0.64 where

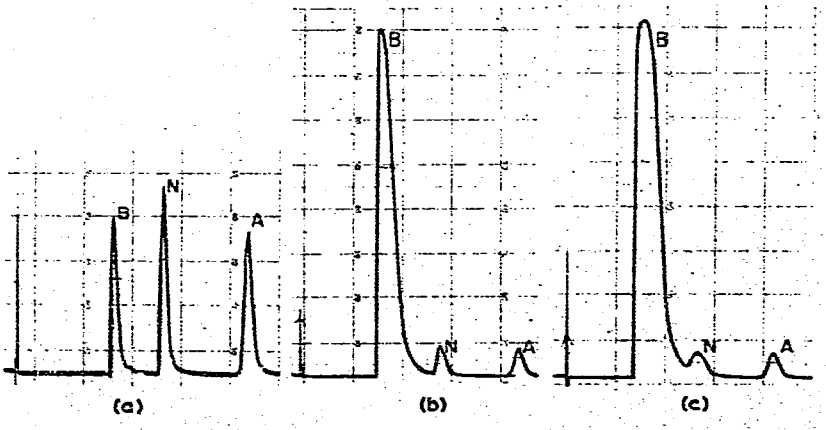

Fig. 8. Chromatograms demonstrating mass overload. Column, 25 cm × 4.6 mm I.D.; flow-rate 1 ml/min. Solutes: B = benzene; N = naphthalene; A = anthracene. (a) B 180 μg, N 9 μg, and A 0.3 μg; (b) B 8.1 mg, N 9 μg, and A 0.3 μg; (c) B 16.9 mg, N 9 μg, and A 0.3 μg.

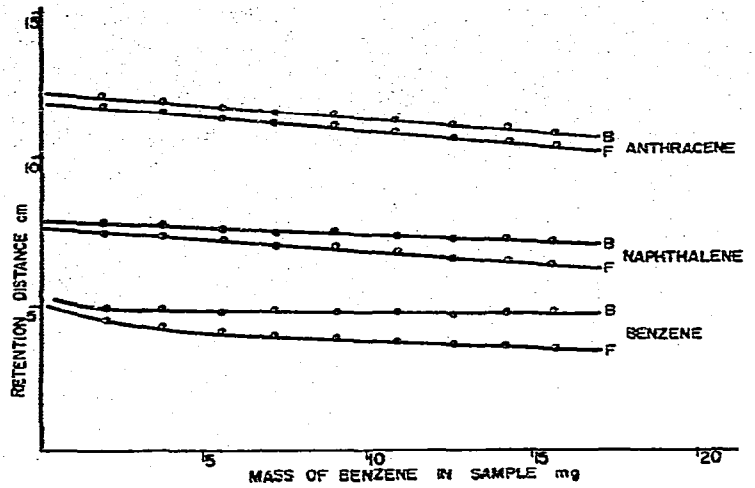

Fig. 9. Graph of retention distance of benzene, naphthalene, and anthracene against mass of benzene contained in sample injected. B = Back of peak; F = front of peak.

UV absorption is still linearly related to concentration of solute. From these chromatograms retention distances of the front and back inflection points (at 0.607 of the peak height) were measured and plotted against the mass of sample injected. The results obtained are shown in Fig. 9. In a similar way and under identical conditions the mass overload from naphthalene was investigated using a mixture of naphthalene and anthracene and progressively increasing the mass of naphthalene in the mixture from 1.1–19.1 mg. This work was done using the refractomonitor detector operated at sensitivities 1–128 × since the absorptivity for naphthalene is relatively high and the UV detector could not be used. A graph similar to that for benzene was obtained from these experiments for and is shown in Fig. 10.

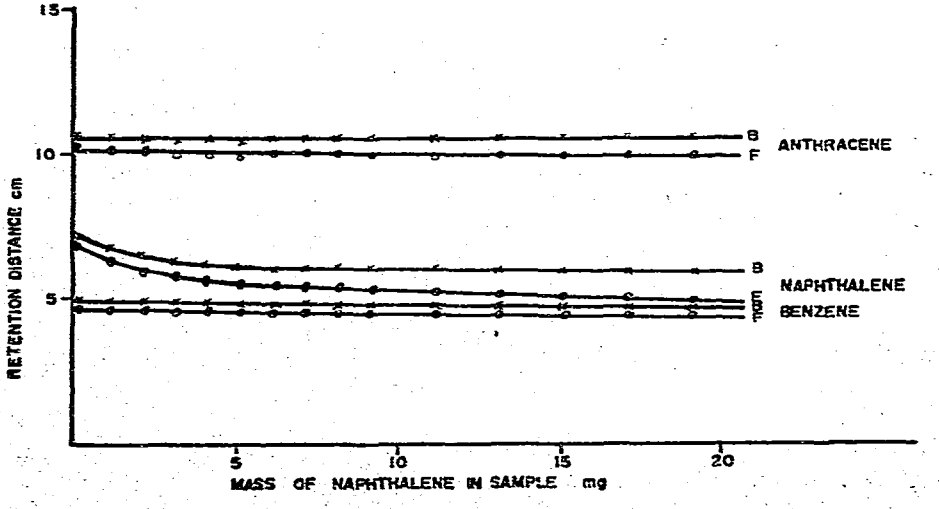

Fig. 10. Graph of retention distance of benzene, naphthalene, and anthracene against mass of naphthalene contained in sample injected. B = Back of peak; F = front of peak.

The second mobile phase (5% (v/v) diethyl ether in heptane) was employed with solutes anisole, benzyl acetate and acetophenone using the same conditions as given above.

The reference chromatogram with no overload was obtained using 19.8 μg of anisole, 44.6 μg of benzyl acetate and 20.5 μg of acetophenone. The sample volume was again kept constant at 200 μl and the mass of benzyl acetate was progressively increased over the range from 1.1–30 mg and a plot of retention distances of front and back inflection points of the elution curves against the mass of benzyl acetate injected is shown in Fig. 11. The UV monitor operated at sensitivities ranging from 0.02–0.64 × was used as the detector.

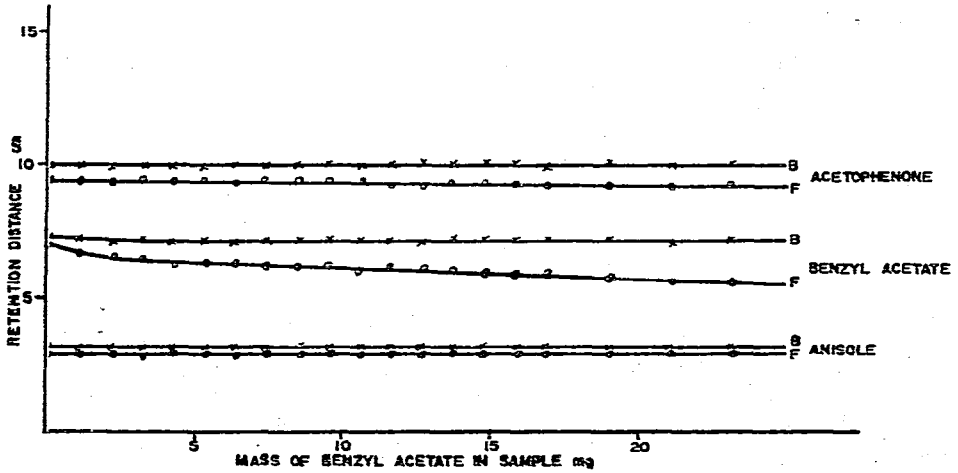

Fig. 11. Graph of retention distance of anisole, benzyl acetate, and acetophenone against mass of benzyl acetate contained in sample injected. B = Back of peak; F = front of peak.

Discussion. The chromatograms demonstrating the effect of mass overload in Fig. 8 show a normally loaded column to the left whereas the center and right-hand side chromatograms are for the same sample but are overloaded with 8 mg and 16 mg of benzene, respectively. The three mass overload effects are clearly demonstrated on both chromatograms. In both chromatograms the benzene peaks have significantly broadened, as would be expected from the massive charges employed, and exhibit gross asymmetry together with long tails. It is obvious that the concentrations of benzene in both the mobile and the stationary phases have reached those levels where the distribution isotherm is no longer linear. It is also clearly seen that the retention times of all three solutes have been significantly reduced. As discussed previously, this results from both the deactivation of the adsorbent by the excessive amount of benzene in the column together with the increased polarity of the solvent as the mobile phase is no longer pure heptane but a solution of benzene in heptane. In Fig. 9 the retention distance of the front and rear of each peak, measured at 0.607 of the peak height, for benzene, naphthalene and anthracene is shown plotted against mass of benzene injected. The change in retention is clearly indicated, the maximum effect being for the solute anthracene and the minimum for the overloaded solute benzene. It should be

noted that there is little change in band width of the most retained solute, anthracene, with increase in mass of benzene injected. There is, however, a significant increase in the band width of the naphthalene peak and a massive spread in the band width of the benzene peak. It is also clearly demonstrated that the short 25-cm column can cope with a charge of 16 mg and still achieve an effective separation from napthalene at a level of 0.056% and anthracene at a level of 0.002% of the original mixture. The naphthalene peak, however, will be significantly contaminated with benzene as it is eluted in the tail of the benzene peak.

In Fig. 10 the effect of mass overloading of naphthalene is shown where the retention distances of the front and rear of each peak for naphthalene and anthracene, measured at 0.607 of the peak height, are plotted against mass of naphthalene injected. It is seen that again the retention times of all peaks are reduced but not to the same extent as with benzene overload. This indicates that, as the distribution coefficient of the naphthalene with respect to the stationary phase is larger than that of benzene, the deactivation effect on the silica gel is greater but the concentration of naphthalene in the mobile phase will be small. It follows that the greater change in retention resulting from overload of the solute benzene results more from the increased polarity of the mobile phase due to the high concentration of solute than to the excess of solute deactivating the silica gel. In Fig. 11 the effect of mass overload is demonstrated for more polar solutes chromatographed using a polar mobile phase. The curves represent the retention distances of the front and rear of the peaks for the solutes anisole, benzyl acetate and acetophenone and are very similar to the curves shown in Fig. 10 for the mass overload of naphthalene using heptane as the mobile phase. It is seen that there is little change in the retention of anisole and acetophenone although the band width of acetophenone increases significantly as the loading of benzyl acetate is increased. The band width of the benzyl acetate peak exhibits the expected band broadening towards lower retention but again it is interesting to note that the retention distance of the rear of the band for the overloaded peak remains sensibly constant.

CONCLUSION

The loading capacity of a column is dependent on the column radius, solute distribution coefficients, packing density, adsorbent surface area, column length and particle diameter. The loading capacity of the column can be increased by adjustment of any of these factors but will only be achieved at the expense of scope, speed of separation, solvent consumption and, sometimes, partial resolution. Although sample consumption will increase as the loading capacity of the column increases, the solvent required per unit mass of solute can remain constant and thus solvent economy expressed in these terms may not be affected. The maximum sample feed volume that can be employed for any given column, solvent or solute concentration can be calculated by a simple equation from the k' value and efficiencies of the respective solute peaks, the separation ratio between solute peak of interest and its nearest neighbor, and the column dead volume. Excessive sample feed volume will result in the retention distances of the peak front remaining sensibly constant, while the retention distances of the rear of all peaks will progressively increase as the volume of sample is made larger. The ultimate effect of increasing the sample volume will be a transition from elution development to frontal analysis. The effect of mass overload is more complicated as it

involves operating under conditions where the adsorption isotherm is non-linear and further, owing to the massive load of solute, the adsorbent becomes partially deactivated and the polarity of the mobile phase is increased. As a result a simple explicit equation cannot be deduced to predict the effect of mass overload. The net effect of mass overload, in contrast to volume overload, is to reduce the retention distance of the front of all peaks as the mass of solute in the sample is increased while the retention distances of the rear of all solute peaks decrease to a lesser degree. The reduction in retention distance of the peak front is greatest for the solute present in the largest amount.

REFERENCES

1 A. Klinkenberg, in R. P. W. Scott (Editor), *Gas Chromatography 1960*, Butterworths, London, p. 182.
2 R. P. W. Scott and P. Kucera, *J. Chromatogr. Sci.*, 12 (1974) 473.
3 H. Purnell (Editor), *Gas Chromatography*, Wiley, New York, 1962, p. 86.
4 R. E. Majors, *Anal. Chem.*, 44 (1972) 1722.