Journal of Chromatography, 125 (1976) 43–57

CHROM. 9570

SAMPLE LOADING AND EFFICIENCY IN ADSORPTION, PARTITION AND BONDED-PHASE HIGH-SPEED LIQUID CHROMATOGRAPHY

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SUMMARY

With the advent of liquid chromatography columns of extremely high efficiency the effect of sample load on the height equivalent to a theoretical plate (HETP) of the column can be more clearly studied. Previously, the inefficiency of the columns masked the peak broadening due to sample load and deterioration of performance from this cause was only seen to occur at loadings of the order of 100 μg/g of adsorbent. It has now been found that the HETP is a linear function of sample load above about 1 μg/g for microparticulate silica adsorbents which have been slurry packed to give columns with minimum HETP values of between 0.02 and 0.05 mm. The degree of dependence has been found to be a function of both the capacity ratio of the solute and the surface area of the adsorbent.

The effect of linear velocity and particle size will be indicated and the relevance of this to the design of preparative liquid chromatography columns will be discussed.

Addition of both physically and chemically bonded stationary phases to silica adsorbents is common place in high-speed liquid chromatography and the effect of these changes on the capacity of the columns is an important consideration which will also be outlined.

INTRODUCTION

The relationship between the efficiency of liquid chromatographic (LC) columns and the weight of the applied sample is of considerable importance. It is important in two different ways, the first, so that maximum efficiency is attained in analytical separations, and secondly, in that a thorough understanding of the relationship is necessary to carry out preparative separations successfully.

One of the first investigations into the effect of sample load on efficiency was reported by Snyder[1] who, from his experimental data on adsorption chromatography, plotted the now familiar type of curve shown in Fig. 1. In this the efficiency (measured as the height equivalent to a theoretical plate (HETP)) and the corrected retention volume per gram are both plotted against the weight of sample applied per gram of adsorbent. From this type of graph, a value for the linear capacity of the adsorbent

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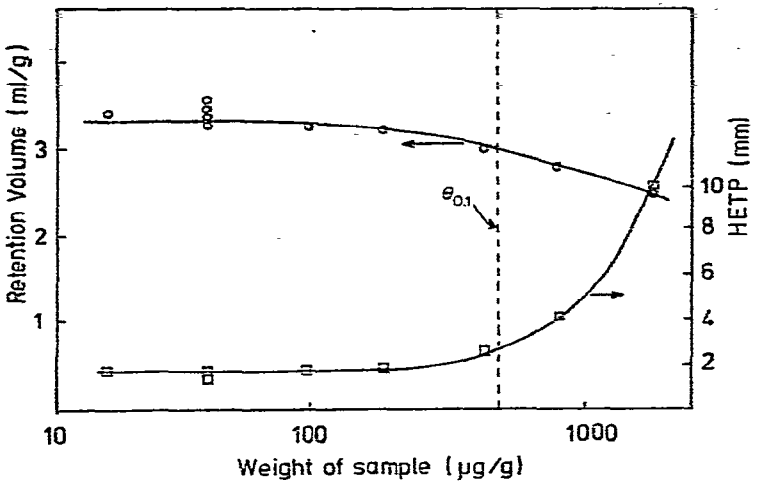

Fig. 1. Retention volume and HETP values plotted against weight of sample per gram of a silica adsorbent. Re-drawn from Snyder[1] with slight modification.

($\theta_{0.1}$) is found where the retention volume per gram has decreased by 10% from its constant value at low loadings. It can also be seen that at this loading the HETP has started to rise and has increased by a similar amount. It now appears that this correspondence was fortuitous and with the vastly improved LC columns now available the effect of sample load on efficiency can now be more fully investigated. It has, in fact, been found that the HETP increases at considerably lower loadings than previously realized and it will also become apparent that the conventional linear–logarithmic plot of the data should be supplemented, if not superceded by a linear–linear plot.

Previously, the HETP–load relationship has only been applied to adsorption chromatography, but here we will also consider the effect of the sample load on the efficiency of columns used in the liquid–liquid and bonded-phase modes of chromatography.

EXPERIMENTAL

The silicas studied in this investigation are all commercially available except for the two samples prepared by the Wolfson Liquid Chromatography Unit in Edinburgh[2] (WLCU-6 and WLCU-10). The particle sizes and surface areas are collected together in Table I.

The silicas were packed by a slurry method using 15% methanol in methyl iodide as the slurrying medium. About 2 g of the silica were mixed with 12 ml of the mixture, placed in an ultrasonic bath for 2 min and forced into stainless-steel columns (145 × 5 mm) with acetone at 3500 p.s.i. The Spherisorb ODS column was packed by the high-viscosity slurry method using cyclohexanol followed by acetone in a similar manner[3].

Chromatography in the adsorption mode was carried out using *n*-hexane or 5% or 8% methylene chloride (50% saturated with water) in *n*-hexane. An efficiency *versus* linear velocity curve was plotted for each column and the loading experiments

TABLE I

PROPERTIES OF SILICA ADSORBENTS

Adsorbent	*Particle size (μm)*	*Surface area (m^2/g)*	*Supplier*
Spherisorb 5W	5	200	Phase Separations (Clwyd, Great Britain)
Spherisorb 10W	10	200	Phase Separations
Spherisorb 20W	20	200	Phase Separations
Partisil	10	400+	Reeve Angel (Maidstone, Great Britain)
LiChrosorb	10	400	E. Merck (Darmstadt, G.F.R.)
WLCU-6	6	140	Experimental batch
WLCU-10	10	200	Experimental batch
Spherisorb ODS	10	—	Phase Separations

were normally carried out at about the minimum in this curve. Liquid partition chromatography was performed on the Spherisorb ODS 10 column by coating the packing with β,β-oxydipropionitrile (BOP) using the method of Kirkland and Dilks[4] and the chromatography was carried out with BOP saturated *n*-hexane as eluent. Reversed-phase chromatography on the same column was carried out using 40% methanol in water as the mobile phase.

The LC equipment used in the study was fairly standard. A Haskel pressure intensifier pump was used for organic eluents and an Orlita DMP 1515 metering pump for the aqueous methanol mobile phases. In order to detect both small and large amounts of solute a variable wavelength UV photometer (Cecil Model 212 or Perkin-Elmer LC-55) was used as detector. Thus for nitrobenzene, detection at 254 nm allowed from 10 ng to 1 μg to be easily detected, but detector overload was a problem above this level and hence the test was carried on at 320 nm in order to avoid this. The photometer outputs were fed into an Autolab Model 6300 digital integrator and then displayed on a standard potentiometric flat bed recorder. Efficiencies were calculated both by measuring the peak width at half height and also by measuring the

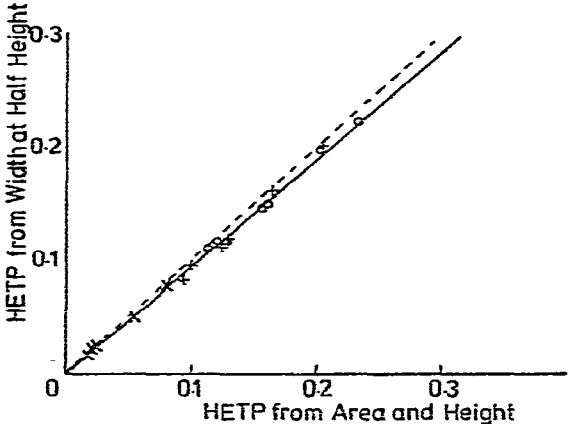

Fig. 2. The relationship between HETP values calculated as indicated. The dashed line is the expected line if the two methods gave the same answer.

area and peak height[5]. Fig. 2 shows the relationship between the two calculated values of the HETP, that from the area being consistently about 10% higher.

Samples were made up in the appropriate mobile phase in 10-ml measuring flasks and an unretained solute was added in order to calculate the partition ratios (k' values) for the retained solutes. In some cases, especially when the detector was used at higher wavelengths, the unretained solute was not detected and a suitable retained solute was added as a standard of know k' value. Anthracene was used in this manner in the adsorption experiments and similarly acetone in the reversed-phase systems.

RESULTS AND DISCUSSION

Adsorption

As has already been mentioned, each column was tested immediately after packing by chromatographing a test sample of tetrachloroethylene and phenetole at different pressures and hence plotting a HETP–linear velocity curve. Fig. 3 shows four curves obtained for Partsil-10 and the three Spherisorb silicas. The other three silicas gave strictly comparable data, LiChrosorb-10 and WLCU-10 being very close to the 10-μm curve and WLCU-6 similar to the 5-μm curve. The data show that the columns were well packed, giving the predicted minimum HETP value of between two and three particle diameters. The gradients of the curves also show the effect of increasing resistance to mass transfer as the particle size increases, the curve for the Spherisorb 20W being considerably steeper than those for the 5- or 10-μm particles.

In order to test the effect of the sample load on the efficiency, a linear velocity was chosen at or about the minimum of the curve and the test carried out. Table II

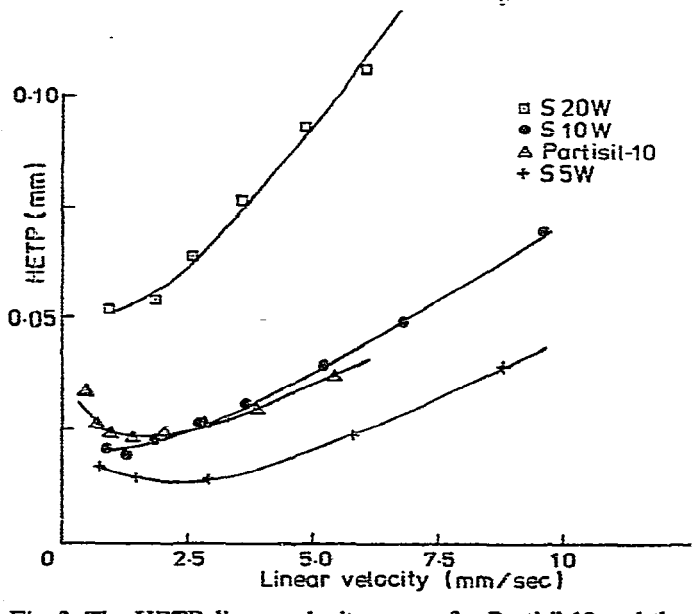

Fig. 3. The HETP–linear velocity curves for Partisil-10 and the three samples of Spherisorb.

TABLE II

PARAMETERS FOR COLUMN TESTS

Adsorbent	*Weight (g)*	*Linear velocity (cm/sec)*	*Solute*	*k'*	*Methylene chloride in mobile phase (%)*
Spherisorb 5W	1.58	0.221	phenetole	1.00	5
Spherisorb 10W	1.63	0.130	phenetole	1.00	5
Spherisorb 20W	1.70	0.182	phenetole	0.85	5
Partisil-10	1.24	0.246	phenetole	1.24	5
LiChrosorb-10	1.06	0.148	phenetole	0.87	8
WLCU-6	1.50 (estimated)	0.151	phenetole	0.90	5
WLCU-10*	0.98	0.250	phenetole	2.10	0
Spherisorb ODS 10	1.66	0.254	nitrobenzene	1.00	5

* The column length was 100 mm in this case.

shows the velocities chosen, the weights of adsorbent in the column and also the solutes used to compare the different silicas. The data from these experiments will normally be shown as linear–linear plots since it is considered more useful in this form; however, conventional linear–log plots will also be given in some cases.

Fig. 4 shows the data obtained for the three silicas of 10-μm particle diameter. The gradients seem to correspond to the surface areas of the silicas, that of Spherisorb being 200 m^2/g while the other two have values of about 400 m^2/g.

The general trend of the data is fairly obvious, but exact quantitative comparisons are not possible since the experimental conditions were not exactly the same for each test. As will be seen from later data on Partisil-10, when the mobile phase was changed to 8% methylene chloride in *n*-hexane, the slope of the plot for phenetole ($k' = 0.9$) had a considerably lower value than that for phenetole with 5% methylene chloride in *n*-hexane as eluent ($k' = 1.24$). In fact, with the higher-polarity mobile phase, nitrobenzene ($k' = 2.20$) gave a line of lower gradient than phenetole at low

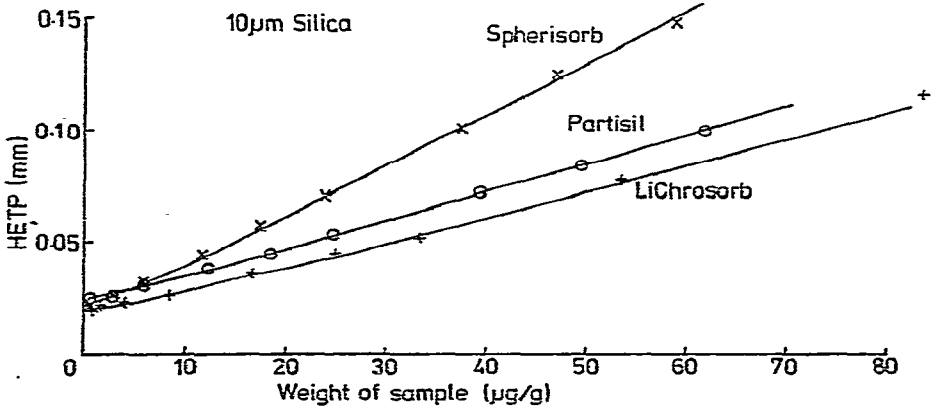

Fig. 4. HETP-load curves for LiChrosorb-10, Partisil-10 and Spherisorb 10W. Conditions are shown in Table II.

TABLE III

ADSORBENT PARAMETERS, PARTITION RATIOS, CURVE GRADIENTS AND RELATIVE CAPACITIES FOR 10-μm SILICAS

Adsorbent	*Surface area (m^2/g)*	*Solute*	*k'*	*Gradient (mm)*	*Relative capacity (μg/g)*
Partisil-10	400 ±	Phenetole	1.24	1.25	83
LiChrosorb-10	400	Phenetole	0.83	0.93	107.5
Spherisorb 10	200	Phenetole	0.99	2.29	44
Spherisorb ODS 10	100	Nitrobenzene	1.00	3.47	28

polarity. This immediately complicates the understanding of the effect of sample load on column efficiency since, in order to compare different adsorbents meaningfully, the effect of different solutes, different adsorbent activities and different k' values will have to be taken into account. Snyder[6] discusses these effects in more detail and it certainly seems that further work is necessary before even limited conclusions can be arrived at.

Table III collects the relevant data for comparison of the various silicas of 10-μm mean particle diameter. The gradient of the plots in Fig. 4 are shown, the units being millimetre per microgram per gram, which becomes millimetre if the loading is measured as gram per gram. This value for the gradient of the curve is not particularly useful on a practical basis, and its reciprocal is more relevant. The latter is in fact the amount of sample (in μg/g) which, when applied to the column will increase the HETP of the column by 1 mm. However, with HETP values of considerably less than 1 mm, the final parameter will be more useful if it refers to an increase of 0.1 mm and it will be designated the relative capacity for the adsorbent at the particular partition ratio. Further definition of the chromatographic conditions will also be necessary if valid comparisons of adsorbents are to be made, but this is outside the scope of the present paper. The final column in the table lists the relative capacities of the adsorbents calculated as indicated above. Also included in the table are the data from Spherisorb ODS 10 which under suitable conditions (see later) can act as

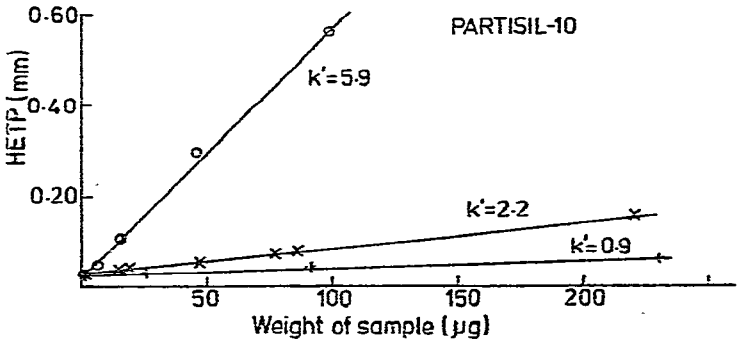

Fig. 5. HETP–load curves at different k' values for Partisil-10 on a linear–linear plot. Conditions as shown in Table II, except the mobile phase contained 8% methylene chloride. Solutes as shown in Table IV.

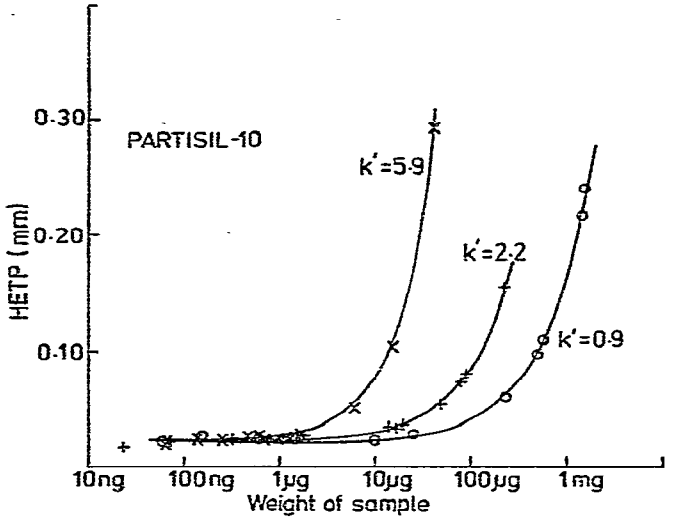

Fig. 6. HETP–load curves at different k' values for Partisil-10 drawn on a linear–log plot. Conditions as shown in Table II, except the mobile phase contained 8% methylene chloride. Solutes as shown in Table IV.

an adsorbent with surface area of slightly less than 100 m^2/g. The relative capacity in this case having, as expected, an even lower value than those of the other silicas.

It has already been suggested that differences in k' values could be affecting any correlation between the surface areas and gradients and Fig. 5 amplifies this statement. It shows the effect of k' on the efficiency–load plot. This linear–linear plot contrasts sharply with the linear–log plot of the same data in Fig. 6, and the rapid decrease of efficiency at higher sample loads as the k' value is increased is very noticeable. The relative capacities and those for similar data on a Spherisorb 20 column are collected in Table IV. Strict comparison between the two adsorbents is again not possible, since the mobile phases were different, that used to obtain the Partisil data being more polar and having a higher water content. As mentioned above, this will lead to an increase in the value of the relative capacities of solutes on Partisil. Some deviation from linearity was evident, particularly with the Spherisorb column but this was possibly due to effects of sample volume and/or concentration. This aspect will be discussed in the next section of this paper.

TABLE IV

RELATIVE CAPACITIES FOR PARTISIL-10 AND SPHERISORB 20 EACH TESTED WITH THREE DIFFERENT SOLUTES

Partisil-10		*Solute*	*Spherisorb 20*	
k'	*Relative capacity (µg/g)*		*k'*	*Relative capacity (µg/g)*
0.90	480	Phenetole	0.75	66
2.20	140	Nitrobenzene	2.00	24
5.90	17	Methyl benzoate	5.00	8

Both sets of data indicate an approximate inverse relationship between log k' and the relative capacity, but again further work is necessary before conclusions can be drawn.

It should be mentioned at this point that the measured increase in the HETP for the solutes on the various columns was not accompanied by a corresponding decrease in the k' value of the solute. Slight decreases were observed but these only occurred at loadings of about 100 μg/g. An example are the data for LiChrosorb-10; at 1 μg/g the k' value was 0.83 while at 84 μg/g the value was 0.82. The data for Spherisorb 20W illustrate the slight decrease that did occur; at 100 ng/g the k' value was 4.9 whilst at 100 μg/g it had dropped to 4.5.

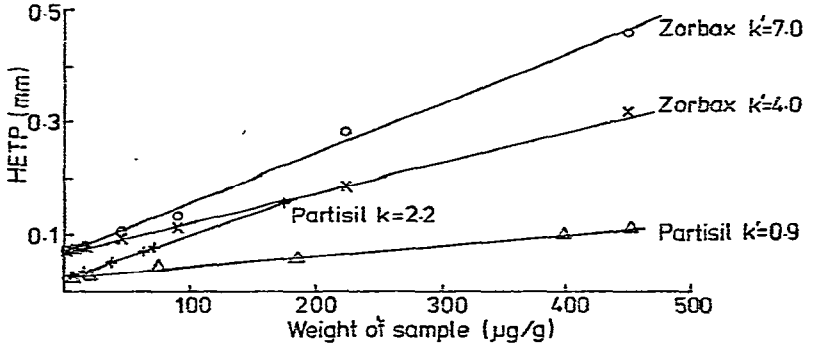

Fig. 7. Comparison of the data obtained by Kirkland[7] on Zorbax and the present data on Partisil-10. Kirkland's data were obtained using 50% and 100% water-saturated methylene chloride as mobile phase and 3-phenylpropanol as solute at a linear velocity of 0.61 cm/sec.

It is convenient at this juncture to consider other published data on the effect of sample size on the efficiency of modern LC columns. The most extensive are that of Kirkland[7] using porous silica microspheres. Using a different column configuration (250 × 3.2 mm), he tested his columns with various solute–mobile phase systems and some of his data obtained under comparable conditions are shown in Fig. 7 along with some of the present data on Partisil-10. The results indicate that the porous silica microspheres (Zorbax-Sil) behave in a fashion similar to the other silicas studied. The effect of the slightly smaller surface area of the microspheres (350 m^2/g) appears to be offset by the lower activity of the silica such that the lines for Zorbax-Sil at $k' = 7$ and for Partisil at $k' = 2.2$ have much the same gradient. The higher value of the HETP to which Kirkland's data converge is most probably due to the higher linear velocity he used to obtain his measurements.

Other workers in LC have also measured the linear capacity of various adsorbents, notably Scott and Kucera[8], Majors[9] and Endele *et al.*[10]. Scott and Kucera reported $\theta_{0.1}$ values for Partisil-10 and LiChrosorb-10 as being of the order of a few hundred micrograms per gram based on an increase in HETP value of 10%. However, they did not find that at low loadings the HETP was independent of k' and so based the increase on two different values of the HETP. Thus from their data on Partisil-10, the HETP of a solute of k' 1.98 (naphthalene) at a loading of 350 μg was 0.11 mm. This corresponds quite well with the value derived from the present data

of 130 μg since Scott and Kucera's columns contained about three times as much adsorbent. The figures for benzene ($k' = 1.06$), however, do not agree and this is most probably due to injection problems which are exaggerated at low values of k'. It is unfortunate that relative capacities could not be calculated from their data and so further comparison is not possible.

A similar calculation on LiChrosorb-10 from this paper is difficult, since they were unable to pack a good enough column of this material. Major's data on LiChrosorb also do not allow a direct comparison to be made since he was using a drop of 10% in k' as the criterion of overloading and this occurs at a much higher loading (1.4 mg/g) than was studied in the present work. If Partisil-10 and LiChrosorb-10 do, as indicated in Fig. 4, behave similarly, it is evident that the HETP value at this loading was very high although the author does not comment on this.

Endele *et al.* prepared their own silica and reported the effect of sample size on the chromatographic performance. Although, again, no strict comparison is possible, replotting the data for nitrobenzene ($k' = 6.3$) and benzene ($k' = 0.32$) leads to estimates of the relative capacities for these two solutes of 19 and 55 μg/g, respectively. These values again agree well with those collected in the present study. However, they did not find that the HETP was independent of k' at low loadings, which can be explained by the relatively inefficient column used for the test but, on the other hand, they did find that at higher sample loads benzene gave the lower value of HETP. Godbille and Devaux[11] also studied silica gel of 20–50-μm diameter in a preparative column and as part of their study plotted HETP–load curves for two solutes and again found that the data, although levelling off at a loading of 100 μg/g, were linear above this value. The relative capacities were 175 and 114 μg/g for dibenzyl and phenol, respectively. These compare to the value found for LiChrosorb-10 (108 μg/g) in the present work, this material being the equivalent of the Merckosorb used by Godbille and Devaux.

One difficulty in comparing other workers' data is the effect of sample volume on the efficiency. Table V shows two sets of results from experiments on two columns at two quite different loadings. It is immediately obvious that at low loadings it is important to use a minimum sample volume but that, as the load increases, the contribution of the sample volume to the plate height is swamped by the effect of the concentrated sample. This is a possible reason for the variable behaviour of the col-

TABLE V

THE EFFECT OF SAMPLE VOLUME ON THE HETP AT LOW AND HIGH LOADINGS OF PHENETOLE ON TWO DIFFERENT COLUMNS

WLCU-6 (sample, 1.77 μg)			*Spherisorb 20 (sample, 120 μg)*		
Volume (μl)	*Concentration (mg/ml)*	*HETP (mm)*	*Volume (μl)*	*Concentration (mg/ml)*	*HETP (mm)*
0.25	7.08	0.0147	1	120	0.265
0.5	3.54	0.0136	3	40	0.228
1.0	1.77	0.0141	10	12	0.199
2.5	0.71	0.0140	25	4.8	0.180
6.2	0.29	0.0152			
25	0.07	0.0203			

umns at higher loadings since the effect of diluting the sample from 1 to 3 μl beads to a drop in the plate height of about 14%. This effect has been commented on by various workers and it is obviously important when concentrated samples are being used.

The opposite effect which occurs at low sample loadings has perhaps not been noticed to the same extent but this is explained by the very low plate heights achieved here and by the fact that other workers reported results obtained either with slightly inferior columns or at higher velocities. Kirkland[7], in fact, reported very little effect on the plate height as the injection volumes were increased from 1 to 200 μl as long as the k' value was greater than 4. At k' values of less than this increases in HETP were evident above 20 μl, but this was ascribed to difficulties in sample application.

We have now seen the effect of surface area and partition ratio, k', on the efficiency of columns of silica as the load is increased. It has already been pointed out that the adsorbent activity plays an important part in determining the relative capacity as also does the nature of the silica surface. In particular, as Snyder[6] has indicated, the homogeneity of the surface strongly influences the peak shape and hence the value of the HETP. Another possible source of band spreading is the pore size and distribution, but the effect of these variables is outside the scope of this paper although it is hoped to carry out investigations into the effect of these variables on the efficiency of adsorbents in the near future. Two other variables that were investigated were particle size and linear velocity. These parameters need to be well chosen particularly if preparative chromatography is to be carried out so that maximum throughput can be achieved.

In the first instance the effect of particle size was studied using the three samples of Spherisorb silica. A column of each was tested under the conditions shown in Table II, and the results were plotted as shown in Fig. 8. The relative capacities are 63.5, 44.8 and 37.1 μg/g for the 5-, 10- and 20-μm materials, respectively, and do not appear to be related to the particle size in any obvious manner. The effect of this on the choice of particle size for a preparative separation can best be shown by the following discussion. We will assume a separation requiring 1000 theoretical plates and start at a loading of 10 μg/g. In order to generate these plates we will require a certain length of column which can be calculated using the date of Fig. 8. So at this loading, the HETP values of the three columns are 0.029, 0.039 and 0.073 mm, and so we will require 29-, 39- and 73-mm lengths, respectively, to generate 1000 theoretical plates. From the HETP–linear velocity data acquired when the columns were tested immediately after packing we can now find the pressure required to achieve the linear velocity at which the load–HETP data are valid, that is, the velocity at which the data in Fig. 8 was measured. Then, assuming a maximum allowed pressure, which for convenience we will set at 100 p.s.i., we can calculate the length of each column which at 100 p.s.i. will give the same linear velocity. All this leads us to the conclusion that we can use columns of 58, 255 and 456 mm containing 5-, 10- and 20-μm particles at 100 p.s.i. and still have HETP values of 0.029, 0.039 and 0.073 and loads of 10 μg/g. However, it is now evident that, owing to the increased column length, we have now too many theoretical plates in the 10- and 20-μm columns and can thus increase the sample load and still achieve the separation. We can, in fact, work at HETP values of 0.029, 0.128 and 0.228 mm on the respective columns and using Fig. 8 again we find that the amended loadings are 10, 43 and 60 μg/g. These essentially are the figures we require and although different column lengths containing different amounts

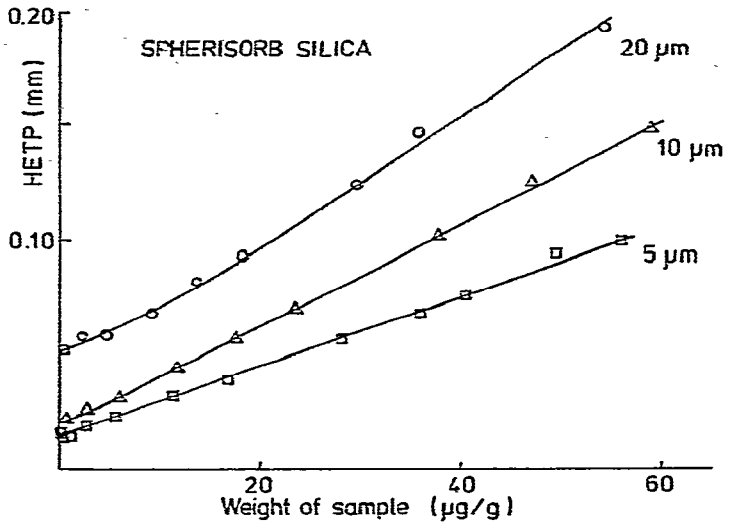

Fig. 8. The effect of particle size on the HETP–load curves. Conditions as shown and the solute was phenetole.

of silica are used, the apparent increase in total load due to this is exactly compensated for by the increased time required for elution. The different linear velocities used in the measurement of the HETP–load curves also lead to a slight amendment of the final figures but this is a minor one, and we then find that the maximum loadings for the three different particle sizes are in the ratio 1:3:6. This conclusion is limited by the data on which it is based but nevertheless it does appear that the use of larger

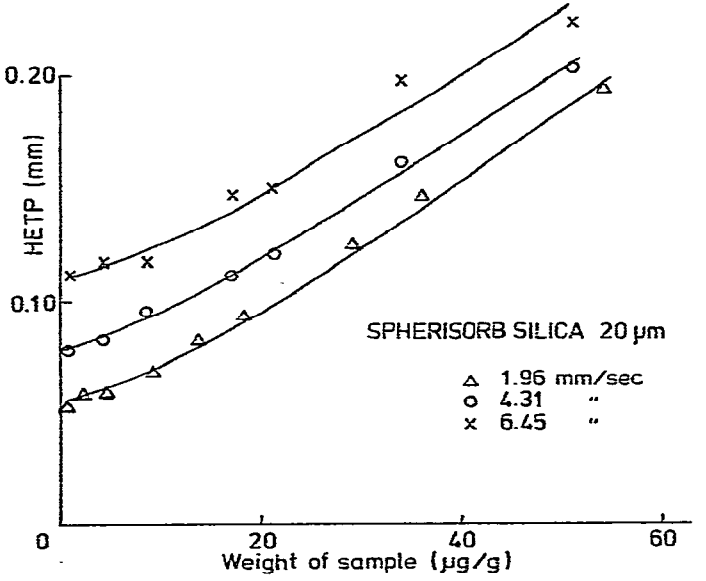

Fig. 9. The effect of linear velocity on the HETP–load curves. Conditions as shown and the solute was phenetole.

particles will give greater throughput when used under overload conditions. The effect of an increase in the linear velocity on the HETP–load curve is shown in Fig. 9. The Spherisorb 20W column was used and tested at 1.96, 4.31 and 6.45 mm/sec. If we assume a separation requiring 1000 theoretical plates (an HETP value of 0.145 mm), we can see from Fig. 9 that the maximum sample weights are 37, 29 and 17 μg/g, respectively. Now these data were measured for phenetole which has a k' value of one and therefore elutes in twice the time of an unretained peak. For the column used, which was 145 mm long this gives retention times of 148 and 51 sec at the low and high velocities, respectively. Hence, the amounts of phenetole that could be chromatographed at the two velocities would be 37/148 and 17/51 μg/sec, which are equivalent to 15 and 20 μg/min. So, under these conditions, it is possible to increase throughput merely by increasing the flow-rate and hence linear velocity.

It is tempting to extrapolate these results on the effect of particle size and linear velocity to even larger particles and faster velocities but this is not really possible without further experimental data. The essential conclusion is that it is not necessary to use 5-μm particles to get maximum efficiency in preparative chromatography since under high load conditions, as we have just seen, we gain nothing by so doing. Minimum flow-rates again are not required since we have shown that the advantage gained in increased sample load at these velocities is lost because of the longer times required for elution.

Partition and reversed phase

The effect of sample load on the efficiency of liquid–liquid partition columns has only briefly been mentioned by workers in the field. Karger and Berry[12] stated that the HETP for progesterone ($k' = 4$) did not change when the weight injected was increased from 1 μg to 1 mg. However, the column was rather inefficient (HETP = 1.4 mm) and thus any contribution to the HETP from the sample weight would not have been apparent. A preliminary investigation was therefore carried out using Spherisorb ODS 10 as the column packing. This is a suitable material since,

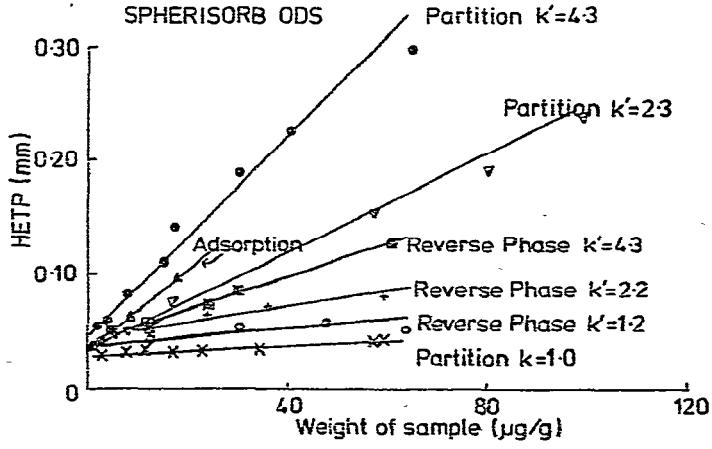

Fig. 10. HETP–load curves for Spherisorb ODS used in the adsorption, reversed-phase and partition modes of LC. Solutes and conditions as Table VI.

even though the silica surface has been modified, there are some surface hydroxyl groups left and consequently it can still function as an adsorbent and as a support for a liquid phase. Fig. 10 shows the results of the tests on a column of Spherisorb ODS 10 in the three modes of LC. It is interesting to note that the column was not quite as well packed as the unmodified silica columns were, a state of affairs which seems to occur fairly frequently with reversed-phase columns and that from the k' value for nitrobenzene (1.0) about half the surface area is still available for adsorption. The column was tested as before in the adsorption mode and then the mobile phase changed via three intermediate solvents to 40% methanol in water and the same procedure carried out with three different phenols. Finally, the column was dried out with methanol, the methanol removed with dry methylene chloride and the silica coated with about 8% BOP, as indicated in the experimental section. Three different solutes were then used to test the behaviour of the column in the partition mode. Table VI collects the relevant data together as well as the derived values for the relative capacities from the graphs.

TABLE VI

COLUMN PARAMETERS AND CALCULATED RESULTS FOR THE SPHERISORB ODS 10 COLUMN USED IN THE ADSORPTION, REVERSED-PHASE AND PARTITION MODES OF CHROMATOGRAPHY

Mode	*Mobile phase*	*Solute*	k'	*Relative capacity (μg/g)*
Adsorption	5% methylene chloride	nitrobenzene	1.0	28
Reverse phase	40% methanol in water	phenol	1.2	254
		p-cresol	2.2	116
		3,5-xylenol	4.3	66
Partition	BOP-saturated *n*-hexane	methyl benzoate	1.0	693
		acetophenone	2.3	43
		dibutyl phthalate	4.3	22

The results for the reversed-phase and partition systems are quite different. In the reversed-phase mode the relative capacities seem to be directly related to the k' values since, when changing k' by a factor of two (from *p*-cresol to 3,5-xylenol), the relative capacity changes by a factor of $\frac{1}{2}$ and similarly with phenol and the xylenol, the ratios in this case being 3.6 and 1/3.8. The effect of increasing the k' value in the partition mode is, on the other hand, much more drastic, and it seems probable that this is due to adsorption effects which are absent at low k' values (methyl benzoate) but become much more important as the retention increases. This is supported by the relative capacities for acetophenone and dibutyl phthalate which are very similar to that for nitrobenzene measured in the adsorption mode. It is now evident from these data that further work needs to be done, but we can draw some useful conclusions from these rather limited data. As with the other types of chromatography discussed here, for maximum efficiency minimum sample weight is required and under these conditions the three modes behave similarly, but at higher sample loads, reversed-phase materials, if applicable, will be the materials of choice.

CONCLUSIONS

The effect of sample load on the efficiency of modern LC columns packed with silica adsorbents is strongly dependent on the sample size. Only at loads of less than 1 μg/g of adsorbent is the HETP independent of partition ratio, adsorbent activity and adsorbent surface area. Above this sample load, the efficiency of the column generally decreases with decrease in surface area of the adsorbent and also decreases rapidly with increase of the partition ratio. Adsorbents of lower activity are effected to a lesser extent as the sample load is increased. A new measure of the adsorbent capacity, the relative capacity is proposed, the parameter being that sample weight per gram of adsorbent which increases the HETP of the column by 0.1 mm from the minimum value. The relative capacity is found by plotting a graph of HETP against sample weight, measuring the gradient of the resulting line, multiplying by 10 and taking the reciprocal of the result. In order to standardize the measurement it is suggested that the solute used for the test be nitrobenzene and the mobile phase be 5% methylene chloride (50% saturated with water) in *n*-hexane. The testing of columns at different velocities should also be carried out under the same conditions with a small sample (about 250 ng) of nitrobenzene as the solute. Nitrobenzene is used because it is retained under these conditions and is easily detected at this level since it adsorbs strongly in the UV at 254 nm.

On a practical basis, we can now predict that in order to achieve maximum efficiency when carrying out analytical separations the smaller the sample weight the better. The volume of the sample should also be kept below 5 μl. These conditions should be applied in adsorption, liquid–liquid partition and reversed-phase chromatography since, as soon as the sample load for an individual component exceeds 10 μg/g, efficiency is lost. Reversed-phase systems appear to be affected to a lesser extent than the others and this may well be due to the absence of adsorption effects in this mode of operation.

In preparative work, the throughput per unit time is of paramount importance and the results reported here indicate that there is no necessity to use the 5- or 10-μm materials to improve the throughput. It appears that, under high load conditions, the 20-μm materials will give the best performance. It is also evident that there is no need to work at minimum linear velocities and it was found that increasing the velocity by a factor of more than 3 only meant that the applied load had to be reduced by a factor of just over 2 and that overall more samples could be processed in a given time at the higher velocity. The volume of the sample was also found to be important when chromatographing large amounts of material. Large volumes of dilute solutions gave better efficiency than small volumes of more concentrated ones. This was the reverse of the case at very small sample loads when small sample volumes are best.

ACKNOWLEDGEMENTS

The author wishes to thank Professor J. H. Knox for helpful discussions about this paper, Perkin-Elmer for the loan of an LC-55 variable wavelength UV monitor and Phase Separations for the gift of the Spherisorb ODS packing material. The Science Research Council is also thanked for a grant during the tenure of which this work was carried out.

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