Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Effect of Loading on Separation Efficiency Using Steric Exclusion Chromatography

T. A. Maldacker[a]; L. B. Rogers[a]
[a] Department OF CHEMISTRY, PURDUE UNIVERSITY, LAFAYETTE, INDIANA

To cite this Article Maldacker, T. A. and Rogers, L. B.(1971) 'Effect of Loading on Separation Efficiency Using Steric Exclusion Chromatography', Separation Science and Technology, 6: 6, 747 — 758
To link to this Article: DOI: 10.1080/01496397108066948
URL: http://dx.doi.org/10.1080/01496397108066948

SEPARATION SCIENCE, 6(6), pp. 747–758, December, 1971

Effect of Loading on Separation Efficiency Using Steric Exclusion Chromatography

T. A. MALDACKER and L. B. ROGERS

DEPARTMENT OF CHEMISTRY
PURDUE UNIVERSITY
LAFAYETTE, INDIANA 47907

Summary

Resolution and resolution-per-unit-time have been studied for columns from 4 to 21 mm i.d. The effects of sample size, particle diameter, and linear flow rate were determined. Greatest resolution was obtained for the largest diameter column, and it was attributed to a loading effect. In a related study, efficiency was examined as a function of column diameter for a constant amount of gel and a constant retention time. The narrower columns gave more plates, but their HETP values were also greater.

INTRODUCTION

Several recent papers have been concerned with optimizing conditions in gel permeation (*1, 2*), liquid–solid adsorption (3, 6, 8), liquid–liquid (*4, 7*), and gas–liquid (*9, 10*) chromatography. One author (*11*) has made a study of maximum allowable sample size if overloading was to be avoided in gel permeation chromatography (GPC). The present paper demonstrates the utility of larger diameter columns for analytical purposes, so as to minimize loading effects for a given amount of sample.

Optimum GPC columns have been predicted to be long and narrow (*2*), and data attempting to show the benefit of decreasing the ratio of column diameter to particle size have been collected from several

sources (*1*). Column diameter studies at constant loading in liquid–solid chromatography have demonstrated optimum column diameters to be in the 2 to 4 mm i.d. range (*6, 8*). Using coated controlled-surface-porosity supports, 2 mm i.d. columns were shown to give superior results (*7*), though other have found highest efficiencies in relatively wide (7 mm i.d.) columns (*4*). In GLC, 4 and 11 mm i.d. columns were compared, and for samples below 30 μg the narrower column performed best, but above 50 μg the wider column was superior (*9*).

In the present work, two different approaches were followed. First, the sample size was held constant for several columns, all of the same length but of different diameters, while determining resolution as a function of linear flow rate. This demonstrated that wider columns were more efficient for a given sample size. While overloading is a widely recognized phenomenon, the unexpected feature of our data was the very small sample size at which the overloading effect was still observable. In the second, efficiency studies were done using columns of different diameters and different lengths containing the same amount of gel and having the same absolute flow rates. This approach normalized the amount of column support as well as the retention time and indicated how one could most efficiently use a given amount of gel.

EXPERIMENTAL

Reagents

The stock solution for samples used in the resolution study was made from ethylene glycol, pentaethylene glycol (Aldrich Chemical Co., Milwaukee, Wis.), and water mixed in the ratio of 1:1:5 by volume. It was maintained in a rubber-stoppered bottle under nitrogen.

For the efficiency study, 5 μl portions of 5 *M* NaCl were used.

The gel used in this work was Sephadex G-25 (Pharmacia Fine Chemicals, Piscataway, N. J.). For the resolution study, particle-size cuts of 10–40, 20–44, and 44–80 μ were used. For the efficiency study, a 20–80 μ cut was used. When swollen in water, the particle diameter increased approximately 1.5 times.

For all columns, ethylene glycol came out at the total retention volume (i.e., it was retained completely) whereas pentaethylene glycol (PEG, mol. wt. = 238) came out between the void volume and the total retention volume, having a distribution ratio of 0.7.

Columns

The 4 through 10 mm i.d. columns were constructed from Pyrex glass tubing and Beckman Teflon fittings. For the 15-mm i.d. column, Swagelok brass fittings were used. The 21-mm i.d. column was constructed from brass pipe fittings.

All of the columns were equipped with tee fittings that allowed injections to be made directly into the gel bed without interrupting the flow. Column lengths were 16.5 cm for the studies of resolution vs. linear flow rates and 26, 51, and 70 cm for the efficiency studies.

Glass-wool plugs were used to support the gel bed. The columns were packed by first filling them with water and then introducing a thin slurry of gel from which the fines had been removed. Water was then allowed to drain from the bottom of the column.

Apparatus

A Milton Roy Minipump provided flows up to 3.2 ml/min and a Durrum Instrument Co. peristaltic pump provided flows up to 4.2 ml/min.

Hamilton syringes of various capacities were used for sample injection.

A Sargent SRL recorder was used to record the outputs from a RefractoMonitor from Laboratory Data Control, Waterbury, Conn., and a Model R4 differential refractometer from Waters Associates, Framingham, Mass. A splitter, designed to control linear flow through the detectors, was constructed using Swagelok fittings and a Nupro valve.

Calculations

Values for the distribution ratio, K, were obtained from the equation:

$$K = \frac{V_r - V_0}{V_i} \tag{1}$$

Resolution values, R, were calculated using:

$$R = \frac{2(V_{r_2} - V_{r_1})}{(W_1 + W_2)} \tag{2}$$

and HETP values, H, from:

$$H = \frac{L}{16[(V_r - V_0)/W]^2} \tag{3}$$

where V_r is the retention volume of the peak, V_0 is the void volume, V_i is the interstitial volume, W is the peak width at the baseline obtained by drawing tangents to the sides, and L is the column length in millimeters.

RESULTS

Column Diameter

Figure 1 shows the results of plotting resolution vs. linear flow for column diameters ranging from 4.2 to 21 mm i.d. using a sample size of 10 μl on 20–44 μ G-25 gel. For all columns, the resolution at a given linear flow rate increased with column diameter up to the limit of 21 mm which was convenient to study. It should be noted that data for both 6 and 10 mm i.d. columns came from three different columns, and that

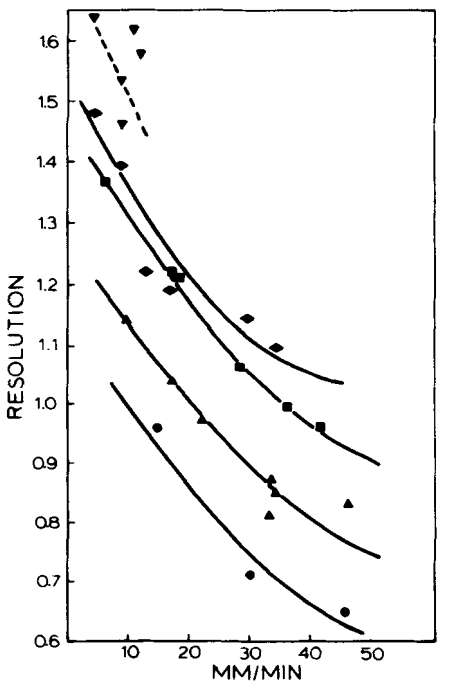

FIG. 1. Resolution as a function of linear flow for 4.2 (●), 6 (▲), 10 (■), 15.5 (◆), and 21 (▼) mm i.d. columns, using 10 μl samples and 20–44 μ G-25.

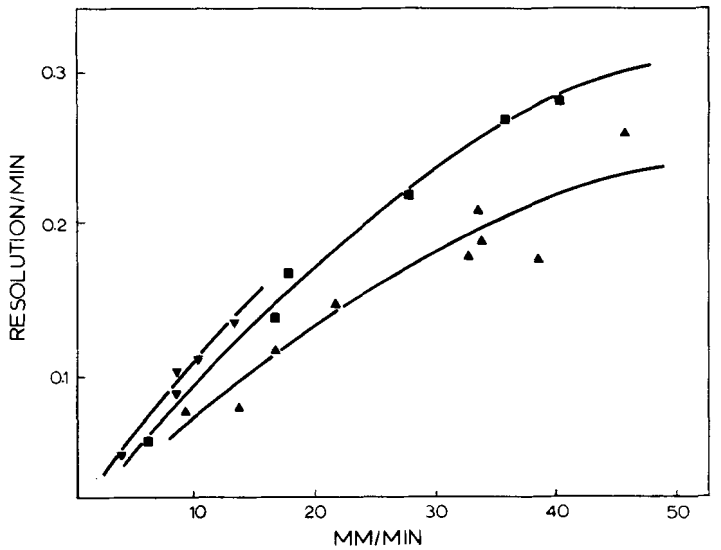

FIG. 2. Resolution/min as a function of linear flow for 6 (▲), 10 (■), and 21 (▼) mm i.d. columns using 10 μl samples and 20–44 μ G-25.

data for 21 mm i.d. came from two columns. The scatter for the 10-mm i.d. columns is much less than for the other two, suggesting that there may be an optimum column diameter that will give a homogeneous packing for a given particle size.

The fact that resolution steadily increased with increasing column diameter can be qualitatively attributed to the expanding cross-sectional areas. This resulted in narrower sample bands moving down the column and more efficient solute percolation through the pores. The eluted peaks were always sharper for the wider columns when everything else was equal.

Stefano and Beachall have examined column-diameter effects at constant loading for liquid–liquid systems (*4*) and have found that, up to 7 mm i.d., columns became more efficient. Beyond this no improvement was seen. This was attributed to an "infinite column diameter," whereby past a certain diameter, the solute never came in contact with the column walls. This concept was advanced earlier by Knox and Parcher (*5*).

Figure 2 shows the results of plotting resolution per minute vs. linear flow rate for the 6-, 10-, and 21-mm i.d. columns. Even though R de-

creased significantly as the linear flow rate increased, R/min increased. (R/min is the product of R and $1/t$, where t was taken as the retention time for the ethylene glycol.) Numerically, $1/t$ increased faster than R decreased, so R/min increased. Likewise, at low flow rates the three curves converged because the resolution values were numerically small relative to those of the retention times. Figure 2 suggests that faster linear flow rates in large diameter columns will give better resolution per unit of time for a given sample size.

Sample Size

In this study the sample sizes were not unrealistically large for analytical purposes. For example, a 10-μl sample contained approximately 1.5 μl each of ethylene glycol and PEG. Figure 3 shows the effect of sample size on resolution for both the 6- and 21-mm i.d. columns, and Table 1 contains data to show the extent to which the effective plate volume on each column was loaded. In obtaining values for Table 1, effective plate heights for the PEG were first calculated. Then the plate volume available for a species was obtained. PEG had a K equal

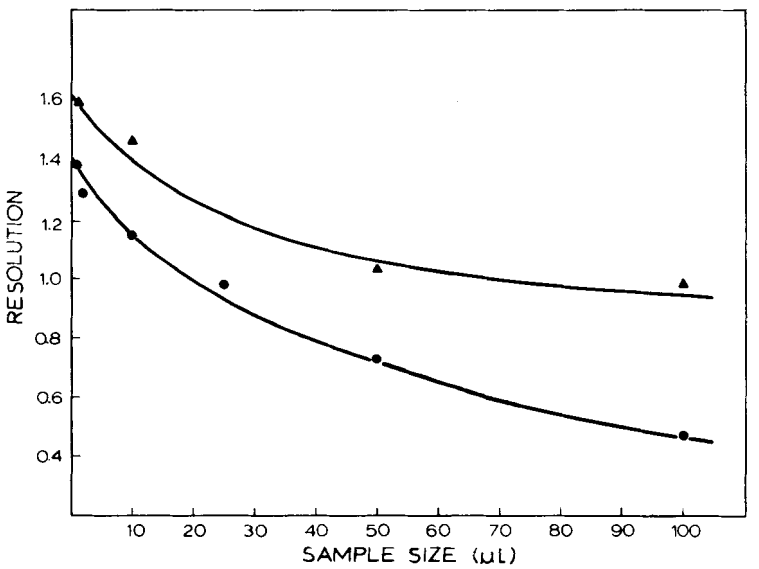

FIG. 3. Effect of sample size on resolution for 6 (●) and 21 (▲) mm i.d. columns for a flow of 9.2 mm/min using 20–44 μ G-25.

TABLE 1

Effective Plate number, HETP, and Corrected Plate Volumes for Pentaethylene Glycol

Column diameter (mm)	Sample size (μl)	Linear flow (mm/min)	Effective plate #	Effective HETP (mm)	Plate volume (μl)	Corrected plate volume (μl)	Resolution for PEG-ethylene glycol
6	1	9.3	134	1.23	35	25	1.40
6	10	9.6	104	1.60	45	32	1.14
21	1	9.6	160	1.03	346	242	1.60
21	10	9.2	137	1.20	415	290	1.46

to 0.7 so that only 70% of the interstitial volume was permeated by it. Using 10 μl samples, this resulted in corrected plate volumes of 32 and 290 μl on the 6- and 21-mm i.d. columns, respectively.

The volume of a theoretical plate on the 6-mm i.d. column was 20 times greater than the 1.5 μl of PEG in the 10-μl sample. Nevertheless, overloading obviously had occurred as evidenced by the significant improvements in HETP and resolution that were realized either by going to the 21-mm i.d. column or by decreasing the sample size from 10 to 1 μl. It should also be noted that values for the effective HETP and the resolution for the 1-μl sample on the 6-mm i.d. column were close to those obtained for the 10 μl sample on the 21-mm i.d. column. This was to be expected since the larger column had approximately twelve times greater volume. Thus, the extent of loading was similar for both columns.

In addition, the total volume of the 2.1 × 16.5 cm column is about 57,000 μl of which approximately half is intersitial volume. Yet, as can be seen in Table 1, increasing the sample size from 1 to 10 μl significantly increased the plate height. It is obvious that very small samples must be used in gel exclusion chromatography if one is to reach the full resolving potential of the column.

Particle Size

Figure 4 shows the beneficial effect of decreasing the average particle size. Going from a 20–44 μ (32 μ average) to a 44–80 μ (62 μ average) particle size, the change in resolution was 0.7 units at 10 mm/min. This is comparable to the change in resolution of 0.5 units at the same flow rate when the column diameter approximately doubled from 10 to 21 mm i.d. for constant particle size. In all cases, increasing the ratio of column diameter to particle diameter resulted in greater resolution. The effect of particle size distribution was not considered.

Though the ultrafine 10–40 μ cut gave the greatest resolution, there was a drawback in that, at linear flows much above 20 mm/min, the required column pressure became too great for the Teflon fittings and leakage occurred.

Efficient Gel Use

In a related study, it was determined how one could most efficiently use a given amount of gel as a function of column diameter. This would be of practical importance to those using column packings available in

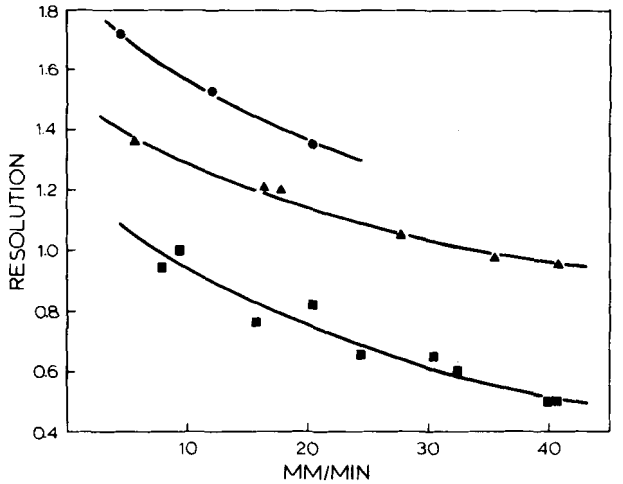

FIG. 4. Resolution as a function of linear flow on a 10 mm i.d. column using 10 μl samples with particle size distributions of 10–40 μ (●), 20–44 μ (▲), and 44–80 μ (■), measured in the dry state.

only small amounts at high cost. In this case, all of the columns contained the same amount of gel and had the same flow rate in milliliters per minute so that retention times were the same on all columns. Table 2 shows that, even though the 6-mm i.d. column was operating at the highest linear flow rate and had the highest HETP, it generated the most plates due to its greater length.

TABLE 2

Effect of Column Diameter on Effective HETP and Effective Number of Theoretical Plates Using a Constant Total Gel Volume of 20 ml and a Constant Volume of Eluent

Column		Linear flow	Retention	Number of	Effective
i.d. (mm)	Length (cm)	(mm/min)	volume (ml)	effective plates	HETP (mm)
6	70	12.5	18.4	585	1.2
7	51	9.3	18.6	456	1.1
10	26	4.5	18.5	359	0.7

However, if one were to make the 10-mm i.d. column as long as the 6-mm i.d. column, it would then have approximately 960 plates, assuming that plate number increased linearly with column length. Even if operated at the same linear flow rate as the 6-mm column, the 10-mm column would still give superior separations, as Fig. 1 shows. Hence, narrow columns make the best use of a given amount of gel, but for a given length, wider columns are best, due to a lower relative loading for a given amount of solute.

Other Studies

The influence of linear flow rate through the detector cell was shown to be negligible by installing a splitter at the exit of the 21-mm i.d. column. Resolution values were obtained with flows through the detector of 3.2 and 0.3 ml/min, while maintaining a constant linear flow through the column. No change in resolution was found.

The peak width at the baseline was examined as a function of pentaethylene glycol concentration on the 10-mm i.d. column to determine at what sample-weight to support-weight ratio loading effects would no longer be significant. The study employed the 10-mm i.d. column, 10 μl samples of solution, and a linear velocity of 9.2 mm/min. The amounts of PEG ranged from 0.02 to 3.0 mg. For comparison, a 10-μl sample represented in Fig. 1 contained approximately 1.5 mg PEG. It was found that peak width decreased only slightly below 2 mg of solute. For example, between 3 and 2 mg, peak width decreased from 55 to 48 mm, while it decreased to only 45 mm for 0.1 mg of solute. Since the column was made up from 2.6 g of dry G-25, a favorable sample-to-dry-support-weight ratio would be 0.7×10^{-3}.

DISCUSSION

Kirkland has shown that for a 1000 $\times$ 2.1 mm liquid–liquid column, sample size could be varied from 1 to 100 μl for a given weight of solute without affecting the HETP (*12*). Thus, changes in resolution vs. sample size in Fig. 2 are probably due to solute amount rather than sample volume.

We have shown that the smaller particle size cuts give improved resolution. Considering that a G-25 bead swells approximately 1.5 times in water (*1*), the average swollen particle diameter of a 20–44 μ G-25

cut would be 48 μ. This gives extremes in our column-diameter to particle-diameter ratios of 83 to 440, which is well above the highly inefficient 10 to 30 ratio range proposed by Horne et al. (*13*). Hamilton found a continuous increase in resolution in ion-exchange columns as the particle diameters decreased from 55 to 25 μ. No improvement was seen for 20 μ particles (*14*). Likewise, Stewart et al. (*3*) found that while 44–56 μ silica gel was superior to larger sizes, decreasing the particles to 28–36 μ hurt performance. Thus, a practical particle size limit may be in the 25–40 μ range for liquid work.

Moore has presented data for peak-width changes with concentration. He used a constant sample size of 800 μl on a $\frac{3}{8}$ in. $\times$ 4 ft Styragel column having a total volume of approximately 60 ml. Loading effects began at concentrations ranging from 1 to 10 mg/ml. This range is equivalent to 0.013 to 0.133 mg/ml of column packing. We have found that peak width continued to decrease with sample amount until the signal was lost in the background noise. The fact that peak area could not be kept constant for the smallest samples may have influenced this trend somewhat. We believe a practical maximum of 0.7 mg PEG per gram of dry G-25 should be used to avoid serious overloading. Since G-25 swells to approximately 5 ml/gram of dry gel, the samples should be no greater than 0.14 mg/ml of column material. This agrees well with Moore's finding (*11*). For packings that have smaller internal volumes, as appears to be the case for one type of Bioglas (*15*), correspondingly smaller samples would be required.

We have shown that for a given linear velocity and sample size, wider columns can give superior resolution. At the same time, it is known that narrower columns dilute the sample to a lesser degree and are thus capable of being used with smaller samples for a given limit of detection. However, wider analytical columns are still of practical importance in cases where larger amounts of eluted samples are needed for further analysis.

Acknowledgment

Supported in part by the U.S. Atomic Energy Commission under contract AT(11-1)-1222.

REFERENCES

1. J. C. Giddings and K. L. Mallik, *Anal. Chem.*, *38*, 997 (1966).

2. J. C. Giddings, *Anal. Chem.*, *40*, 2143 (1968).
3. H. N. M. Stewart, R. Amos, and S. G. Perry, *J. Chromatogr.*, *38*, 209 (1968).
4. J. J. Stefano and H. C. Beachall, *J. Chromatogr. Sci.*, *8*, 434 (1970).
5. J. H. Knox and J. F. Parcher, *Anal. Chem.*, *41*, 1599 (1969).
6. L. R. Snyder, *Anal. Chem.*, *39*, 698 (1967).
7. J. J. Kirkland, *J. Chromatogr. Sci.*, *7*, 7 (1969).
8. L. R. Snyder, *J. Chromatogr. Sci.*, *7*, 352 (1969).
9. W. J. A. Vanden Heuvel and G. W. Kuron, *J. Chromatogr. Sci.*, *7*, 651 (1969).
10. W. J. de Wet and V. Pretorius, *Anal. Chem.*, *32*, 1396 (1960).
11. J. C. Moore, *Separ. Sci.*, *5*, 723 (1970).
12. J. J. Kirkland, *J. Chromatogr. Sci.*, *7*, 361 (1969).
13. D. S. Horne, J. H. Knox, and L. McLaren, *Separ. Sci.*, *1*, 531 (1966).
14. P. B. Hamilton, *Anal. Chem.*, *30*, 914 (1958).
15. C. H. Lochmüller and L. B. Rogers, *Anal. Chem.*, *41*, 173 (1969).

Received by editor May 10, 1971