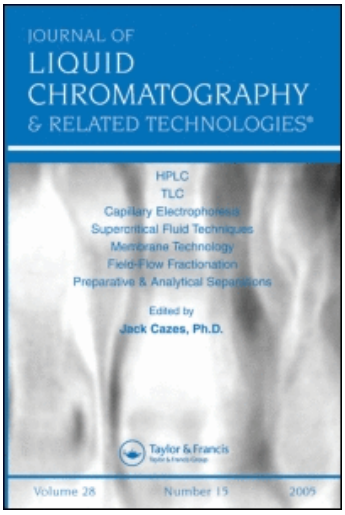

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Liquid Chromatographic Preparative Packing Utility as a Function of Particle Shape

John A. Perry[a]; Ted J. Szczerba[a]
[a] Regis Chemical Company, Morton Grove, Illinois

To cite this Article Perry, John A. and Szczerba, Ted J.(1993) 'Liquid Chromatographic Preparative Packing Utility as a Function of Particle Shape', Journal of Liquid Chromatography & Related Technologies, 16: 6, 1371 — 1383
To link to this Article: DOI: 10.1080/10826079308020959
URL: http://dx.doi.org/10.1080/10826079308020959

JOURNAL OF LIQUID CHROMATOGRAPHY, 16(6), 1371-1383 (1993)

LIQUID CHROMATOGRAPHIC PREPARATIVE PACKING UTILITY AS A FUNCTION OF PARTICLE SHAPE

JOHN A. PERRY* AND TED J. SZCZERBA
Regis Chemical Company
8210 Austin Avenue
Morton Grove, Illinois 60053

ABSTRACT

The "equal-cut-point" approach (12) has been applied to comparing the relative utilities for preparative liquid chromatography of five irregular and three spherical packings. Packed with these, the variation of peak width with loading was first established and plotted, for columns of given length. From these plots, the loading that produced a given peak width was found for each column. The packings then received purely technical ratings, taken as the ratios of these loadings to the minimum loading observed. The three highest purely technical ratings--without regard to packing cost--were attained by the 5-micron irregular (4.72), the 10-micron irregular (4.04), and the 10-micron spherical (3.09). To express value (that is, performance per cost), the technical ratings were divided by the costs of the packings. The most valuable packings were found to be, first, the 10-micron irregular, value 9.45; second, the 40-micron irregular, value 7.01; third, the 20-micron irregular, value 6.54; and fourth, the 5-micron irregular, value 5.20. The irregular packings showed much more value than the spherical--the fourth-ranking 5-micron irregular at 5.20 was almost 3 times more valuable than the best of the sphericals, the 10-micron, at 1.78. This study was conducted under mass overload; at the end of the paper, volume overload is shown as inherently unsuited for such studies.

*Present address: Riis Terrasse 13, 2007 Kjeller, Norway.

INTRODUCTION

In many studies of preparative liquid chromatography (1-8), simple questions do not get asked and simple answers are hard to obtain. One might wish, for instance, to know which particle size to have in a preparative column. Considering this question after having reviewed several such studies (9-11) and done further work of their own (4), Snyder et al. concluded that if preparative column length be unrestricted, as generality requires, then "no single `rule of thumb' [can] guide choice [of particle size]." In the usual laboratory, however, the length of a preparative column is fixed. Here, one might wish for a simple means of choosing preparative particle size. Such was described in the first report on the equal-cut-point (ECP) approach, which allows otherwise-equivalent columns to be compared for not only the effect on loading of variation in some chosen parameter but also economic value (12).

A first statement of ECP principles was presented in Fig. 2 of the first report (12). In actual ECP practice, for each column the half-height peak width is first determined and plotted as a function of the logarithm of solute loading. This is the loading curve of that column. Then, from the loading curve for the least efficient of the columns to be compared, a reference preparative peak width is determined. Usually, this reference peak width is taken as 150% of the peak width observed at minimum solute loading. A horizontal reference line at the level of this reference peak width is then drawn across each loading curve. At each intersection of the reference line with a loading curve, the equal-cut-point loading capacity of each column is then read off the x-axis. Dividing the loading capacity of each column by that of the least efficient yields a series of technical ratios by means of which to rank the columns on a purely technical basis: the larger the technical ratio, the greater the relative loading capacity of the column. This ranks the columns (that is, the packings) on a technical basis, but there is also an economic basis to be considered: packings differ in cost.

If the numerator and denominator of each loading-capacity ratio be divided by the respective monetary costs of the packing, a technical/economic ratio that combines the technical and the economic considerations is obtained. The technical/economic ratio expresses value. ("Value," defined as in an English dictionary (13), here expresses packing loading capacity per packing volume monetary cost.) The packing of highest value is the one of choice.

In the study reported here, the ECP approach was applied to finding which combination of particle size, shape, and cost to use in preparative liquid chromatography. The question of particle shape alone seems not to have been addressed elsewhere.

MATERIALS

For this study, dibutylphthalate (DBP), purchased from Aldrich (Milwaukee, WI, U.S.A.), was used as the solute. HPLC grade solvents were used throughout.

The packings used were ODS-bonded, had 100 A pore diameters, and, except for the 5-micron irregular, which was obtained from the PQ Corporation (Conshohocken, PA, U.S.A.), were all obtained from Regis. The irregular [Biochrom] packings had specific surface areas of 320-400 square meters per gram; were 5, 10, 20, 40, and 80 microns in particle diameter; and cost $10.50 (PQ price), $4.35, $2.85, $1.50, and $1.02 per gram, respectively. The spherical [Rexchrom] packings had specific surface areas of 200 square meters per gram; were 3, 5, and 10 microns in particle diameter; and each cost $19.00 per gram.

The eight 25 cm long x 4.6 mm I.D. columns used had been packed at Regis. Each of the five that contained irregular packings held 3.5 plus or minus 0.2 grams of packing; each of the three that contained spherical packings held 3.7 plus or minus 0.2 grams of packing.

METHODS

As mobile phase, methanol/water (80:20, v/v) was used at a flow rate of 1.0 ml/min. Throughout, elution was isocratic. The amounts of DBP charged to each column in 50-ul volumes varied by a factor of over 7000, ranging from 2 to somewhat over 10 000 micrograms DBP/g of packing, specifically, 1.43, 14.3, 1430, 3570, 7140, 10 700, and 14 300 for the irregular packings; and, because of higher packing density, 1.00/1.06 or 0.94 of these amounts for the spherical packings.

RESULTS

Because the loading curves for the two types of particles overlap severely at the higher loadings, for clarity they are first shown by themselves; and then, for comparison, with each other. The loading curves for the 3-, 5-, and 10-micron spherical particles are shown in Figure 1; for the 5-, 10-, 20-, 40-, and 80-micron irregular particles, in Figure 2; and for both types, in Figure 3.

All the packings display two distinct ranges, a constant resolution range, and a preparative range.

The constant resolution range extends from about 1.5 micrograms solute per gram of packing (MSGP) to about 150 MSGP. In the constant resolution range, peak width (and thus resolution) remains essentially unchanged despite increases in loading of over two orders of magnitude. For analytical work, this range has great potential: With essentially no loss in resolution in this range, far-larger-than-normal samples may be injected in order to emphasize and reveal trace constituent peaks that cannot normally be observed.

The preparative range extends from about 200 MSGP up to an upper loading limit (ULL), beyond which each given loading curve becomes essentially vertical and chromatography is no longer in effect.

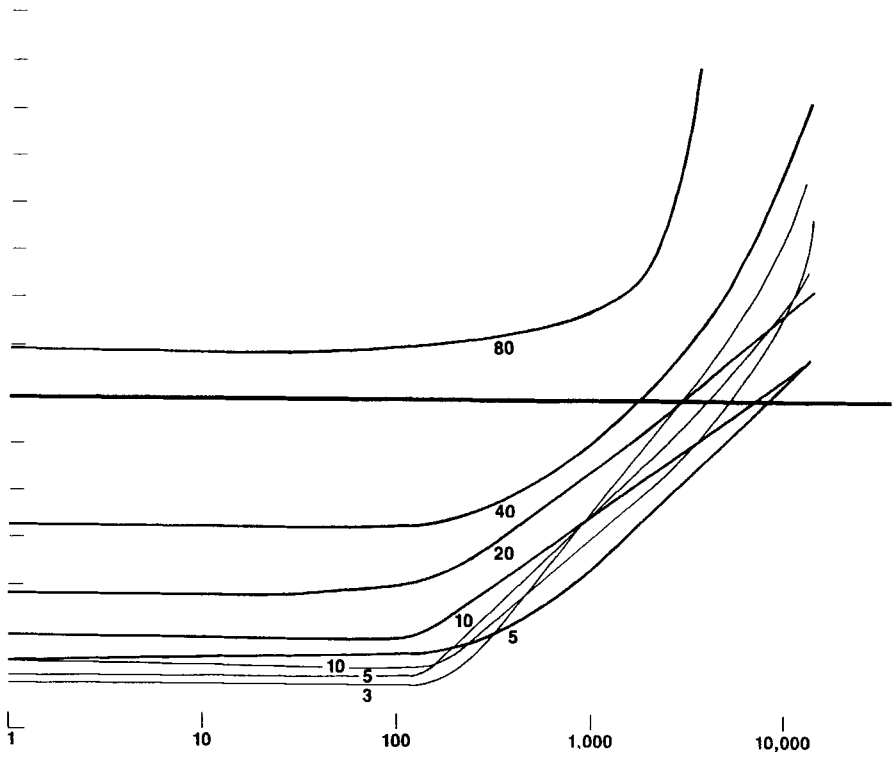

Figure 1. In the constant resolution range (1.5 to 150 micrograms solute per gram of packing (MSGP)), spherical packings show narrower peaks than irregular and are therefore obviously better for analytical use. In the preparative range (above 200 MSGP), the curves for the spherical particles reveal a much more sharply decreasing utility than the irregular (see Figure 2) and also cross each other. In consequence, among the spherical particles the 10-micron are best for preparative chromatography.

In the constant resolution range, spherical packings show narrower peaks than irregular.

At the upper limit of the constant-resolution range, the loading curves for the spherical particles break sharply upwards. Whereas the 3-micron particles show an almost linear increase in peak width above a loading of about 300 MSGP, the 5- and 10-micron loading

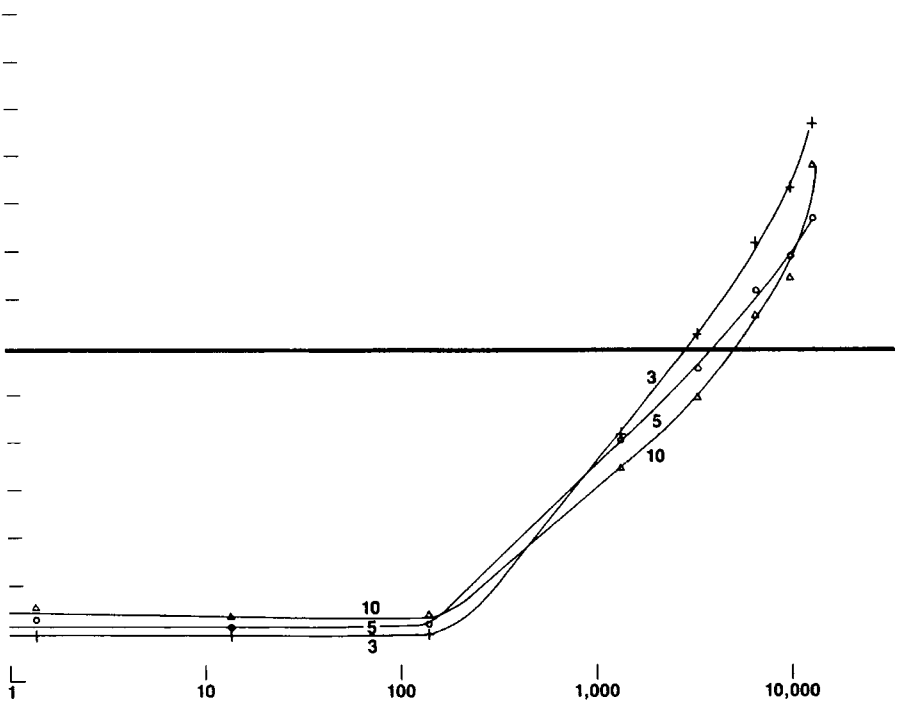

Figure 2. The 80-micron particles are so chromatographically inefficient that they cannot be usefully compared to either the smaller irregular or the spherical particles. However, particularly in the preparative ranges the loading curves for the smaller irregular particles may be instructively compared with the spherical. It can be seen that the irregular particles are useful for preparative chromatography for loads even greater than 50000 MSGP.

curves break less sharply. All three curves cross; among the spherical particles from 500 to about 7000 MSGP, the 10-micron particles are the most efficient. For either the 3- or the 10-micron spherical particles, the ULL is about 15000 MSGP. The 5-micron ULL was not established in this study, although it is probably just beyond the region investigated.

In contrast to the loading curves for the spherical particles, those for the irregular particles do not break upwards so sharply beyond the constant resolution range and are

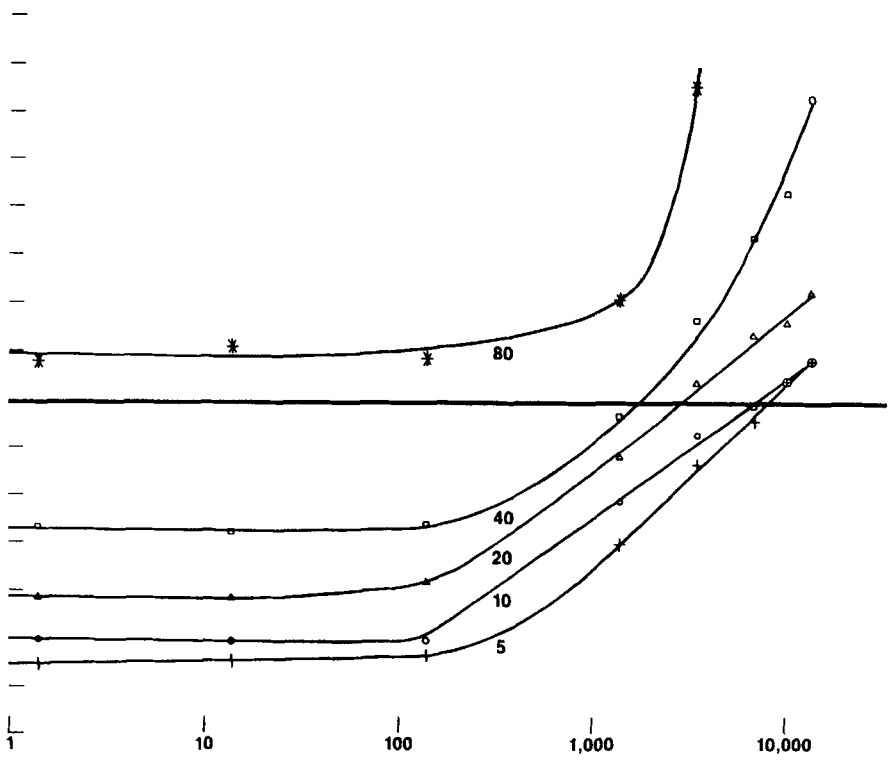

Figure 3. Even at quite low loadings, in preparative chromatography the irregular particles tend to become more efficient than the spherical. By 500 MSGP, the 5-micron irregular particles are more efficient than any of the spherical; and by 4000, the 10-micron.

less uniformly limiting. The curve for the 80-micron particles shows an extended "constant-resolution" range; but a severely limited preparative range that cuts off at about only 5000 MSGP--at least an order of magnitude less than the ULLs of the smaller irregular particles. In contrast, the 5-, 10-, and 20-micron irregular particles should afford chromatography beyond 50000 MSGP--loadings at least three times greater than those tolerable by the spherical particles.

As can be seen in Figure 3, the irregular particles begin to become more efficient than the spherical at quite low preparative loadings. By 500 MSGP, the 5-micron irregular is more efficient than any of the spherical; and by 4000, the 10-micron.

The peak width value for the reference line that is imposed on the figures was taken as 150% of the 40-micron minimum peak width. (A line at 150% of the 80-micron minimum peak widths would have intersected none of the other loading curves, thus was unusable as a reference line.) The intersections of the reference line with the loading curves allows the relative performances of the particles to be expressed quantitatively.

DISCUSSION

In the upper part of Table I are presented the ECP solute loadings (the loadings that produce peak widths equal to 150% of the 40-micron, constant-resolution-range peak width). (The lower part of Table I is discussed following the Table.) These ECP solute loadings are expressed on an absolute basis in the first row, a relative basis--the technical rating--in the second.

In receiving high solute loading and yet maintaining a given resolution, the 5- and 10-micron irregular particles excelled. The three highest purely technical ratings--without regard to packing cost--were attained by the 5-micron irregular (4.72), the 10-micron irregular (4.04), and the 10-micron spherical (3.09).

Greater specific surface area must underlie the relatively strong showing of the irregular particles against the spherical. The irregular particles have specific surface areas of 320-400 square meters per gram; the spherical, about 200. In good agreement to the 1.6-to-2.0 times greater specific surface area of the irregular packings can be seen the greater irregular/spherical ECP loading ratios: 1.3 for the 10-micron (1.3 = 4.04/3.09), 2.0 for the 5-micron (2.0 = 4.72/2.36).

TABLE 1

Packing Performance Expressed as ECP Solute Loading and as Value.

ECP Solute Loadings, Absolute and Relative

Solute Loading						
micrograms DBP/gram packing						
Particle Diameter						
microns						
Spherical			Irregular			
3	5	10	5	10	20	40
Absolute						
3,100	4,200	5,500	8,400	7,200	3,100	1,780
Relative (normalized on 40-micron)						
Technical Rating						
1.74	2.36	3.09	4.72	4.04	1.74	1.00

Relative Value

Dollars per gram, at uniform packing density						
20.14	20.14	20.14	10.50	4.95	3.08	1.65
Relative ECP loading/packing cost						
0.0864	0.117	0.153	0.450	0.816	0.564	0.606
Relative value, normalized on 3-micron spherical						
Technical/Economic Rating						
1.00	1.36	1.78	5.20	9.45	6.54	7.01

As mentioned earlier, there is also the matter of what it costs to fill the column. For equal cost, the packing with the better performance--here, the greater ECP solute loading--has greater value. Or, for equal loading, the packing with the lower cost has greater value. One expresses value by dividing performance by cost.

(Packing density is relevant in assessing packing cost. A greater weight of spherical packing is required to pack a column. About 3.7 grams spherical packing per column volume was used here, versus 3.5 grams of irregular: the spherical packings are about 1.06 times more dense. But packings are sold on a weight basis, so the packing costs must be adjusted for density. Instead of citing $19.00 per gram for the spherical, we use 1.06 x $19.00 to get a density-adjusted cost of $20.14.)

The relative packing values can now be expressed quantitatively. The overall technical/economic rating is given in the last line of Table 1. The 10-micron irregular packings (which had received the second-highest purely technical rating) were found the most valuable, receiving a technical/economic rating of 9.45; among the others, the 40-micron ranked second (7.01), the 20-micron third (6.54), and the 5-micron (which had received the highest purely technical rating) fourth (5.20). The irregular packings showed much more value than the spherical--the fourth-ranking 5-micron irregular at 5.20 was almost 3 times more valuable than the best of the sphericals, the 10-micron, at 1.78 (even on the purely technical basis, the 10-micron spherical had received only the third-highest rating).

Note added in review. A referee has suggested that mass and volume overloads are separate manners of overload, that each is important, that only one should be involved in a given investigation, and that this work not only involves both but also, therefore, does not inform.

We shall argue not only that the type of overload involved in this work is essentially mass. We shall also show that volume overload is inherently unsuitable for comparing the preparative capabilities of columns.

Mass overload. One of the three basic assumptions of any form of chromatography is that the sample is initially deposited on the first theoretical plate. It follows that except conceivably for radioactive detection, actual injection of any detectable sample inevitably involves mass overload.

In this work, the columns were subjected to increasingly heavy mass overload at constant injection volume. This volume was 50 microliters, a convenient but not particularly large volume, even for analytical work, certainly with columns 25 cm in length, as these were. (Perspective on this point is gained by considering that a 5-cm, 4.6-mm I.D. column packed with particles 3 microns in diameter could (and can) be injected with a 20-microliter sample volume and yet yield a chromatographic efficiency of not quite 120,000 plates per meter (14).)

The systematically increased mass overload used in this work allowed an equally systematic comparison of the column capabilities for chromatography under such conditions.

Volume overload. In contrast, as we shall now show, volume overload is simply not usable for these purposes.

Volume overload as a variable was first investigated by Klinkenberg and Sjenitzer, who referred to it as "feed volume" (15). In either volume overload or feed volume, the sample is introduced in increasing volumes at constant concentration. Larger volumes are obtained by substituting one stream of the mobile phase for another that contains a given concentration of the sample. The feed or injection volume is varied by varying the

period during which the sample-containing mobile phase is substituted for the original, pure mobile phase.

As the Klinkenberg-Sjenitzer figures reveal, each chromatographic peak consists of two concentration gradients, first increasing, then decreasing. The more efficient the column, the sharper the gradient. Increasing the feed volume (or volume overload) does not change the slope of these two concentration gradients but, rather, merely inserts an increasingly wide plateau between them. (With this technique, analytical sensitivity can be conveniently maximized without particularly affecting chromatographic efficiency. See, for instance, ref. 16.)

As feed volume and/or volume overload is increased, the initial concentration gradient "looks" to the column like the beginning of a chromatographic peak and is characterized by the same retention volume. A delay ensues during which the constant-concentration part of the feed is washed toward the end of the column. Finally, the terminal concentration gradient is eluted. The duration of the constant-concentration part of the feed, and thus the width of the corresponding "peak", is the adventitious choice of the chromatographer. As such, it is not a parameter--nor for the same reason is volume overload a technique--by means of which the preparative capabilities of columns can be systematically compared.

REFERENCES

1. J. E. Eble, R. L. Grob, P. E. Antle, L. R. Snyder, J. Chromatogr., 384: 45 (1987)
2. Y. S. Kim, B. W. Sands, J. L. Bass, J. Liq. Chromatogr., 10: 839 (1987)
3. J. H. Knox, J. Chromatogr. Sci., 15: 352 (1977)
4. L. R. Snyder, G. B. Cox, P. E. Antle, Chromatographia, 24: 82 (1987)
5. L. R. Snyder, G. B. Cox, P. E. Antle, J. Chromatogr., 444: 303 (1988)

6. L. R. Snyder, J. W. Dolan, G. B. Cox, J. Chromatogr., 483: 63 (1989)

7. L. R. Snyder, G. B. Cox, J. Chromatogr., 483: 85-94 (1989)

8. L. R. Snyder, G. B. Cox, J. Chromatogr., 511: 404-5 (1990)

9. J. H. Knox, H. M. Pyper, J. Chromatogr., 363: 1 (1986)

10. J. E. Eble, R. L. Grob, P. E. Antle, L. R. Snyder, J. Chromatogr., 405: 51 (1987)

11. A. Jaulmes, C. Vidal-Madjar, H. Colin, G. Guiochon, J. Phys. Chem., 90: 207 (1986)

12. J. A. Perry, T. J. Szczerba, J. Chromatogr., 404: 267-272 (1989)

13. Webster's Third New International Dictionary, Unabridged; Merriam, Springfield, Massachusetts, 1961, **value**, definition **2**.

14. J. A. Perry, L. J. Glunz, T. J. Szczerba, and V. L. Hocson, LC, 1: 40-41 (1983)

15. A. Klinkenberg, F. Sjenitzer, Chem. Eng. Sci., 5: 258 (1956)

16. J. A. Perry, J. D. Rateike, T. J. Szczerba, J. Chromatogr., 389: 57-64 (1987).

Received: June 9, 1992
Accepted: October 9, 1992