Journal of Chromatography, 484 (1989) 267–272
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 801

PREPARATIVE PACKING UTILITY AS A FUNCTION OF PARTICLE SIZE

JOHN A. PERRY* and TED J. SZCZERBA
Regis Chemical Company, 8210 Austin Avenue, Morton Grove, IL 60053 (U.S.A.)

SUMMARY

The idea is still current, if not indeed prevalent, that more efficient packings overload more quickly than less efficient, and that therefore they are less useful in preparative liquid chromatography. In the light of new measurements made for this purpose, we have reassessed the terms such as overload, loadability, and dynamic capacity. These terms are self-referent, refer only to the ideal behavior of a given packing at zero load, and are not useful for comparing the relative preparative utilities of packings that differ merely in particle size. An "equal-cut-point" approach is proposed as a better method for this comparison.

Given an equal-cut-point approach, a 20-μm column is seen to be roughly 15 times more productive than an 80-μm column, roughly 4.5 times more productive than a 40-μm column, all of equal length. If the costs of the packings are taken into account, the 20-μm column is seen to be almost 10 times more valuable than an 80-μm column, that is, more productive per dollar paid for the packing; and about 3 times more valuable than a 40-μm column.

INTRODUCTION

Preparative liquid chromatography[1–6] continues to attract rapidly increasing interest. In the practice of preparative liquid chromatography (LC), among the more important parameters is the particle size of the preparative column. Although smaller particles produce more efficient columns at analytical loads, the use of smaller particles for preparative loads has been viewed skeptically on the grounds that smaller particles overload more quickly than larger. This view may have had its origin in a study by De Jong *et al.*[1], who in their conclusions reported finding "higher loadabilities ... for coarser particles".

Particle size was one of the parameters considered in a thorough review of and model for preparative LC methodology[7]. Combining this study with other recently preceding ones[8,9], Snyder *et al.*[10] proposed a comprehensive model to make it "possible to draw a number of general conclusions relating to optimum conditions for preparative HPLC". In the fourth and last of these conclusions, however, they stated, "... there is currently no single 'rule of thumb' to guide (the) choice (of particle size). ... the optimum particle size for any given case can best be determined only from a knowledge of the exact circumstances surrounding the separation"[10].

0021-9673/89/$03.50

As is proper in theory, each relevant parameter is varied over the full range of its pertinent continuum. However, most practitioners do not have the luxury of choosing the values of their parameters from a continuum in which, for instance, column length can take on any value. For most practitioners, parameter values refer not to a continuum but to components that can be purchased from catalogues. For many, preparative column length is 25 cm, perhaps extendable *in extremis* to 50 cm; and the range of available preparative pressures and flow-rates is set by the pumps at hand (and they, probably analytical). Our study refers to this limited laboratory in which a real question is, "In the preparative column I am about to order, what particle size should I specify?".

In this study, we have remeasured peak widths over a wide range of loadings, correlated the results, and rephrased the findings. We present here these findings from both the earlier, "overloading" point of view, and a new and simpler one that might be called, "equal-cut-point".

EXPERIMENTAL

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.

Columns and equipment

The four columns used were 25 cm × 4.6 mm I.D., and had been packed at Regis with irregular, 100-Å pore-diameter, ODS-bonded silica particles 10, 20, 40, and 80 μm in diameter.

Procedures

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-μl volumes varied by a factor of over 7000, ranging from 2 to somewhat over 10 000 μg DBP/g of packing, specifically, 1.43, 14.3, 1430, 3570, 7140, 10 700, and 14 300.

Shown in Fig. 1 for each particle size is the variation of peak width (measured at half-height) as a function of solute loading.

RESULTS AND DISCUSSION

The term "overload" was early defined in gas chromatography as an increase in peak width 10% over that characteristic of zero load. One can also approach the problem by measuring the number of theoretical plates produced by a given column as a function of increasing load, then noting that load at which the number of theoretical plates decreases by a chosen fraction. If the loading is expressed in load/g of packing, one has the term, *specific loadability*[1]. We have been unable to find where the terms "capacity" and then "dynamic capacity" were introduced; here, for our isocratic elutions we use "specific dynamic capacity" to mean the load per gram of packing at which is observed a peak width 50% greater than that found at essentially zero load. All such terms express a certain increase in peak width caused by a corresponding

TABLE I

SPECIFIC DYNAMIC CAPACITY AS A FUNCTION OF PARTICLE DIAMETER

Particle diameter (μm)	*Specific dynamic capacity (μg DBP/g packing)*
10	2000
20	3400
40	5000
80	9600

increase in sample load, differ mutually only in degree, and are arbitrary in choice of degree of peak broadening.

In Table I, the *specific dynamic capacity* just defined is listed for each of the four particle sizes used. It can be seen that the specific dynamic capacity increases with increasing particle size, in agreement with the idea alluded to in the Introduction, that coarser particles show higher loadabilities. To show the relative magnitude of the increase, we can divide the capcities by the smallest one measured; the result is shown in Table II. The 80-μm particles show 4.8 times higher specific dynamic capacity, or loadability, than the 10-μm particles.

Nevertheless, we *are* talking about the chromatographic efficiencies of columns that differ mutually only in the sizes of the particle contained, and thus about the efficiencies of these particles. If the smaller particles are admittedly more efficient at loadings that approach zero, then if they overload more quickly, surely the curves must cross (an active concept: at the ASTM meeting in Baltimore, MD, U.S.A. as late as October 1988 and in the discussion following a paper on preparative LC, that the curves cross was given voice by a speaker). However, whether the curves cross can be seen in Fig. 1. Although theory may predict they would eventually coincide[1], the curves do not cross.

How is it, then, that as just shown in Tables I and II, more efficient packings *do* overload more quickly? It comes from entrapment in language. Terms such as overload, specific loadability, and dynamic capacity are self-referent, only; they refer to the ideal behavior of a given packing, its chromatographic efficiency at zero load. These terms are not useful for comparing the preparative utility of one packing with another that differs from the first only in particle size. A different approach is needed, one that actually compares such packings.

TABLE II

RELATIVE SPECIFIC DYNAMIC CAPACITIES

Particle diameter (μm)	*Restated dynamic capacity (μg DBP/g packing)*
10	Unit
20	1.7
40	2.5
80	4.8

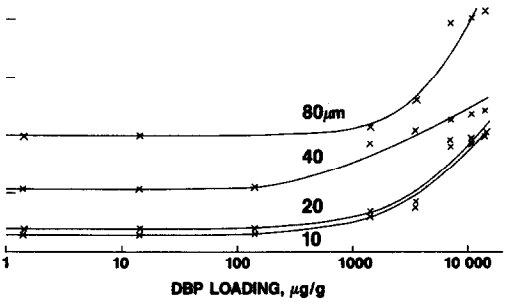

Fig. 1. Loading curves showing peak width as a function of sample loading for particles of diameters of 10, 20, 40 and 80 μm [Biochrom 100 Å (ODS)]. If a more efficient packing were to show a larger peak width for a given loading than a less efficient one, the curves would have to cross. Here the curves do not cross.

Let us suppose that we have two columns that differ only in the particle size of the packings. Let us set up our separation, cut points and all, on the column that contains the coarser particles. Then we replace the column with the coarser particles by the one with the finer particles. Now, using the larger charge required by the more efficient column to produce the peak width that corresponds to the cut points already established with the less efficient column, we repeat the separation. The operations with the two columns are shown in diagram in Fig. 2, wherein vertical lines indicate the cut points established with the coarser-particle column. In Fig. 2, the peaks are tracings from chromatograms produced for this paper; and the vertical lines indicate the loading corresponding to the specific dynamic capacity of the coarser-particle column. Let us call this the "equal-cut-point" approach.

Listed in Table III are the loadings determined by the two approaches: the specific dynamic capacity and the equal cut point. In Table IV are listed the

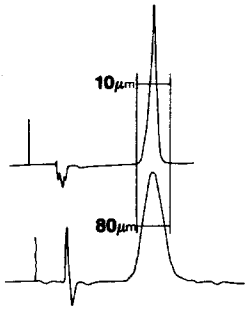

Fig. 2. Figure suggesting diagrammatically that a separation first be set up with a less efficient column that contains coarser particles, and then that the less efficient column be replaced by one that is more efficient. The vertical lines correspond to a 50% increase in peak width of the solute peak from the less efficient column. The vertical lines are taken as cut points. Let enough sample be charged to cause the peak from the more efficient column to have a width equal to those cut points. The data, obtainable from the loading curves in Fig. 1, listed in Table IV, show that under the conditions tested here, columns that contain 20-μm particles are more both productive and valuable than those that contain either 80- or 40-μm particles.

TABLE III
PREPARATIVE CAPACITY AS A FUNCTION OF METHOD OF EXPRESSION

Particle diameter (μm)	*Capacity (μg DBP/g packing)*	
	Specific dynamic	*Same cut point*
10	2000	23 000
20	3400	22 000
40	5000	5000
80	9600	1500

intra-column ratios of these loadings. The specific dynamic capacity ratios were shown earlier, in Table II. The equal-cut-point ratios show that, given the identical experimental operations, a 20-μm column is roughly 15 times more productive than an 80-μm column, roughly 4.5 times more productive than a 40-μm column. These measurements do not show much improvement in using 10-μm particles rather than 20-μm.

There is also the matter of packing costs. More efficient packings cost more. If we divide the ratios of Table IV by the costs of the respective packings, we produce ratios that express value. The value ratios (see Table V) show that a 20-μm column is almost 10 times more valuable than an 80-μm column, that is, more productive per dollar paid for the packing; and about 3 times more valuable than a 40-μm column. Again, the value ratios show no advantage in replacing a 20-μm column by a 10-μm.

TABLE IV
RELATIVE PACKING CAPACITIES

Particle diameter (μm)	*Capacity (μg DBP/g packing)*	
	Specific dynamic	*Same cut point*
10	Unit	15.3
20	1.7	14.7
40	2.5	3.3
80	4.8	Unit

TABLE V
RELATIVE PACKING VALUES

Particle diameter (μm)	*Value (capacity/dollar)*	
	Specific dynamic	*Same cut point*
10	Unit	4.1
20	4.1	9.5
40	8.7	3.1
80	18.	Unit

Note that the value ratios hold without regard to the cost or selling price of whatever product is being purified.

Pressure is simply not considered in the equal-cut-point approach, in which the packings to be compared are contained in columns of equal length. Columns, of course, need not be of equal length. In the Knox–Pyper review[7], pressures were held equal, columns could have any length and contain particles of any size. Performance (g/h of purified product) then does not depend on particle size. Consider these two points of view.

In columns of equal length and inner diameter, the pressure required for a given flow-rate and mobile phase varies inversely as the square of the particle diameter of the packing. Compared to the 80-μm column, the 20-μm column requires (80/20) squared times as much pressure per length: 16 times as much. Just let the 80-μm column be long enough to require that pressure, and it will do as well as the 20-μm. (Merely choose to use 16 80-μm columns or 4 40-μm and proportionately more pure solvent and column hardware, rather than one 20-μm.) In actual practice, the more efficient packing, easily accommodated by the pressure capabilities of current equipment, is the one of choice.

REFERENCES

1 A. W. J. de Jong, H. Poppe and J. C. Kraak, *J. Chromatogr.*, 209 (1981) 432–436.
2 A. W. J. de Jong, J. C. Kraak, H. Poppe and F. Nooitgedacht, *J. Chromatogr.*, 193 (1980) 181–195.
3 K. P. Hupe and H. H. Lauer, *J. Chromatogr.*, 203 (1981) 41–52.
4 G. Cretier and J. L. Rocca, *Chromatographia*, 16 (1982) 32–38.
5 R. W. Stout, J. J. de Stefano and L. R. Snyder, *J. Chromatogr.*, 261 (1983) 189–212.
6 M. Verzele and C. Dewaele, *LC · GC*, 3 (1985) 22–28.
7 J. H. Knox and H. M. Pyper, *J. Chromatogr.*, 363 (1986) 1–30.
8 J. E. Eble, R. L. Grob, P. E. Antle, G. B. Cox and L. R. Snyder, *J. Chromatogr.*, 405 (1987) 82–96.
9 A. Jaulmes, C. Vidal-Madjar, H. Colin and G. Guiochon, *J. Phys. Chem.*, 90 (1986) 207.
10 L. R. Snyder, G. B. Cox and P. E. Antle, *Chromatographia*, 24 (1987) 82–96.