Journal of Chromatography, 295 (1984) 81–87
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,723

MINIATURIZATION OF THE PARTICLE SIZE AND LOW DISPERSION LIQUID CHROMATOGRAPHY

LOW VISCOSITY SOLVENT UPWARD PACKING PROCEDURE*

M. VERZELE
Laboratory of Organic Chemistry, State University of Ghent, Krijgslaan, 281 (S.4), B-9000 Ghent (Belgium)
(First received January 19th, 1984; revised manuscript received February 16th, 1984)

SUMMARY

Dispersion can be reduced by particle miniaturization since this affects both the column length and the plate number. With smaller silica gel-based particles (particle diameter 2–5 μm) it is important to use spherically shaped particles and to use the low viscosity solvent upward packing technique.

INTRODUCTION

Column choice in modern high-performance liquid chromatography (HPLC) is rather confusing with the advent of open hole capillary columns, packed capillary columns, microbore columns, high speed and super speed HPLC, etc. A common denominator of these developments is that they all tend to produce lower dispersion. Low dispersion liquid chromatography (LDLC) has thus recently attracted more attention. One way to achieve low dispersion lies in the miniaturization of the particle size of the column packing material. This is the subject of the present paper.

THEORETICAL AND DISCUSSION

In HPLC, the following expression is applicable

$$C_i V_i = C_c V_c$$

where C_i, C_c = concentrations of injected, and collected samples, V_i, V_c = volumes of injected and collected samples. Since dispersion obviously results in a volume increase or concentration decrease, it could be defined as the ratio C_i/C_c or V_c/V_i. The trouble with this approach is that C_i and V_i can be varied over a fairly large range without affecting C_c or V_c. The ratio V_c/V_i can therefore be varied arbitrarily

* Presented at the *Workshop on Low Dispersion Chromatography, Amsterdam, January 19–20, 1984.*

0021-9673/84/$03.00

by changing C_i and V_i. It is therefore not possible to define dispersion as a ratio. An alternative is to use the absolute volume of solvent in which the chromatographic peak is eluted or the bandwidth volume as measured on the baseline of the chromatogram. This can be the conventional bandwidth between peak tangent intercepts on the baseline.

The concentration of the sample compound in the eluted band or peak changes greatly, from zero to its maximum value and back to zero. In the collected peak the concentration of the sample compound is averaged over the whole bandwidth volume. The chromatographed compound, on stream or collected, is however dispersed over the whole bandwidth. Dispersion looked at in this way is the same as the bandwidth volume.

With this idea a very simple theoretical approach revealing the factors affecting dispersion in HPLC can be developed. Bandwidth, B, as derived from the plate number (N) equation is

$$N = 16\,(V_R/B)^2$$

or

$$B = 4V_R \cdot 1/\sqrt{N}$$

With $V_R = V_0(k' + 1)$ and $V_0 = \rho\pi r^2 L$ this leads to

$$B = \rho 4\pi r^2(k' + 1)L \cdot 1/\sqrt{N} \qquad (1)$$

where the symbols have their usual meanings, ρ is the fraction of the total column volume available to the solvent, usually around 0.5. The factors affecting band volume are therefore the partition factor, k', the column radius, r^2, the column length, L, and the plate number, $\sqrt{N}$. k' is a thermodynamic parameter, the influence of which is often offset by gradient elution which results in small k' values for all peaks; r and L are arbitrary factors chosen by the chromatographer, only N is a column parameter. For comparison purposes it is better to reduce eqn. 1 for k'. The dispersion, D, is then given by:

$$D = B/k' + 1 = \rho 4\pi r^2 L \cdot 1/\sqrt{N} = 4V_0/\sqrt{N} \qquad (2)$$

The dispersion, D, as defined above is the bandwidth volume for a non-retained peak with $k' = 0$. A difficulty is that both V_0 and N are not easily determined with accuracy. The value of N should be the same for all peaks but it rarely is, retention, polarity, flow-rate and molecular weight can significantly influence N. Anyway the D value calculated with V_0 and N for a retained, chromatographically ideal compound should be identical with the experimentally determined bandwidth volume of a non-retained peak. If this is not so, the extra-column effects are large. D is therefore an indication of the column and system quality and can be used for system comparison purposes. For this, N should be determined at optimum flow-rate. Columns with

equal V_0 give equal dispersion if N is the same. This can hardly be expected in practice. For a 1-mm internal diameter (I.D.) microbore column and a 4.6 mm I.D. normal column the difference in V_0 per unit length is a factor of 21. For equal V_0 values, the 4.6-mm column would have to be 21 times shorter, *e.g.*, 1.2 as against 25 cm. It cannot be expected that the plate numbers for such columns will be the same. For equal lengths, a 1-mm I.D. column should give 21 times lower dispersion and therefore 21 times better sensitivity compared to a 4.6-mm column. However, a 1-mm I.D. column requires smaller and therefore less sensitive detector cells. Moreover, the sample size should remain the same and this too is not always possible. Thus, the column radius has the strongest influence on D.

In the above equations L and N are of most interest since they are affected by the particle size of the packing material. If smaller particles can be packed as effectively as larger ones, the dispersion can be diminished by particle miniaturization. As the value of N per unit length increases, L can be shorter for a particular analysis and this too will reduce dispersion.

Reducing dispersion in this way finally leads to peaks which are so narrow and rapidly eluted that the hardware (dead volumes) detector volume and electronic speed of detection of most HPLC instruments are no longer capable of handling them[1]. Instrument parameters can strongly influence dispersion. However, in the present paper this aspect will not be discussed. The lowest dispersion is not necessarily the most desirable. An open hole capillary of 5–10 μm I.D. would possibly give the lowest dispersion, even if the plate number per unit length is not so good. However, the time element becomes important in this case. A higher sensitivity and lower detection limits are not necessarily the result of lower dispersion.

Optimization of L

The choice of column length depends only on the desired plate number. Experience shows that most separations can be carried out with columns producing not more than 5000–10,000 plates. This led to the first generation of successful columns having lengths of about 25 cm, packed with 10-μm irregular derivatized silica gel. Majors[2] was the first to show the potential of fully porous 10-μm silica gel particles in such columns. At the time the proposed column I.D. was 2 mm. Why the larger I.D. of 4–5 mm then became most popular is not clear.

Modern packing procedures and packing materials are much improved and reduced plate heights, h, of 2–2.5 or chromatographic efficiencies (CE = $100/h$)[1] of 40–50% are more generally attainable even for the smaller particle sizes[3–5]. The desired column lengths as a function of particle size are thus easily calculated (Table I). Theoretical dispersion values for the different columns are also calculated. In Table I the retention volumes for $k' = 10$ illustrate the solvent consumption for the different column types. Dispersion is smaller by a factor of 5 when comparing column lengths of 25 and 5 cm. The sensitivity with the short column is also increased by the same factor. On such short columns it becomes important to inject smaller volumes than the usual 10 μl via the sample loop injector. This again increases the sensitivity for the shorter column. In practice, the sensitivity is therefore usually around 6–10 times larger on a 5-cm than on a 25-cm column.

Improvement of D by decreasing the column length is only possible in practice if equal chromatographic efficiency can be achieved with the smaller particle sizes.

TABLE I

PARTICLE SIZES AND COLUMN LENGTHS PRODUCING 10,000 PLATES WITH CORRESPONDING DISPERSION VALUES (D)

Conditions: columns of 0.46 cm I.D. except the last (0.3 cm I.D.); packing material, silica gel with $V_0 \approx 1$ ml per 10 cm in the 0.46-cm I.D. columns; $k' = 10$ for the last peak of interest.

Particle size (μm)	*Column length (cm)*	*Retention volume for k' = 10 (ml)*	*B for k' = 2 (μl)*	*D (μl)*
10	25	27.50	300	100
5	12.5	13.75	150	50
3	7.5	8.25	90	30
2	5	5.50	60	20
3	7.5	3.50	38.4	12.8

This is the case, but it is easier to obtain a CE value of around 45–50% with 5- and 10-μm particles than with 2–3 μm particles. The best column length as far as dispersion is concerned is then the shortest. For practical purposes, the conditions given in the second and third lines of Table I are to be preferred.

Optimization of N

To counter dispersion the N value per unit length should be the highest possible. This points to the smallest particle size. Material of particle size 2 μm has only recently been introduced; 3- and 5-μm materials are however rapidly becoming popular. It is important to remember that spherically shaped particles are essential for the highest N values.

Irregular and spherically shaped silica gel particles have been compared previously[1]. Spherically shaped particles smaller than $d_p = 10$ μm gave higher plate numbers than irregularly shaped particles. However, this may not generally be accepted, and the choice between irregular or spherical particles is therefore still controversial. From the recent literature, spherical materials are said to pack more reproducibility[6], more tightly[7] or give more stable columns[8]. They do not produce more plates than irregularly shaped materials under identical conditions[9–11]. There is a difference, as stated above. It is important, however, to mention that this assertion is based on results with octadecylated materials. For 10-μm particles the difference between irregularly and spherically shaped packing materials is minor. The highest CE value found, 70% ($h \approx 1.4$), was even obtained with an irregularly shaped reversed-phase material: 10-μm RSiL-C_{18}-HL-D. In general, however, 10-μm spherical materials give more easily optimized results than do 10-μm irregular particles. For 5-μm particles the difference is more pronounced and for 3-μm particles it is very great. The best 25 × 0.46 cm column packed with 5-μm irregular octadecylated silica gel (5-μm RSiL-C_{18}-HL-D) produced 21,000 plates for a retained polycyclic aromatic hydrocarbon. With spherical 5-μm ROSiL-C_{18}-HL-D (RSiLs and ROSiLs from Alltech – RSL) the maximum plate number obtained at our laboratory under similar conditions is 30,000 plates (CE = 61%). For 2- and 3-μm ROSiL-C_{18}-HL-D, the

highest CE values obtained are nearly 55%. These values are deduced for plate numbers based on peak widths measured at half-peak height and for pyrene at $k' \approx 5$. Smaller particles not only lead to higher efficiency but they also enable high solvent flow-rates since the *H vs. u* curve remains (practically) flat in the C region[12]. The differences between spherical and irregular silica gel particles mentioned above show that even the best packing methods are not really optimal for small particles. The CE values of 55, 61 and 70% (reduced plate height, *h*, of 1.8, 1.6 and 1.4 respectively) for 2–3, 5 and 10 μm particles show that there is probably room for further improvement, with the smaller spherical particles. The packing of columns is far from being completely mastered or even well understood.

Low viscosity solvent upward packing method

The efficient packing of columns is a matter of technique. The columns must be packed at high speed. This should not be confused with rapid procedures, often said to be necessary because of slurry instability. Speed is needed to pack the material tightly. The particles have to enter the column at high velocity in order to overcome frictional forces at the walls. Friction between the particles is even more important and therefore the particles must have as smooth a surface as possible. Commercial spherical silica gels do vary in this respect. It can easily be shown experimentally that a column packed at low pressure and then placed under high pressure is not as efficient as a column packed from the start at the higher pressure. Speed in this context has nothing to do with carrying out all packing steps rapidly. The slurry should be stable for a reasonable time and this is so in the particle size region 2–10 μm with the usual solvents. This is true for polar and for apolar packing materials. The packing speed is related to the pressure applied as stated, but also to the viscosity of the slurry solvent and to the particle size (permeability). Larger particle sizes, low viscosity slurry solvents and higher pressures therefore give more readily the best results. For 10-μm silica gel packings and for polar derivatized 10-μm silica gel packings, the conditions are thus not so critical. A downward packing procedure in methanol–water (90:10) can be used. For more apolar packing materials (octadecylated silica gel) with larger particle sizes a downward tetrachloromethane slurry method will also readily give optimum results. Also balanced density and viscosity slurries give good results with such materials. For smaller particle sizes, upward packing and low viscosity solvents as slurry medium are better. For 5-μm octadecylated silica gel, acetone with upward packing can be used. The acetone has to be dry and of the highest quality. Drying the acetone, *e.g.*, on molecular sieves, possibly leads to formation of traces of diacetone alcohol. This drastically reduces the packing quality. The same effect is observed when adding a trace of water or of an alcohol to the acetone. With 2- and 3-μm packing materials, the solvent has to be even less viscous than acetone. Pentane or diethyl ether are recommended. This leads to "low viscosity solvent upward" packing. This is in direct contradiction with balanced density and viscosity slurry packing methods. For the 2-, 3- and even 5-μm derivatized silica gel packings these last two methods rarely lead to optimum results. Upward "low viscosity solvent" packing is better. Apparently, the viscosity of the solvent must be adapted to the particle size. For 5-μm particles, acetone is best while pentane, for example, can give less good results. For smaller particles pentane is better. The viscosities in cP of some solvents at 20°C are: *n*-pentane, 0.23; diethyl ether, 0.23; acetone, 0.32; acetonitrile, 0.37; methanol, 0.60; tetrachloromethane, 0.97.

Heating the slurry will reduce the viscosity and should therefore be beneficial. This is indeed the case. It should be mentioned that the frictional forces in high speed packing procedures increase the system temperature much more than is generally realized. When packing upwards, the top of a column can easily show a temperature rise to 60°C. If the packing procedure does not yield a CE value of 40–50%, heating the slurry and packing equipment may help.

I have not yet tried even less viscous solvents like butane, dimethyl ether or even supercritical CO_2, but it seems worthwhile to try these as slurry medium in the low viscosity solvent upward packing method.

In the above context it is obvious that the inner walls of the chromatographic tubes should be polished, thus reducing friction between the particles and the column wall during the packing procedure.

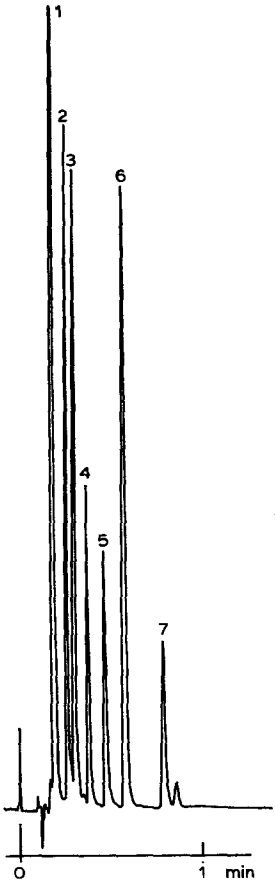

Fig. 1. Low dispersion chromatogram obtained on a Varian LC 5020 chromatograph with 1.7-μl detector cell and detector response 50 msec. Column: 4 × 0.5 cm packed with 2-μm ROSiL-C_{18}-HL-D. Mobile phase: acetonitrile–water (75:25) at 2 ml/min. Pressure: 216 atm. Detection at 300 nm. Injection via a 10-μl Valco 7000-p.s.i. injector with partial fill. Peaks: 1 = *p*-nitroaniline; 2 = *p*-nitroanilide of C_5 fatty acid; 3 = *o*-nitroanilide of C_6 acid; 4 = *p*-nitroanilide of C_7 acid; 5 = *p*-nitroanilide of C_8 acid; 6 = *p*-acetylanilide of C_{10} acid; 7 = *p*-nitroanilide of C_{10} acid.

Silica gel and derivatized silica gel have been studied for a long time at this laboratory. The results are still not absolutely predictable and every new batch of packing material, even when produced under standardized conditions, has to be tested chromatographically. One quality criterion of the packing material is the nature of its slurry in tetrachloromethane. For all materials this must be translucent, almost transparent. Those showing aggregation or visible particles should be discarded. Suspensions in acetone are less translucent but they must be homogeneous and stable. If they flocculate easily or if an inhomogeneous deposit on the flask wall (rivulet or delta formation) can be seen, the CE value will also be less than expected. To test this, a clear glass bottle (50 ml) is filled with a slurry of 4 g stationary phase in 20 ml tetrachloromethane or acetone. The glass wall above the solution must present a homogeneous picture, without rivulets or "river deltas".

Examples

A chromatogram illustrating that particle miniaturization leads to low dispersion is shown in Fig. 1. More examples and a detailed discussion of the use of 2-μm particles can be found in ref. 12. In the 4 × 0.5 cm column used for Fig. 1 the V_0 value is only 330 μl. The plate number for optimized conditions is 8000–10,000 and the calculated D value is therefore about 13–15 μl. From the data in Table I of ref. 12, this is also the experimentally observed value.

CONCLUSIONS

Smaller particles (particle diameter 2–5 μm) lead to less dispersion because they yield a higher plate number per unit length and this in turn allows the use of shorter columns. This again reduces dispersion.

Smaller particles can only be used if the column packing procedures are adapted by using the low viscosity solvent upward packing technique. The superiority of spherical over irregular silica gel particles becomes more obvious with the smaller particle sizes.

ACKNOWLEDGEMENTS

Thanks are due to the Ministerie voor Wetenschapsbeleid, the Nationaal Fonds voor Wetenschappelijk Onderzoek (NFWO) and the Instituut tot Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw (IWONL) for financial help.

REFERENCES

1 M. Verzele, J. Van Dijck, P. Mussche and C. Dewaele, *J. Liquid Chromatogr.*, 5 (1982) 1431.
2 R. Majors, *Anal. Chem.*, 44 (1972) 1722.
3 J. Giddings, *Dynamics of Chromatography*, Part I, Marcel Dekker, New York, 1965.
4 P. Bristow and J. Knox, *Chromatographia*, 10 (1976) 279.
5 K. Unger, *Porous Silica*, Elsevier, Amsterdam, 1979, p. 184.
6 J. Asshauer and I. Halasz, *J. Chromatogr. Sci.*, 12 (1974) 139.
7 G. Manius and R. Tscherne, *Int. Lab.*, 1, No. 2 (1981) 18.
8 J. Kirkland, *J. Chromatogr. Sci.*, 10 (1972) 593.
9 G. Laird, J. Jurand and J. Knox, *Proc. Soc. Anal. Chem.*, 11 (1974) 111.
10 K. Unger, W. Messer and K. Krebs, *J. Chromatogr.*, 149 (1976) 1.
11 R. Ohmacht and I. Halasz, *Chromatographia*, 14 (1981) 216.
12 C. Dewaele and M. Verzele, *J. Chromatogr.*, 282 (1983) 341.