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SPHERICAL SILICA GEL OF 2 μm PARTICLE SIZE FOR REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Performance data and applicability of 2-μm spherical octadecylated silica gel (2-μm ROSiL-C_{18}-D) are presented. Short columns (25–70 mm) with conventional internal diameter (*ca.* 5 mm) generate 200,000 to 270,000 theoretical plates per metre. Even with 2-μm particles, a reduced plate height of 2 d_p or a chromatographic efficiency of 50% can thus be obtained. The H/u plot for such 2-μm particles is flat over a very wide range in the C term portion. Analysis time can be shortened considerably through the double effect of this very high efficiency per unit length and the high solvent rate, because of the absence of a minimum in the H/u curve. With such short columns, solvent consumption per analysis is also considerably reduced. The instrument requirements are discussed. The performance of 4 × 0.5 cm I.D. columns, packed with 2-μm material, generating up to 1000 plates/sec is illustrated with some practical examples. In addition to their remarkable speed, these packed columns also give a tenfold increase in sensitivity over conventional columns.

INTRODUCTION

Over the past decade, much effort has been directed towards increasing the speed and efficiency of high-performance liquid chromatography (HPLC). At present, most HPLC analyses are carried out on 15 to 25 × 0.4 to 0.5 cm I.D. columns, packed with 5- or 10-μm diameter particles. The versatility and efficiency of reversed-phase columns have become widely recognised. Separations requiring 5000–20,000 theoretical plates can be performed on such columns in 5–20 min. The most recent improvement in chromtographic performance is due to the general introduction of 3-μm silica gel particles for adsorption, partition, and reversed-phase partition chromatography. The necessary technological development of columns and instruments has also been described extensively[1–7]. As a result, 3-μm silica gel column packing materials have become commercially available.

In the present contribution, we discuss the use of even smaller, 2-μm, spherical silica gel particles for reversed-phase chromatography. Several suggestions and experiments have been published on the use of 1- to 3-μm materials in the straight-phase mode. As the result of a theoretical study on LC, Knox and Saleem[8], suggested that the use of 2-μm particles would be optimal. With semi-empirical equations and

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by experiment, it was demonstrated by Halász *et al.*[9] that the minimum possible particle size in HPLC is between 1 and 3 μm. Halász states: "It is surprising, however, that the optimum particle size ... is *ca.* 2 μm in all of the tables". He emphasises the adverse effects of temperature gradients, possibly generated through friction when such small particles are subjected to high solvent pressure. Halász *et al.*[10] and Kirkland *et al.*[11] have used 3-μm particles in the straight-phase mode, and Unger *et al.*[12] showed a separation on 1.8-μm Al_2O_3 with high efficiency in the first eluted peak.

That smaller particles, in the 1- to 3-μm range, have not been the subject of closer investigation sooner is partly due to several comments in the literature emphasising expected difficulties. Knox and Saleem[8] state: "formidable technological problems will have to be solved before columns, 10 cm in length, containing 2-μm diameter particles can be operated without loss of peformance". They also suggest that the take-off for the eluent to the detector should be located centrally in the column and should not exceed 50% of total eluent. This, of course, infers serious technical problems. As recently as 1981, Poppe *et al.*[13] stated: "It can be concluded that ... the prospects for HPLC with particles well below 5 μm do not look very promising, because of thermal effects" and "smaller column diameters ... would avoid this effect".

A second set of reasons slowing down the trend toward smaller particles is connected with technological problems of producing such materials and of using them in chromatography. The main problems in this context are: (a) sizing the particles is difficult because conventional techniques are no longer applicable; (b) derivatising is not so easy, because washing and filtering of such small particles are very tedious; (c) packing a column with these materials is difficult. Because of the high surface energy of the small particles, particle bridging is possible; (d) chromatography must be carried out with specially designed or modified instrumentation in order to avoid extra-column effects.

In the present paper we show how these difficulties can be overcome in a straightforward manner.

EXPERIMENTAL

Equipment

All chromatographic experiments were performed on a Varian 5020 Liquid Chromatograph with a modified Varichrom UV-50 detector (cell volume 1.7 μl, time constant 250 msec) and with a Varichrom UV-100 (cel volume 2 μl, time constant 50 msec). The data were recorded with a Varian Vista data system or a Varian A25 recorder. Two types of injector were used: a Valco 7000 p.s.i. 10-μl external sample loop (Houston, Tx, U.S.A.) and a Rheodyne 1-μl injector.

Packing material

The 2-μm material is a spherical ROSiL silica gel (Alltech-RSL) (S = 200 m^2/g, pore diameter 80 Å, octadecylated to 13% organic content by thermogravimetric analysis and endcapped). Particle size evaluation by microscopic viewing becomes more difficult for smaller particles. Silica gel particles in the range 1–3 μm cannot be focused sharply, probably because of Brownian movement. However, the particles in queston, 2-μm ROSiL-C_{18}-D, were estimated to conform to their claimed

size (Reichert microscope, magnification × 1000, British Standard Graticule 3406, Part 4: 1963). The chromatographic particle size, deduced from flow-rate, viscosity and back-pressure data, was 2 ± 0.1 μm.

Columns

The columns, prepared in the laboratory from 316 stainless steel, were 25, 40, 50 or 60 mm long and had a 5-mm bore. They were fitted with modified Valco fittings and adapted porous stainless-steel fritted disks (upper frit, 2-μm pore size, 0.7-mm thick; lower frit, 0.5-μm pore size, 1.7-mm thick).

Packing procedure

The columns were packed by a slurry technique with upward flow under the following conditions: slurry concentration, 25% w/w in liquid pentane, pump, Haskel; packing pressure, 600 kg/m^2.

RESULTS AND DISCUSSION

Performance data and extra-column effects

With the new 2-μm reversed-phase silica gel it is possible to generate 200,000 to 270,000 theoretical plates per metre. Up to now, the maximum efficiency for 3-μm materials, published in the chromtographic literature, was 150,000 to 170,000 theoretical plates per metre[2–4]. Due to the large back-pressure, the maximum column length possible with 2-μm particles in the reversed-phase mode is only 7 cm.

Typical plate numbers for 2.5, 4, 5, and 6 cm × 0.5 cm I.D. columns are, respectively, 6000, 8600, 10,650 and 12,000 (for triphenylene with k *ca.* 7); eluent, acetonitrile–water (3:1) at 1.2 ml/min (= 1.7 mm/sec). For a 4 × 0.5 cm column under these conditions the pressure drop is 120–130 atm. According to Bristow and Knox[14], we calculated reduced plate heights (h) of 1.90–2.35. According to Verzele *et al.*[7], this amounts to a chromatographic efficiency (CE) value of *ca.* 50%. The resistance factor (φ) was 980–1030. These columns therefore meet the criteria established by Knox for well-packed, efficient columns.

In order to establish whether our efficiency data were not overinfluenced by extra-column effects, we employed the approximation method of Huber, as described by Abbott *et al.*[15], which estimates losses in performance from observed efficiencies over a range of retention times. Some data for a 4 × 0.5 cm I.D. column are given in Table I, from which we can conclude that the UV-50 system is adapted to the high efficiency of the column for compounds eluted with a k value of 5 or more only if a *ca.* 10% loss in efficiency is tolerated. Peak volumes for fast-eluted peaks are indeed very small, and further adaptation of instrumentation is necessary. Table I also shows that the detector cell volume is less important than the detector time-constant and dead-volumes from injector and tubing connections. With the UV-100 detector and a 50-msec time-constant the intrinsic column efficiency (N_c) is practically reached even for peaks with lower k value. The column efficiency, calculated according to Abbott *et al.*[15], is the efficiency after deduction of extra-column effects. These extra-column effects, expressed as σ^2_{ec}, are negligible for the Varian UV-100 detector and for the columns used in the present study.

TABLE I

COLUMN PERFORMANCE AND EXTRA-COLUMN EFFECTS

Compound	*k*	*N**	*Peak width 4 σ (μl)*	*N***	*Peak width 4 σ (μl)*
Naphthalene	2.5	6,763	66	10,054	54
Biphenyl	3.2	7,234	77	10,666	63
Fluorene	3.8	8,153	81	10,461	72
Phenanthrene	4.2	8,320	89	10,632	79
Fluoranthene	5.7	9,489	106	10,790	99
Pyrene	6.3	9,445	116	10,810	108
Triphenylene	7.3	10,449	126	10,849	124
		$\sigma_{ec}^2 = 10^{-4}$ ml^2		$\sigma_{ec}^2 \approx 0$	
		$N_c = 11{,}000$		$N_c = 11{,}000$	

* Experimental conditions: Varian 5020 chromatograph, UV-50 detector with laboratory-modified 1.7-μl cell and 250 msec time-constant; Valco 10-μl injector (2-μl partial fill); Varian Vista data system; column, 4 × 0.5 cm I.D.; packing material, 2-μm ROSiL-C_{18}-D; eluent, acetonitrile water (3:1); flow-rate, 1.2 ml/min; ΔP, 120 atm; $N = 5.54\,(t_R/w_{1/2})^2$.

** Experimental conditions: Varian 5020 chromatograph; Varian UV-100 detector with 2-μl cell and 50 msec time-constant; Rheodyne 1-μl injector; Varian Vista data system; column and eluent as above.

H/u relationship

In Fig. 1 the plate height, H, of a 4 × 0.5 cm I.D. column, filled with 2-μm ROSiL-C_{18}-D particles is evaluated as a function of the rate of elution. This Van Deemter curve is fundamental in chromatographic theory and is usually expressed mathematically as $H = A + B/u + Cu$. The B term, or diffusion in the mobile phase, contributes strongly to the total plate height if the rate of elution is less than 1.2 ml/min ($u = 17$ mm/sec). Only a few years ago, H/u curves of HPLC data did not even show an upswing at lower elution rates. Therefore, the Van Deemter equation was often simplified to $H = A + Cu$. It is obvious from Fig. 1 that, with the new trend toward smaller particles, this can no longer be done. The relative importance of the B term in Fig. 1 is due to the very small A (Eddy diffusion) and C terms for the 2-μm particles. Indeed, the C term is apparently zero over a wide linear solvent velocity range (1.4–4 mm/sec). Because of this low C term, the total plate height at practical elution rates is determined by the A term ($A = 4.6$ μm $= H_{min}$ in this case). At higher rates (greater than 3 ml/min) the plate number decreases drastically, and peaks show fronting. This is probably due to a temperature effect or thermal friction. In an earlier study of the effect of particle size, Unger *et al.*[12] observed indeed that the increase in plate height with velocity was high for particles less than 4 μm in diameter, and they suggested that these observations might be explained by thermal gradients in the columns as a result of viscous heating. In the introduction to this paper we mentioned friction heating and the reservations it has created in the literature[9,13]. Such discussions were probably the reason for suggestions to thermostat the injector and/or column[2]. The idea of using columns with smaller internal diameters, which would dissipate heat more easily, is also related to this. However, our results show that the range of solvent rates over which there is no adverse effect of friction heating, is so great that this factor need not to be reckoned with in practice.

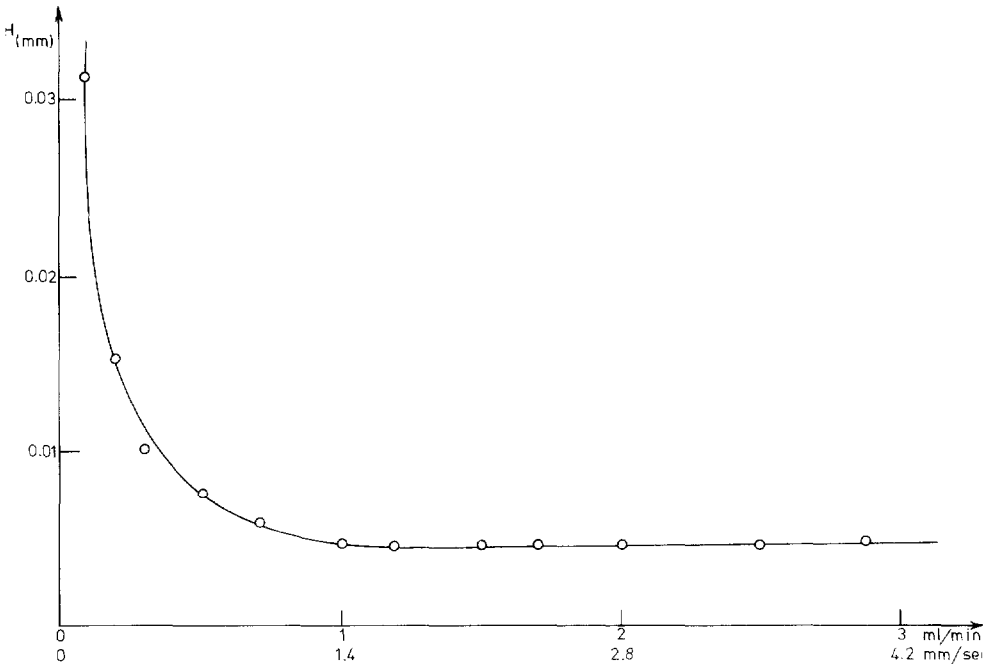


Fig. 1. Column, 4 × 0.5 cm I.D., packed with 2-μm ROSiL-C_{18}-D; mobile phase, acetonitrile–water (3:1); sample, pyrene (k = 6); detector, UV-50 modified (1.7-μl cell, 250 msec time-constant); injector, Valco 10 μl (2-μl partial fill).

Chromatographic speed

To characterize performance of fast chromatographic systems, different expressions can be used. For gas chromtography, Desty *et al.*[16] suggested the use of the number of theoretical plates produced per unit time:

$$Y = \frac{N}{T_R}$$

At first glance, the "plates per second" should increase with smaller capacity factors. However, peaks eluted closer and closer to the dead-volume lead to smaller plate values. This offsets the gain of a smaller t_R value. Better instrumentation (smaller detector cell volumes, smaller dead-volumes, and faster detector response times) can improve this factor. The "plates per second", calculated in this way, therefore compare the whole chromatographic system: the instrument and the column. Most importantly, the "plates per second", calculated in this way, depends strongly on the k value. A value for "plates per second" independent of instrument or product capacity factor and, therefore, comparing only the column and packing material is obtained by measuring the plate number for a well-retarded peak and dividing this through the dead-volume retention time.

$$X = \frac{N}{t_0}$$

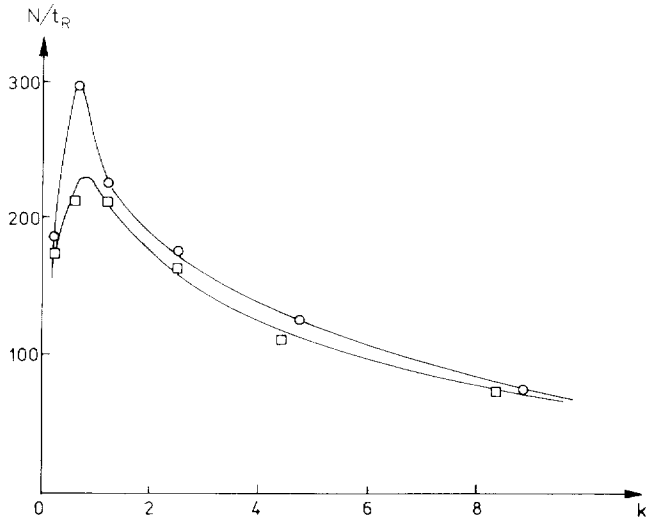


Fig. 2. Column, 4 × 0.46 cm I.D., packed with 2- (□) and 3-μm (○) ROSiL-C_{18}-D; eluent, acetonitrile water (9:1); samples, phenol (k = 0.25), toluene (k = 0.6), *tert.*-butylbenzene (k = 1.4), hexylbenzene (k = 2.5), octylbenzene (k = 4.4) and decylbenzene (k = 8.2).

In our opinion, this way of calculating "plates per second" reflects better the separation power of the column. This value for "plates per second" is constant and better suited for comparative purposes. This has been already proposed by Li *et al.*[6]. The data, $Y = (N/t_R)$, for a 4 × 0.46 cm I.D. column, filled with 2- and 3-μm ROSiL-C_{18}-D are given in Fig. 2. The 3-μm curve shows the maximum N/t_R values obtained at 4 ml/min. The 2-μm curve shows the maximum N/t_R values obtained at 2.5 ml/min (ΔP_{max} = 350 atm). The maximum N/t_0 values obtained for 2- and 3-μm materials and for peaks with $k > 5$ were 837/sec and 779/sec, respectively. Therefore, 2- and 3-μm silica gels are nearly equivalent for high-speed HPLC. However, 2-μm particles have a lower solvent consumption (2.5 ml/min *versus* 4 ml/min). In these experiments, pressure is the limiting factor for the use of 2-μm particles.

Stability of columns packed with 2-μm ROSiL-C_{18}-D

Dissolution of silica gel particles, even when coated with octadecyl groups, is the major reason for the loss in efficiency of liquid chromatography columns over a period of time. After a day's use, a 4 × 0.5 cm I.D. column, packed with 2-μm ROSiL-C_{18}-D, showed a drop in efficiency from 10,500 to 8500 plates. This is a very rapid decrease, which requires explanation.

On first sight, dissolution should only be a matter of surface area, and this is the same for all ROSiL materials, regardless of their particle size. Still, the fact is that the dissolution rate increases for smaller particles. This affects the immobilisation of mobile phase in the pores of larger particles or the greater resistance to mass transfer. This fact is also related to the greatly decreased C term for the smaller particles. For the very small silica gel-based particles, as discussed in the present paper, it is therefore imperative to use a saturating precolumn. We have used a 7 × 0.46 cm I.D.

precolumn, packed with a special batch of 10-μm ROSiL having a very large specific surface area of 800 m^2/g[1,17]. Any other silica gel precolumn will also do, of course.

Applications

Method development. Small-particle systems are suited for routine analysis, but can also be used for research work particularly in developing a chromatographic method, when analysis time can be decreased 5–10 times. The time for determining the optimum solvent composition can be reduced to minutes. At present, optimum isocratic conditions for an analysis are often deduced by running a gradient from low to high eluting power, *e.g.* from 50% methanol in water to 100% methanol. Possible isocratic conditions are then deduced from the solvent composition at which elution of the peaks of interest occurs. With the very fast chromatography provided by short columns and small-particle packing materials, a reverse approach can be used. Isocratic elution at 100, 90, 80, 70, 60 or 50% methanol or acetonitrile in water establishes the optimum solvent composition in a matter of minutes. This is not only due to fast chromatography but also to the speed with which reversed-phase systems are equilibrated. Once the best conditions are established, hundreds of analyses can be performed in one day. An example of such a development is given in Fig. 3. Four

Fig. 3. Fast determination of solvent composition. Experimental conditions: column, 2-μm ROSiL-C_{18}-D (4 × 0.5 cm I.D.); solvent, acetonitrile–water; flow-rate, 1 ml/min; detector UV 300 nm; sample, nitroanilides of fatty acids.

chromatograms are shown in which the solvent composition is changed from 100 to 90, 80 and 70% aqueous acetonitrile.

Sensitivity of analysis. Given the small dead volumes and the high efficiency of the columns under discussion, compounds are eluted in very small volumes. This leads to high mass sensitivity. For comparison purposes, an analysis was performed once on a fast HPLC column (4 × 0.5 cm I.D., 2 μm ROSiL-C_{18}-D) and once on a conventional column (10 μm RSiL-C_{18}-D, 25 × 0.46 cm I.D.) under comparable instrumental conditions (Fig. 4). The sensitivity with the small column was *ca.* 10 times higher.

Practical examples. Some practical examples of fast separations on a 4 × 0.5 cm I.D. column, filled with 2 μm ROSiL-C_{18}-D are presented (Figs. 5 and 6). Fig. 5 shows a separation of α,β-acids in a commercial hop extract. The analysis is 5–10 times faster than before; and *ca.* 30 samples can be analyzed in 1 h. Fig. 6 shows the separation of a synthetic mixture of polycyclic aromatic hydrocarbons. More than ten compounds are separated in less than 1 min and with a solvent consumption of only 2 ml.

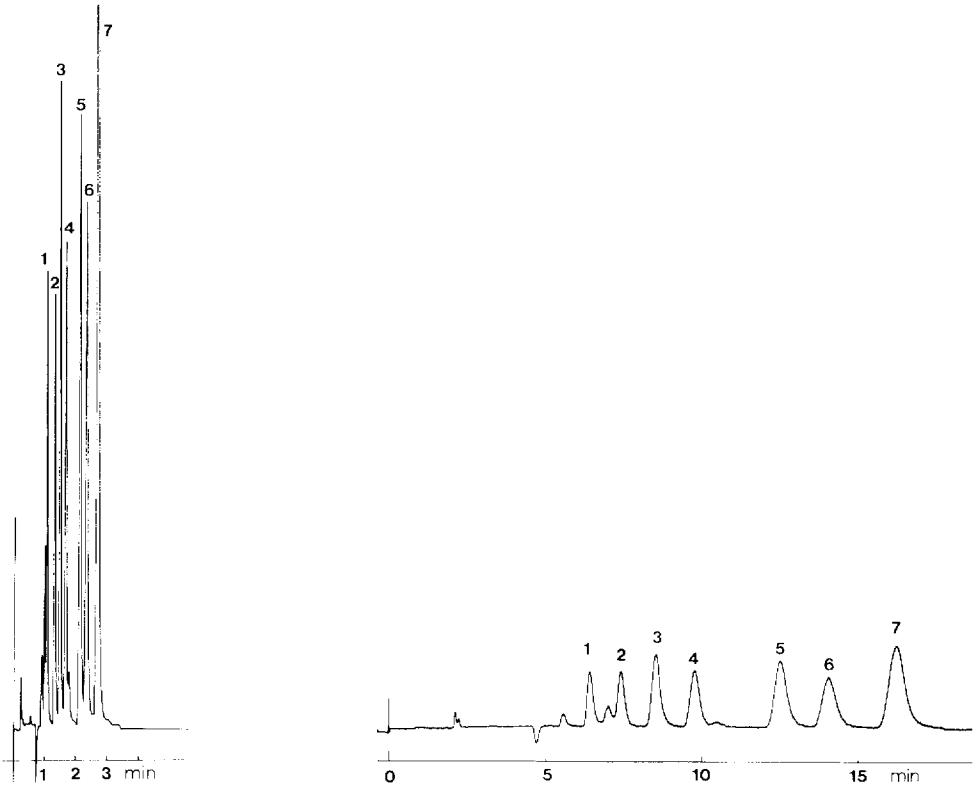
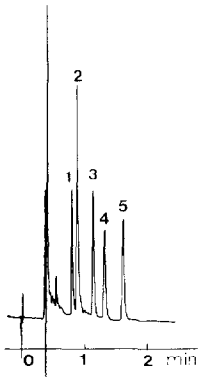


Fig. 4. Analysis of a test mixture on different C_{18}-bonded columns. Experimental conditions: (A) column, 2-μm ROSiL-C_{18}-D (4 × 0.5 cm I.D.); solvent, acetonitrile water (3:1); flow-rate, 1.2 ml/min; ΔP, 130 atm; detector, UV 254 nm at 0.2; (B) column, 10-μm RSiL-C_{18}-D (25 × 0.46 cm I.D.); solvent, acetonitrile water (3:1); flow-rate, 1 ml/min; ΔP, 35 atm; detector, UV 254 nm at 0.2.



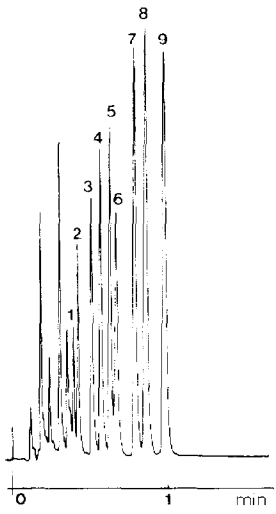


Fig. 5. Column, 4 × 0.5 cm I.D., packed with 2-μm ROSiL-C_{18}-D; mobile phase, methanol water–H_3PO_4 (9:1:0.05); flow-rate, 1 ml/min; pressure, 180 atm; detector, 360 nm; sample, hop extract. Peaks: 1 = cohumulone; 2 = humulone; 3 = colupulone; 4 = lupulone; 5 = internal standard.

Fig. 6. Column, 4 × 0.5 cm I.D., packed with 2-μm ROSiL-C_{18}-D; mobile phase, acetonitrile water (3:1); flow-rate, 2 ml/min; pressure, 216 atm; detector, 254 nm. Peaks: 1 = toluene; 2 = naphthalene; 3 = diphenyl; 4 = fluorene; 5 = phenanthrene; 6 = anthracene; 7 = fluoranthene; 8 = pyrene; 9 = triphenylene.

CONCLUSION

Chromatography on 2-μm spherical octadecylated silica gel packed in 3–5 × 0.5 cm I.D. columns is a practical proposition. It is possible to perform efficient separations (N = 10,000) in 1–2 min with moderate elution rates and low solvent consumption. All separations now performed on 5- or 10-μm particles could advantageously be carried out on 2- or 3-μm particles in correspondingly shorter columns. The advantages achieved are a 5- to 10-fold reduction in analysis time and solvent consumption, and a similar increase in sensitivity. This form of HPLC will probably gain much in popularity in the coming years. The next step would seem to be to reduce column internal diameters to 2–3 mm.

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REFERENCES

1 N. Cooke and K. Olsen, *J. Chromatogr. Sci.*, 18 (1980) 512.
2 N. Cooke, B. Archer, K. Olsen and A. Berick, *Anal. Chem.*, 54 (1982) 2278.

3 J. Di Cesare, M. Dong and L. Ettre, *Chromatographia*, 14 (1981) 257.
4 J. L. DiCesare, M. W. Dong and J. G. Atwood, *J. Chromatogr.*, 217 (1981) 369.
5 J. Di Cesare, M. Dong and F. Vandemark, *Amer. Lab.*, (1981, Aug.) 52.
6 X. Li, G. Zhou, M. Bao, Y. Zhang and P. Lu, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1981) 569.
Chromatogr. Commun., 11 (1981) 569.
7 M. Verzele, J. Van Dijck, P. Mussche and C. Dewaele, *J. Liq. Chromatogr.*, 5 (1982) 1431.
8 J. Knox and M. Saleem, *J. Chromatogr. Sci.*, 17 (1969) 614.
9 I. Halász, R. Endele and J. Asshauer, *J. Chromatogr.*, 112 (1975) 37.
10 I. Halász, H. Schmidt and P. Vogtel, *J. Chromatogr.*, 126 (1976) 19.
11 J. Kirkland, W. Yau, H. Stoklosa and C. Dilkes, *J. Chromatogr. Sci.*, 15 (1977) 303.
12 K. K. Unger, W. Messer and K. I. Krebs, *J. Chromatogr.*, 149 (1978) 1.
13 H. Poppe, J. Kraak, J. Huber and J. van den Berg, *Chromatographia*, 14 (1981) 515.
14 P. Bristow and J. Knox, *Chromatographia*, 10 (1977) 279.
15 S. Abbott, R. Simpson, J. Tusa, P. Achener, F. Klink and T. Alfredson, *Fifth International Symposium on Liquid Chromatography, Avignon, 1980.*
16 D. Desty, A. Goldup and W. Swanton, in N. Brenner, J. Callen and M. Weiss (Editors) *Gas Chromatography*, Academic Press, New York, 1962, p. 105.
17 J. G. Atwood, G. J. Schmidt and W. Slavin, *J. Chromatogr.*, 171 (1979) 109.