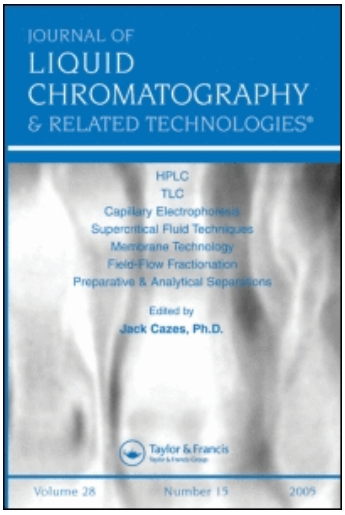

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SPHERICAL VERSUS IRREGULAR-SHAPED SILICA GEL PARTICLES IN HPLC

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ABSTRACT

With optimised packing procedures, spherical shaped silica gel particles produce 1.5 to 2 times more plates in HPLC than irregular shaped silica gel particles. The lowest reduced plate height obtained by us so far is for 5 µm ROSiL-C_{18}-HL-D and is h : 1.62 for k' : 4.5. It is suggested to transform h into 100/h % and to name this the "Chromatographic efficiency", or a % of the ideal 100 % limit. This limit would be an h value equal to the mean particle diameter. Spherical and irregular silica gel particles of 5 and 10 µm particle diameter and with similar physical characteristics have the same permeability in HPLC columns.
Whether a correct column packing procedure is used can be shown by the constancy of plate number and column permeability in function of different packing pressures.

INTRODUCTION

In the present study we evaluate the chromatographic characteristics of spherical silica gel particles in HPLC and compare these with irregular-shaped silica gel particles. The case for or against spherical silica gel in HPLC is indeed not yet settled. Asshauer and Halasz obtain better reproducibility in packing spherical silica gel (1). Manius and Tscherne claim that spherical silica gel gives a tighter bed (2),

which would suggest better column life expectancy. Kirkland (3) finds that spherical particles produce more efficient columns. Laird contradicts this (4) and states "little if any advantage arises from the use of spherical particles rather than broken chips". Unger (5,6) and Halasz (7), as recently as 1981, agree with this last opinion. We are sure many more contributions to these contradictory views can be found in the literature of the last years.
Today, many commercial brochures claim and show by actual chromatograms, that spherical silica gel is superior to irregular-shaped particle silica gel. The general commercial introduction of small sized spherical particles is even one of the most important advances in HPLC of the last years. We report now on our efforts in the field using some reversed phase silica gels, the most used HPLC stationary phases today.

Materials and instruments

All irregular-shaped silica gels tested were of the RSiL series (RSL-Alltech, Eke - B-9731 Belgium). This material is available as such and in most derivatised forms in 5 and 10 µm mean particle size. As spherical silica gel, we used, mainly, ROSiL (RSL-Alltech, Eke - B-9731 Belgium) and, for a restricted number of experiments, Spherisorb (Phase Sep, Queensferry, Clwyd, UK) and Nucleosil (Macherey Nagel, Düren - Germany). Most experiments were carried out with 5 µm ROSiL-C_{18}-HL-D, a spherical silica gel with mean particle diameter of 5 µm, derivatised with octadecyl chains in as high a concentration as possible without polymerisation, and capped with trimethylsilyl functions.

For all chromatographic runs, a Varian 5020 LC instrument (Varian, Walnut Creek - California) was used with a Varichrom variable wave length detector.

Column tubing was Lichroma with ID of 4.6 mm, injection unless otherwise stated, was with a 7000 psi Valco (Houston, Texas) 10 µl sample loop injector. The recorder was a Varian A25 recorder. All chromatographic experiments were carried out isocratically and mostly with acetonitrile-water mixtures as eluent.

Except for the column filling materials, the chromatographic conditions were, therefore, very simple and routine in character.

Chromatographic Efficiency (CE value)

Important points for comparison of column packing materials are the plate number at optimum flow and the permeability of the system. Much used in HPLC is the concept of reduced plate height or reduced parameters in general as introduced by Giddings (8) and in various papers by Bristow and Knox (9). Bristow and Knox (9) recommend as major comparison properties : the reduced plate height (h), the reduced eluent linear velocity (V) and the dimensionless permeability measure (Ø) which is called the column flow resistance. Some years after these recommendations, it turns out that the reduced plate height has found acceptance, permeability is also mentioned sometimes, but the reduced eluent velocity is rarely used. Today, the efficiency of systems is often expressed in plate number per meter although this does not account for the influence of particle diameter. Reduction of the figures seems essential, however, since this allows better comparison of the performance of particles of different sizes and shapes. Unger, in his excellent book on porous silica (6) summarising literature data, mentions that the lowest reduced plate height is about 2 for an unretained solute but that this is easily much higher for retained solutes. This was so in 1979 when Unger's book was published and still is true for

irregular silica gel; but it is not applicable anymore to spherical silica gel as shown in the present paper. Our lowest reduced plate value obtained so far for 5 µm ROSiL-C_{18}-HL-D, is only 1.62 and that is for k' ∿ 4.5 (table 1).

In capillary GC, the theoretical limit of the plate height can be calculated as shown by Ettre (10). In our paper on the static coating technique for glass capillaries (11), we have called this yield actually obtained at optimum flow rate, the "coating efficiency". Expressing the quality of a GC capillary column as a percent of the theoretically obtainable plates has been adopted by the workers in this field, and the notion of coating efficiency has been found quite useful in practice. Ettre (12) has criticised this "coating efficiency" nomenclature, mainly on the ground that factors other than

TABLE 1

	Silica gel	Column cm	Plates	h	CE in %	ΔP (Atm)
1.	5 µ ROSiL-C_{18}-D	25xO.46	25.5OO	1.96	51	96
2.	5 µ ROSiL-C_{18}-D	"	24.15O	2.07	48	98
3.	5 µ ROSiL-C_{18}-D	"	25.8OO	1.93	52	96
4.	5 µ Spherisorb ODS	"	22.OOO	2.27	44	75
5.	5 µ RSiL-C_{18}-D	"	14.OOO	3.57	28	96
6.	5 µ ROSiL-C_{18}-D	"	30.8OO	1.62	61	96

All columns except n° 6 tested on a standard Varian LC 5O2O at 1 ml/min of CH_3CN/H_2O - 75/25. Mixture of polycyclic aromatic hydrocarbons injected with a 1O µl Valco sample loop injector. Column 6 coupled to a self made 1.7 µl small volume detector cell. Plate calculation for the pyrene peak with k' ∿ 4.5.

column qualities affect the plate number. This is, of course, correct in principle. In practice however, it is obvious that the column far outweighs all other factors. Still, it would be better to avoid such criticism and to introduce e.g. a "chromatographic efficiency" or CE value. This is the same as Ettre's utilized fraction of theoretically best performance %, but at optimum flow rate. The CE value can be used as a measure of the system quality.

Something similar could be done with HPLC columns. Bristow and Knox state in their paper (9) that the "test procedure ... should enable one to see at a glance how near the performance of any particular column approaches the theoretical ideal ...". They do not mention what the "theoretical ideal" is. It is, indeed, not clear what the limit of HETP could be in HPLC, but the mean particle diameter of the packing material seems to be an acceptable limit. Halasz, indeed, mentions this for small particles like the ones mostly used with spherical silica gel (13). It is, however, easier to measure the internal diameter of capillary GC columns than to pin point the mean particle diameter of a heterogenous mixture of HPLC packing particles. Still, expressing the quality of a column by a % of the attainable and calling this the Chromatographic Efficiency (CE value) would be useful. A name such as "Packing Efficiency" could again be criticised because it excludes extra-column effects. This Chromatographic Efficiency then is defined as $CE = 100 \frac{dp}{H} = 100/h$. Expressing column plate height or plate number in % of an idealised limit enables one to see at a glance how close the system approaches that ideal. A reduced plate height of 2 is equivalent to a CE value of 50 %. In the above example of h = 1.62 the CE value is then of course 61 %. The CE concept has the

advantage that it automatically measures the quality of an LC system against a 100 % limit. It is a reduced value as recommended by Knox (9) but then, of course, it is only 100 times the inverse of h.

Column permeability

Column permeability is related to the mean particle diameter (dp) solvent viscosity (η) column length (L) flow rate of the solvent (u) and the pressure drop (ΔP). For comparison of different materials, Bristow and Knox (9) proposed the column resistance factor (Ø) which is sort of a reduced permeability value.

$$\emptyset = \left(\frac{dp}{L}\right)^2 \frac{\Delta P . to}{\eta}$$

For a given column, solvent and solvent rate, Ø is proportional to ΔP. Unger (6) mentions that Ø is about twice as high for irregular silica gel particles than for spherical silica gel particles. Unger rightly states however that these "differences cannot be discussed in terms of particle shape because both types of packings also differ in porosity and mean pore diameter". This point of permeability differences between spherical and non-spherical material is not at all clear.
A point hardly mentioned in the literature is that Ø or, therefore, ΔP for otherwise fixed conditions, can be strongly influenced by the packing pressure. We feel that this should not be so. If the densest packing is achieved, additional pressure should not increase the amount of material in the column or should increase it only very slightly and, therefore, Ø and ΔP should be constant regardless of packing pressure; unless the material is compressible and this is an undesirable situation.

This is, indeed, what is observed when the material is good and/or when the packing method is adapted to the material (table 2).

Table 2 shows that 200-250 Bar is insufficient as packing pressure for all ROSiL materials. Bristow mentions that underivatised Spherisorb can be packed at lower pressure (14). Above 250 Bar, permeability and plate number are practically constant for 5 and 10 µm ROSiL-C_{18} and for 10 µm RSiL-C_{18}. Why this is not so for the other materials is unclear. The 3 µm experimental material was probably not hard enough and was crushed under packing pressure. For 5 µ RSiL we do not understand yet why the back pressure goes up as it does with increasing packing pressure. The most obvious reasons could also be crushing under pressure (why then is this absent with 10 µ RSiL ?) or obstruction of leads or filters. Anyway, we believe now that constancy of back pressure and plate number for various packing pressures are indications that good procedures are being used. The inverse situation proves that different approaches should be attempted for that particular packing material.

Amazingly enough, a 15 x 0.46 cm column with 3 µm irregular silica gel phase, RSiL-C_{18} packed upwards at 600 kg gave only a back pressure of 85 kg under the standard conditions of our test. This was reproducible. Together with the data of tables 2 and 4, this indicates that the permeability of spherical silica gel can be either higher or lower than that of a comparable irregular shaped silica gel. Generally, the difference is minor. The same 3 µ RSiL-C_{18} mentioned above, packed downwards in a CCl_4 slurry produces very high back pressures or even totally impermeable columns. We find it very surprising that the packing technique so strongly influences permeability.

TABLE 2

ΔP and N for octadecylated and end-capped materials

Packing pressure in bar	3 μ spher.	3 μ ROSiL	5 μ ROSiL	5 μ RSiL	10 μ ROSiL	10 μ RSiL
200-250	- ( )	- ( )	86(20.300)	86(14.200)	-	20(6.500)
350-400	160(18.000)	160(19.000)	98(25.000)	96(14.000)	28(13.000)	30(8.700)
650-700	- (-)	- (-)	100(23.400)	128(13.420)	-	30(8.500)
750-800	220(13.000)	- (-)	- (-)	- (- -	-(-)	-(-)
950-1.000	230(10.500)	- (-)	96(26.000)	160(8.240)	-(-)	34(7.030)

Figures for 15 x 0.46 cm (3 μ materials) and for 25 x 0.46 cm (others) octadecylated and end-capped materials. Acetonitrile/water 75/25.
Plates for pyrene with k' = 4.5. Back pressure ΔP for 1 ml/min.

In comparing columns and packings, the most difficult point is to be sure that the packing procedure is the best and cannot be further optimised. Comparing performance (which should be very similar) under different packing pressure is one of the few confidence criteria that we know of in this respect.

Packing HPLC columns

Martin and Guiochon (15) have extensively reviewed the literature on packing procedures. A discussion on general terms of packing procedures is also due to Unger (6) and more recently to Majors (16). We tried most of these techniques with irregular silica gels and found that acceptable-to-good results were obtainable with all of them. With a variety of derivatised irregular-shaped silica gels we obtained good results with a simple slurry in dry CCl_4, packing downward at 600-900 kg/cm^2 pressure with a Haskel air-driven pump. With 5 μ RSiL-C_{18}-HL-D, for example, (irregular shaped octadecylated silica gel heavely substituted and end-capped with trimethylsilyl-groups) we obtain, in this way, a maximum of 13.600 plates for k' : 5-8 with polycyclic aromatic hydrocarbons on a 25 x 0.46 cm column. The reduced plate height is, therefore, below 4 for retained solutes; this is good for irregular-shaped silica gel. Packing 5 μ ROSiL-C_{18}-HL-D with the CCl_4 slurry technique gave essentially the same plate number. The reproducibility is also acceptable. Using a CCl_4 slurry and downward packing, therefore, reveals no difference between irregular and spherical silica gel. Whether a result is "good" or not depends on comparison with earlier figures. It suffices to obtain a higher efficiency once in order to classify all earlier "good" results in a lower category. This happened to us with the ROSiL-C_{18} packing. With

the dry acetone upwards packing method of Phase Sep (17), we obtain higher plate numbers. Occasionally a column has more than 20.000 plates. With 5 µm Spherisorb ODS, the plate numbers obtained showed exactly the same tendency : for a 25 x 0.46 cm column, about 12-14.000 with a CCl_4 slurry and downward packing, but above 20.000 with the dry acetone upward packing technique. Having obtained these very high efficiencies a few times, we wanted, of course, to achieve this reproducibly. The striking points in the dry acetone slurry packing technique are the relatively low packing pressure and the speed of packing with the low viscosity solvent. Assuming that this last point was important, we tried systems with even lower viscosity than acetone, such as ether or pentane. In the same line of approach, we packed at elevated temperatures to reduce viscosity. This sometimes leads to a positive effect, but not always ! We also packed columns by blowing in the packing with a nitrogen gas cylinder at 150 kg/cm^2. Reasonable columns are obtained but liquid slurries produce better results. We also tried the latest contributions to the field (18) with precolumns, postcolumns, both pre- and postcolumns to equalise, regulate pressure. We used pressure programmers, different pumps etc. etc. Our conclusion is that every different packing material requires its optimised individualised packing procedure. By different we do not only mean different in brand, but also differently derivatised and even different in particle size. If applicable, forcing the slurry up into columns is better than packing downward. With some packing materials, upwards packing does not work; the columns are only partially filled, and downward packing is the only possibility. With larger ROSiL-C_{18} particles, acetone as slurry solvent gives good results.

TABLE 3

n°	ROSiL-C_{18}-D	Plates	h	CE	ΔP(Atm)
1	8 µm	14.360	2.17	46	50
2	"	13.600	2.29	43	50
3	"	15.300	2.04	41	50
4	"	13.100	2.38	42	50
5	"	14.000	2.23	49	50
6	10 µm	13.240	1.89	53	28
7	"	13.000	1.92	52	30

Performance data for 25 x 0.46 cm columns. Acetonitrile-water 75-25 at 1 ml/min. Varian 5020 LC with Varichrom and 10 µl Valco injector. Calculations on pyrene with k' : 4.5.

With smaller ROSiL-C_{18} particle sizes, pentane is a better slurrying solvent. With an optimised packing procedure the slurry concentration does not seem to be very important. We used mostly 20 % slurry concentrations. The data for some columns packed with larger particle sizes with the upwards acetone slurry packing technique are shown in table 3. The data are again obtained on an unmodified Varian 5020 LC instrument.

Concluding this section on column packing, we feel that this part of HPLC leaves still much to be desired. We understand practically nothing of the mechanism of the procedures. Many methods presented as optimal, work only for one particular stationary phase. Points that are certain about column packing are :

- slurry packing is better than dry packing for very small particles
- the packing has to be dried (for reversed phase at 70-80° because in some cases higher temperature may

cause ignition of the materials) except, of course, when water is used in the slurry medium
- the dimensions and form of the packing vessel are important and have to be adapted to the packing material
- taking off the column from the packing vessel is a most important and delicate step in the procedure
- packing upwards is better if applicable (in this we agree with the reasons given by Bristow (14))
- ultrasonication of the slurry is beneficial.

Points that maybe are beneficial but of which we are not so sure that this indeed is so, are :
- dilute or high (< 10-25 % <) concentration slurry packing
- the use of dried solvents
- the use of very low viscosity solvents
- changing from slurry solvent to another solvent to follow up in the packing procedure

Points which have been claimed to be important but which are overrated or unimportant :
- the use of high viscosity slurries
- the use of balanced density slurries
- the use of very high pressures

H-u curves with ROSiLs

Graphs of the plate height against the eluent rate for various ROSiLs in C_{18} form are shown in Figure 1. The data are for pyrene with k' : 4.5 in acetonitrile-water 75/25 on 25 x 0.46 cm columns for the 5 µm material and on 15 x 0.46 cm columns for the 3 µm material. Chromatographic conditions are standard as explained elsewhere in this text. Band widths are measured at half-peak-height for chart speeds of 2 m/h or with the VISTA 401 - Chromatography Data System of Varian leading to good precision of the needed measurements.

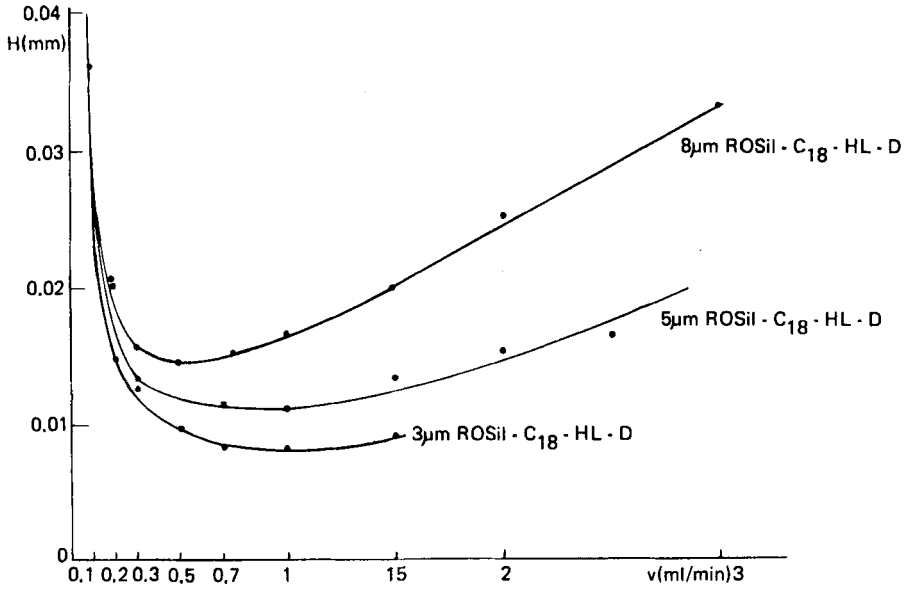

FIG. 1

Van Deemter plots for octadecylated spherical silica gels of different mean particle size. Varian LC 5020 chromatograph with Varichrom detector at 254 nm. Acetonitrile-water, 75-25 at 1 ml/min. Data for pyrene peak with k' : 4.5. 25 x 0.46 cm columns for 5 and 8 µm, 15 x 0.46 cm columns for 3 µm material. Injection through 10 µl Valco sample loop.

The behaviour of the ROSiLs in this context is classical. With the small particle sizes, the solvent speed can be increased by a factor 2 to 3 above the optimum, without adversely affecting column efficiency. This is a most important point for high speed analysis. For plate number measurements of these high performance columns, it is essential to use fast electronic detection. With an older fixed 254 nm wave length detector having a time constant of 3 sec, plate numbers were halved only because of the slow detector response. Even

the "fast" time constant of 0.5 sec of the Varichrom proved to be too slow. Therefore we modified the 2 sec "slow" time constant of the detector to an ultra fast 0.25 sec time constant. Some results are shown in Figure 2.

Figure 2 was reproduced for several other polar and non-polar compounds with similar results. For early, hardly retained peaks, the plate number can be drastically different, depending upon the speed of the electronics of the detector. Some contradictory literature statements concerning this point can probably be ascribed to this effect. Even at higher k' values, around k' : 4.5, differences can easily be 10-20 % in favour of faster detection.

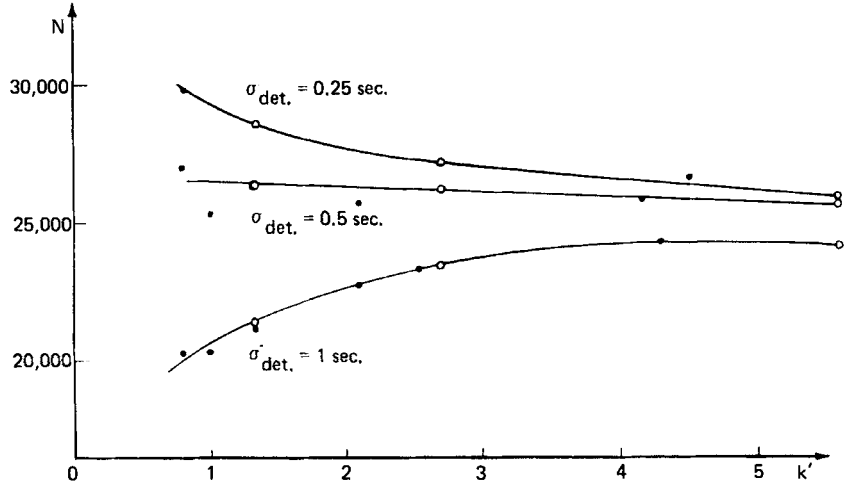

FIG. 2

Efficiencies as a function of detector time constant for the same 5 μ ROSiL-C_{18} column, 25 x 0.46 cm at 1 ml/min with variable mixtures of acetonitrile-water. Open circles : pyrene, shaded circles : naphtalene or anthracene.

Conclusions concerning spherical versus irregular silica gel

A direct comparison of spherical and irregular silica gel is shown in the next table.
Table 4 mentions values for the column resistance factor Ø and the Separation Impedance E of Bristow and Knox (9). It is important to note that these values are for k' : 4.5. For unretained peaks the literature mentions mostly lower h values and therefore more favourable E values. We feel that h values for unretained peaks should not be used as comparison criteria, if only because of their dependence of detector electronics speed as shown in the present paper. This situation is very similar to what has been experienced in GC where it now is customary only to calculate efficiencies for decidedly retained peaks e.g. k' > 3.

TABLE 4

Comparison of spherical and irregular silica gel

	Material	N	ΔP	h	Ø	E
1.	5 μ ROSiL-C_{18}-D	25.000	98	2.00	818	3.272 (2.250)
2.	5 μ RSiL-C_{18}-D	14.000	96	3.57	815	10.387
3.	10 μ ROSiL-C_{18}-C	13.000	28	1.92	1.022	3.779
4.	10 μ RSiL-C_{18}-D	8.700	30	2.87	1.029	9.041

All columns packed upwards at 350-450 kg/cm^2.
Data for pyrene with k' : 4.5 in acetonitrile-water 75/25. All chromatographic conditions standard as explained in the text and exactly equal. Ø is the column resistance factor and E is the Separation Impedance as described by Bristow and Knox (9). $E = h^2.Ø$. The value 2.250 was for optimised conditions using a small volume detector cell.

It has been suggested that further improvement through more efficient injection should be possible. We find, however, that coaxial flow injection, stopped-flow hand-injection or small-loop-volume injection do not improve the performance of a top quality column. A positive effect is only found for these approaches when column performance is not the best attainable to start with.

It should also be mentioned that the efficiency of these high performance columns is decidedly lower when methanol-water mixtures are used as eluent and/or when relative large molecular weight polyfunctional polar compounds are chromatographed. For hop α- and β-acids (humulone etc.) with methanol-water as eluent, the efficiency can be more than halved. A chromatogram of a synthetic mixture is shown in Figure 3.

The back pressure of spherical versus irregular silica gel columns can be higher or lower without obvious reason. Astonishingly enough, permeability can be strongly influenced by the packing technique. In most cases the permeability is about the same for comparable phases. The higher efficiency of spherical silica gel allows to use shorter columns while still achieving sufficient efficiency. This in turn leads to reduced pressure drops, allowing to use smaller particle sizes leading again to better efficiency and further reduced column length. Smaller particle sizes can be effectively used at higher relative eluent rates (flat H-u curve). Thus we come to very fast and very efficient HPLC. This is the most important aspect of spherical silica gel as HPLC packing material.

Because of this point it is possible to replace systematically, classic 25 cm long 10 µm irregular silica gel columns (∿ 5-10.000 plates) by 10 to 15 cm long 5 µm spherical silica gel columns (10-15.000 plates) with

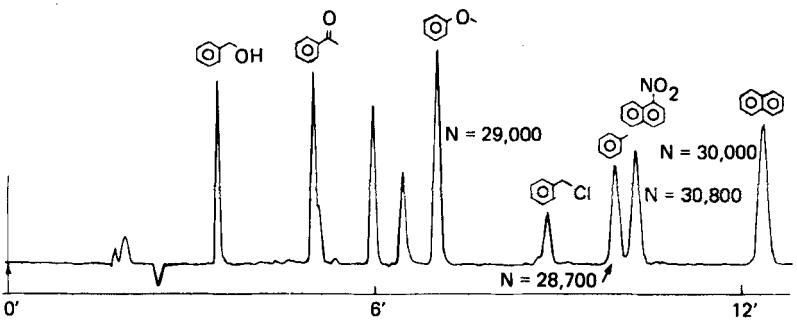

FIG. 3
Polarity mixture on 5 µ ROSiL-C_{18}-HL-D Varian 5020 LC instrument with Varichrom detector and 10 µl Valco sample loop injector. 1 ml/min of acetonitrile-water 60-40 at 120 Bar.

better resolution, better sensitivity and less than half the time of analysis.
Spherical silica gel will not agglomerate on standing for longer periods like can be the case with 3-5 µm irregular silica gel.
We have no experimental evidence about lifetime expectancy differences. Spherical silica gel columns seem to loose fairly rapidly (after 20-30 injections) about 10-20 % of their efficiency.

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