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SPHEROSIL FOR HIGH-PERFORMANCE, MODERATE-PRESSURE LIQUID CHROMATOGRAPHY

C. L. GUILLEMIN, J. P. THOMAS*, S. THIAULT and J. P. BOUNINE*

Rhône-Poulenc Industries, Centre de Recherches d'Aubervilliers, 12 rue des Jardinoux, 93308 Aubervilliers (France)

SUMMARY

The aim of this study was to prove experimentally that an inexpensive high-performance liquid chromatography system for routine control, operating at moderate pressures as predicted by the theoretical optimization model of Martin, Eon and Guiochon, is feasible.

Two improved types of Spherosil, XOA 600 and XOA 800, lead to better selectivities and efficiencies (800 plates/cm), short columns (5-10 cm), that can be operated at moderate pressures (10-30 bar) and reasonable analysis times (5-15 min).

The low pressure drops are due mainly to: the spherical shape of the particles, an average particle diameter of 5-7 μm , a narrow particle-size distribution (90% of the fraction between the limits $\bar{d}_p \pm 2 \mu\text{m}$), a high specific surface area (600 or 800 m^2/g) (the larger the surface area the shorter is the column and consequently the lower the pressure drop) and the linear velocity of the mobile phase fixed at the minimum of the Van Deemter curve.

Numerous examples of separations confirm the potential of this new trend in high-performance liquid chromatography.

INTRODUCTION

Newer developments in liquid chromatography (LC) were made without major concern for financial aspects, the primary aim being to reduce the gap between gas and liquid chromatographic techniques by improving the column efficiency and speeding up separations. As a consequence of the latest column developments using very small particle sizes and generating high pressure drops, very sophisticated and expensive commercial equipment has been built, the prices of which could be detrimental to the development of techniques for routine control purposes.

Under these circumstances there may be a risk that high-performance liquid chromatography (HPLC) could be restricted to research laboratories only, and it is therefore appropriate to consider whether cheaper HPLC systems, necessarily operating at lower pressures, would be possible.

* Rhône-Poulenc Industries, Centre de Recherches Nicolas Grillet, 13, Quai Jules Guesde, 94400 Vitry-sur-Seine, France.

An answer to this problem might be afforded by two improved grades of Spherosil, manufactured especially for HPLC, which give remarkable performances in efficiency, selectivity and analysis time, at relatively low pressure drops and consequently at a lower cost. These two grades are Spherosil XOA 600 and XOA 800, with specific surface areas of 600 and 800 m²/g, respectively.

EXPERIMENTAL

Physical characteristics of Spherosil XOA 600 and XOA 800 and column packing procedure

These two commercially available grades of Spherosil consist of completely porous and spherical silica beads, the physical characteristics of which are given in Table I.

TABLE I
PHYSICAL CHARACTERISTICS OF SPHEROSILS USED IN HPLC

<i>Type of Spherosil</i>	<i>Specific surface area, S (m²/g)</i>	<i>Pore volume, V_p (ml/g)</i>	<i>Particle size distribution, d_p (μm)</i>
XOA 600	600 ± 10%	1	\bar{d}_p between 5 and 7 μm with 90% of the sample at $\bar{d}_p \pm 2 \mu\text{m}$
XOA 800	800 ± 10%	0.6	\bar{d}_p between 5 and 7 μm with 90% of the sample at $\bar{d}_p \pm 2 \mu\text{m}$

TABLE II
PHYSICAL CHARACTERISTICS OF CARBON TETRACHLORIDE AND PERCHLOROETHYLENE SOLVENTS

<i>Solvent</i>	<i>Density (g/cm³)</i>	<i>Viscosity at 20° (cP)</i>	<i>Toxicity, max. exposure (ppm)</i>
Carbon tetrachloride	1.5942	0.97	10
Perchloroethylene	1.6230	0.88	100

The recommended packing procedure is that described by Coq *et al.*¹, which is a variant of the well known balanced density slurry technique described in detail earlier by Kirkland² and Majors³. The slurry of Spherosil in carbon tetrachloride (0.10 g/ml) is rapidly transferred into the column with a Haskel pumping system, adjusted for a pressure of 400 bar.

Because of the toxicity of carbon tetrachloride (10 ppm, OSHA* table), perchloroethylene, which has a lower toxicity (100 ppm) but similar physical characteristics, can also be used (see Table II). The use of dry perchloroethylene as the solvent in the balanced density slurry technique allows one to achieve column efficiencies similar to those normally obtained with carbon tetrachloride: efficiencies of 7000–8000 theoretical plates per 10 cm of column, measured on the naphthalene peak ($k' = 2.35$) using a test mixture of toluene, naphthalene and anthracene eluted with

* Occupational Safety and Health Administration, U.S.A.

dry *n*-hexane as the mobile phase, can be obtained. Perchloroethylene has the disadvantage, however, that the column conditioning time is double that with carbon tetrachloride.

Apparatus

Because of the very short columns used, operating under low pressure drops, a simple and inexpensive apparatus (Prolabo, Paris, France), including a pressurized coil pumping system, was employed. This apparatus is modular and consists of two major parts: the solvent distribution unit and the injector-column module (Fig. 1).

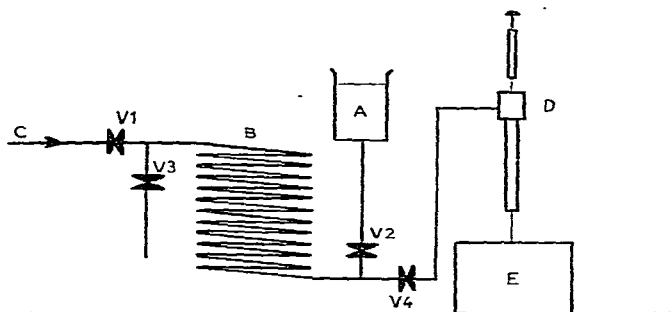


Fig. 1. Scheme of the pressurized coil pumping system of the liquid chromatograph. A = Refilling reservoir; B = coiled reservoir; C = helium inlet; D = injector-column module; E = detector; V₁, V₂, V₃, V₄ = valves.

The solvent distribution unit releases a free pulse and constant liquid flow-rate provided that the pressure drop of the entire system remains constant. For instance, the variation in the pressure drop of the coiled reservoir, during solvent discharge, is negligible with respect to that of the column. On the other hand, this system allows one to obtain the lowest flow-rates without any difficulty. Its capability of delivering mobile phase at a constant flow-rate is illustrated on the chromatogram in Fig. 2, which shows repetitive injections of a phenothiazine mixture carried out on a similar, home-made but fully automated liquid chromatograph. The repeatability of the absolute retention times is better than 1% over a day. The usefulness of this system is confirmed by the various stable, symmetrical and drift-free chromatograms shown later in the application section.

The originality of the injector-column module lies in the absence of union fittings between the column and the septum or valve-type injectors, thus decreasing the cost of maintenance. A stirrup piece maintains both the column and injector leak free up to 100 bar. Three column lengths have been provided for: 5, 10 and 15 cm, with an internal diameter of $\frac{1}{2}$ in. A stainless-steel fritted disc is crimped into the column outlet, whereas a PTFE fritted disc is sealed on to the column inlet after the column has been packed.

Any commercial detectors can be used with this apparatus; in the present study all work was performed with an Altex 254-nm detector.

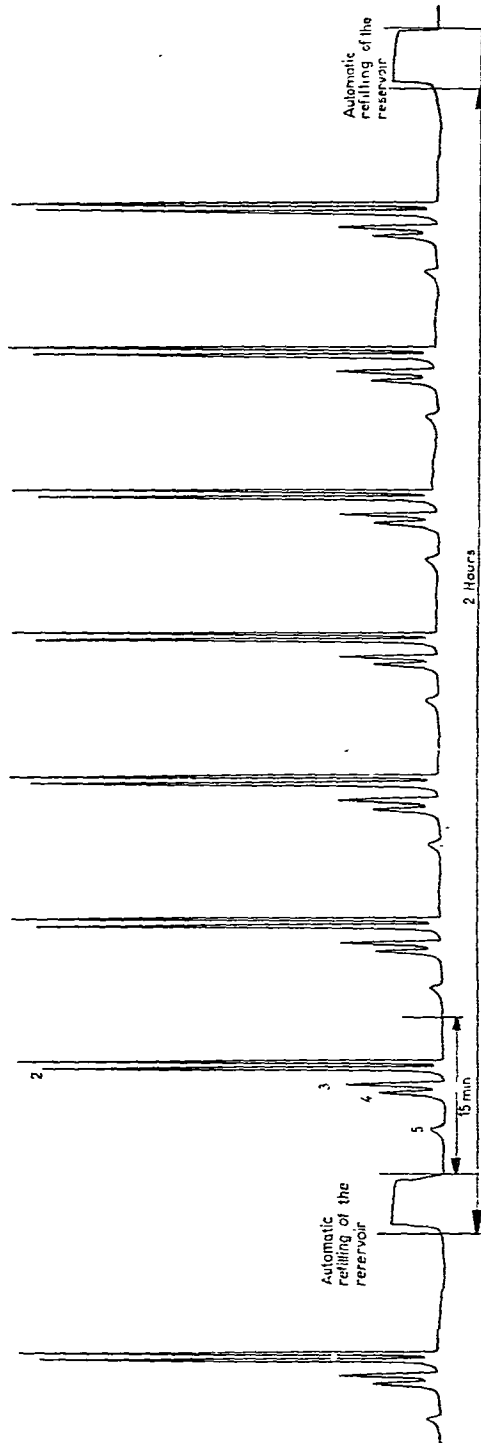


Fig. 2. Chromatograms showing the repeatability of retention data of a phenothiazine mixture when using a pressurized coil pumping system. Injections were carried out on a fully automated home-made chromatograph. Peaks: 1 = levomepromazine; 2 = chlorpromazine; 3 = dimetotiazine; 4 = propi-
cazine; 5 = oxomemazine.

CRITERIA FOR HIGH-PERFORMANCE, LOW-PRESSURE LIQUID CHROMATOGRAPHY

Choice of particle size. Optimization model of Martin, Eon and Guiochon applied to Spherosil

The optimization problem according to Martin *et al.*⁴⁻⁷ consists in the determination of the minimum pressure necessary, or in other words the cost to be paid for achieving a given separation.

Combining the Darcy equation with some classical chromatographic quantities, these authors calculated and plotted the variation of pressure drop against the column length and particle size necessary to achieve in 5 min the elution of a retained compound ($k' = 2$) as a 5000-plate peak. The classical equations used for these calculations are as follows⁴.

$$\text{Darcy equation: } u = \frac{K_0 d_p^2}{\eta} \cdot \frac{\Delta P}{L} \quad (1)$$

$$\text{Resolution: } R_s = \frac{\sqrt{N}}{4\epsilon} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k'}{1 + k'} \quad (2)$$

$$\text{Number of theoretical plates: } N = \frac{L}{H} \quad (3)$$

$$\text{HETP: } H = \frac{2\gamma D_m}{u} + \frac{A}{D_m^{1/3}} \cdot u^{1/3} d_p^{4/3} + \frac{C d_p^2 \cdot u}{D_m} \quad (4)$$

$$\text{Retention time: } t_R = \frac{L}{u} (1 + k') \quad (5)$$

$$\text{Maximum concentration of the peak: } C_{\max} = \frac{4m\sqrt{N}}{\pi \cdot \epsilon_m (1 + k') d_c^2 L \sqrt{2\pi}} \quad (6)$$

Fig. 3 shows the theoretical curves obtained by Martin *et al.* The minimum of the two curves shows that a compound ($k' = 2$) can be separated in 5 min with an efficiency of 5000 plates in an 11-cm column packed with 7- μm particles and operated at 11.5 bar. It follows that high-performance, low-pressure chromatography is theoretically possible.

Some preliminary experimental results having partly verified these theoretical predictions, a systematic study was carried out on experimental batches of Spherosil XOA 600 and XOA 800 with particle sizes between 5 and 15 μm (measured with a Coulter counter apparatus). A test mixture of toluene, naphthalene and anthracene was separated on columns 5 and 10 cm in length packed with the two grades of Spherosil using *n*-hexane at a flow-rate of 1 ml/min. The results were compared with reference to the optimization model of Martin *et al.*

The same general shape is observed for the curves $\Delta P = f(d_p)$ and $\Delta P = f(L)$ (Figs. 4 and 5) in comparison with the theoretical curve (Fig. 3); the minima of these

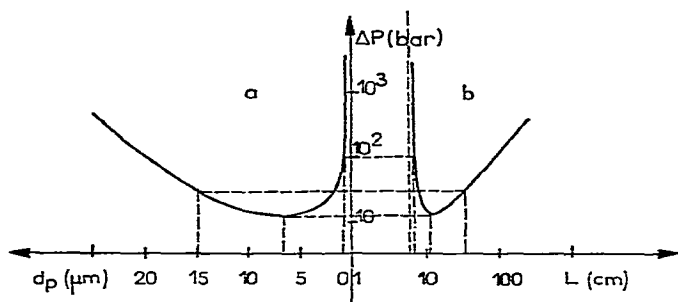


Fig. 3. Economical optimization model of Martin, Eon and Guiochon⁴⁻⁷ (by courtesy of the authors). Variation of pressure drop (ΔP) with column length (L) and particle size (d_p) necessary to achieve in 5 min the elution of a retained compound ($k' = 2$) as a 5000-plate peak.

plots correspond to ΔP , d_p and L values of the same order of magnitude as given by the model.

It can be concluded that high-performance, low-pressure liquid chromatography is feasible, provided that a spherical support with a particle size of $7 \mu\text{m}$ is used in a column with a length of about 10 cm. Both the theoretical and the experimental results clearly indicate that a particle size of about $7 \mu\text{m}$ is optimal; however, as pressure drops are still moderate with a particle size of $5 \mu\text{m}$ (Figs. 4 and 5), and because the column efficiency increases with decreasing particle size, the average particle diameter can be chosen within these two limits.

According to Snyder⁸, the decrease in HETP with particle diameter is governed by the empirical expression

$$H = a d_p^\beta \quad (7)$$

where $\beta \approx 1.8$.

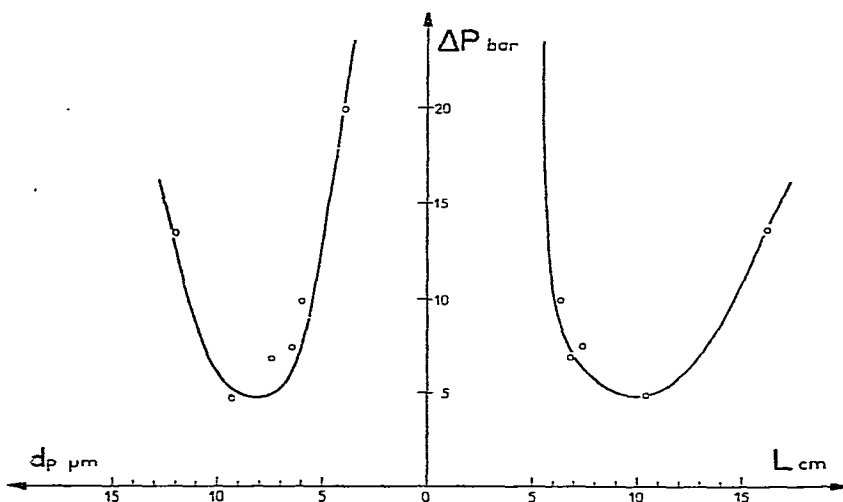


Fig. 4. Application of the Martin, Eon and Guiochon's optimization model to Spherosil XOA 600. Plots drawn from experimental data.

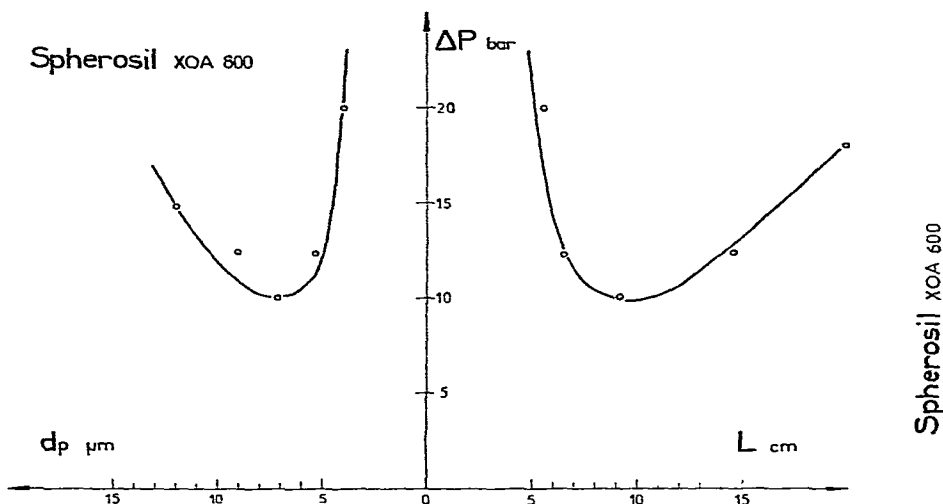


Fig. 5. Application of the Martin, Eon and Guiochon's optimization model to Spherosil XOA 800. Plots drawn from experimental data.

Spherosil XOA 600 and XOA 800 have average particle diameters varying between 5 and 7 μm , with 90% of the sample being within the limits $\bar{d}_p \pm 2 \mu\text{m}$. For instance, if $\bar{d}_p = 5.5 \mu\text{m}$, 90% of the sample will be between 3.5 and 7.5 μm . These characteristics allow excellent column efficiencies, as shown by the different chromatograms presented here. HETPs as low as 12.5 μm or reduced HETPs < 2 can easily be attained.

However, the advantages of Spherosil are due not only to the suitable particle diameter and particle size distribution, but also to the specific surface area, the importance of which has already been demonstrated in gas chromatography⁹.

Influence of specific surface area of Spherosil

As is already known in adsorption chromatography, the chromatographic properties are functions of the specific surface area of Spherosil; for instance, the following expression for retention time can be written¹⁰:

$$t_R = \frac{L}{u} \left[1 + \frac{V_p}{V_0} + K_{ad} \cdot \frac{S(1 - \varepsilon_s)(1 - \varepsilon_m) \rho_s}{\varepsilon_m} \right] \quad (8)$$

with

$$k' = \frac{V_p}{V_0} + K_{ad} \cdot \frac{S(1 - \varepsilon_s)(1 - \varepsilon_m) \rho_s}{\varepsilon_m} \quad (9)$$

As can easily be inferred from eqn. 8, the retention time can be kept constant for given values of u , V_p/V_0 and K_{ad} if the following condition is obeyed:

$$LS(1 - \varepsilon_s) = \text{constant} \quad (10)$$

With columns packed by the technique previously described, one can assume that the inter-particle void fraction, ε_m , is constant to a first approximation, so that

only S and ε_s have to be taken into account. Therefore, an increase in the specific surface area will involve a corresponding reduction in the column length and consequently a decrease in the column pressure drop and analysis time.

One of conditions fixed by the optimization model is an analysis time of 5 min for $k' = 2$ and, because to a first approximation for a given solute k' tends to increase with the corrected specific surface area, as shown in Fig. 6, a column of Spherosil XOA 800 always will be shorter than the corresponding column of Spherosil XOA 600; the shorter the column, the lower is the pressure drop.

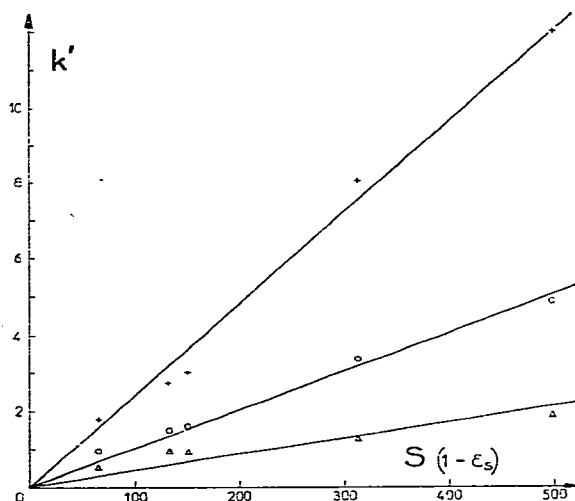


Fig. 6. Plot of capacity factors (k') for benzene (Δ), naphthalene (\circ) and anthracene ($+$) versus the corrected specific surface area of Spherosil, $S(1 - \varepsilon_s)$.

Moreover, it must be pointed out that values of ΔP read at the minima of the curves (Figs. 4 and 5) show some differences that can be explained on the one hand by a larger standard deviation of the particle size distribution for Spherosil XOA 800 than for Spherosil XOA 600 ($\sigma_{800} = 1.40$ and $\sigma_{600} = 1.25$), and on the other hand by the fact that there is slightly more resistance to fluid flow with Spherosil XOA 800 as its pore diameter (40 Å) is smaller than that of XOA 600 (90 Å).

Fig. 6 shows a straight-line relationship for the dependence of k' on the corrected specific surface area, for aromatic hydrocarbons, as expected from eqn. 8. This aspect of the influence of the specific surface area will be briefly emphasized in the applications section.

Choice of the flow-rate of the mobile phase

In modern liquid chromatography, reductions in analysis time are mainly obtained by increasing the flow-rate of the mobile phase, but consequently there is a corresponding pressure drop, according to Darcy's equation (eqn. 1). However, in recent years there has been a trend towards working at lower pressure drops (between 50 and 100 bar^{11,12} or less¹⁰) than before (higher than 100 bar).

To achieve the best routine separation in the minimum time and at the mini-

imum cost, the flow-rate of the mobile phase must be reconsidered, as it affects the equipment costs because of the pressure drop that has to be surmounted and because of the consumption of the mobile phase. As the maximum column efficiency is required when using short columns of Spherosil with a large specific surface area, the linear velocity of the mobile phase should be chosen at the minimum of the HETP = $f(u)$ curve.

The remaining problem consists in proving that running a separation with an optimized flow-rate of the mobile phase does not affect the analysis time too much.

In order to establish the optimal flow-rate to be used, plots of HETP *versus* linear velocities were constructed for different particle diameters of Spherosil XOA 600 (Fig. 7). Remarkably low HETP values (down to 12 μm) were obtained, especially for the 6.5- μm sample, which corresponds to a reduced plate height, $h = H/d_p$, of 1.85. A column with a length of 10 cm may generate up to 8000 theoretical plates ($k' = 2$), which is sufficient in many instances for separations in routine control.

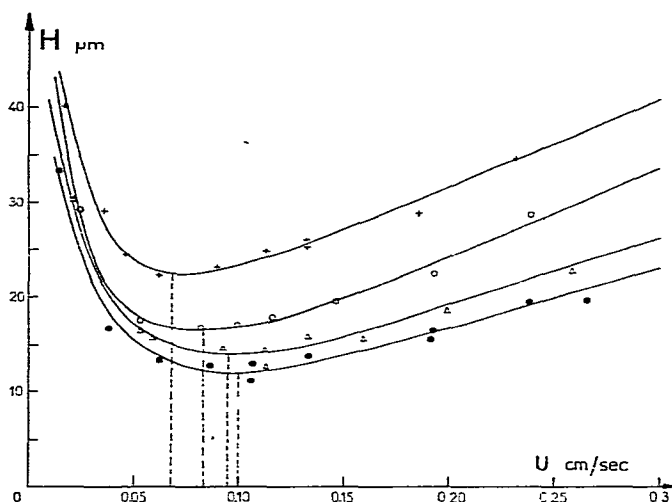


Fig. 7. HETP *versus* linear velocity of the mobile phase for Spherosil XOA 600 of different particle diameters: ●, 6.5 μm ; Δ , 7.5 μm ; ○, 9 μm ; +, 12 μm .

Complete agreement with theory was found for the variation of the linear velocity at the minimum of the curves with respect to particle diameter: in the reduced linear velocity expression $v = ud_p/Dm$ according to Giddings¹³ and Kennedy and Knox¹⁴, the linear velocity is inversely proportional to the particle diameter. Fig. 8 illustrates this expression and gives the optimal value of the flow-rate of the mobile phase with regard to $1/d_p$ for a 4-mm I.D. column. For instance, with a particle diameter of 6.5 μm , the optimal flow-rate is 0.8 ml/min.

In Fig. 9, the experimental results have been plotted in terms of the reduced dimensionless parameters h and v . Theoretically, such a reduced parameter plot should be independent of particle size and allow comparisons between different commercial supports to be made. The limited spread of the experimental results confirms

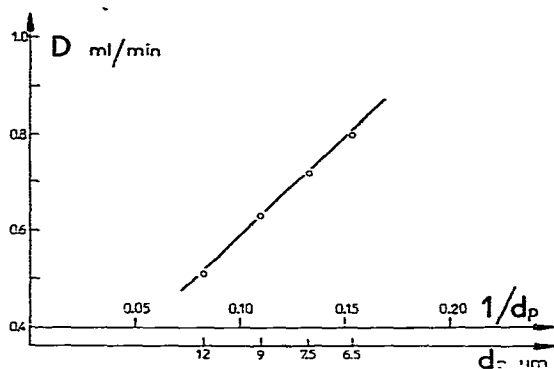


Fig. 8. Plot of optimal flow-rate of mobile phase (D) versus $1/d_p$ for a 4-mm I.D. column.

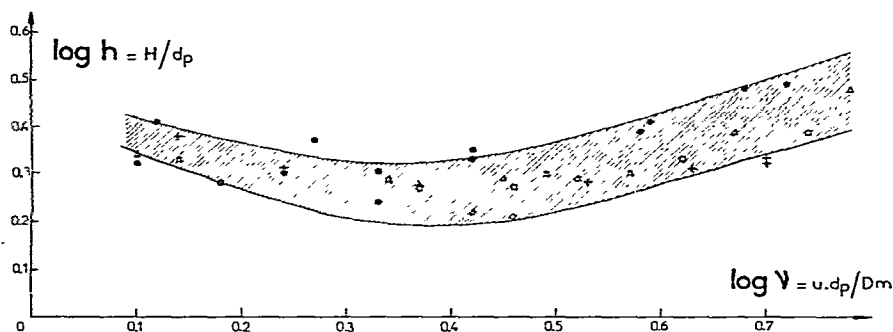


Fig. 9. Reduced plate height versus reduced velocity plots of the data in Fig. 7. Diffusion coefficient (D_m) of benzene in *n*-hexane = $3.2 \cdot 10^{-5}$ cm²/sec.

that there is no systematic influence of particle size and that the packing technique employed is satisfactory for obtaining efficient short columns when using small particle sizes (5–7 μm).

The combined characteristics of short, efficient columns with a low pressure drop, running at the optimal flow-rate of the mobile phase, should give reasonable analysis times. This is illustrated by Fig. 10, which shows a synthetic blend of aromatic hydrocarbons separated on a 4-mm I.D. column, 5 cm in length, packed with Spherosil XOA 800, $\bar{d}_p = 5.3 \mu\text{m}$, with an *n*-hexane flow-rate of 1 ml/min, close to the minimum of the $\text{HETP} = f(u)$ curve.

The efficiency of such a column is 4000 plates (800 plates/cm) measured on the anthracene peak with $k' > 5$. The pairs of peaks benzene–toluene and anthracene–phenanthrene need only a 5-cm column of Spherosil XOA 800 for their separation; also, more than eight peaks could possibly be inserted between the naphthalene (No. 4) and anthracene (No. 6) peaks, according to Kaiser's calculation^{15,16}. In addition to the excellent separation, symmetrical peak shape, a low pressure drop of 12 bar and a consumption of the mobile phase of only 20 ml, the analysis time of 20 min demonstrates that economical high-performance liquid chromatography is feasible.

In general, speeding up an analysis when Spherosil is used will involve shorten-

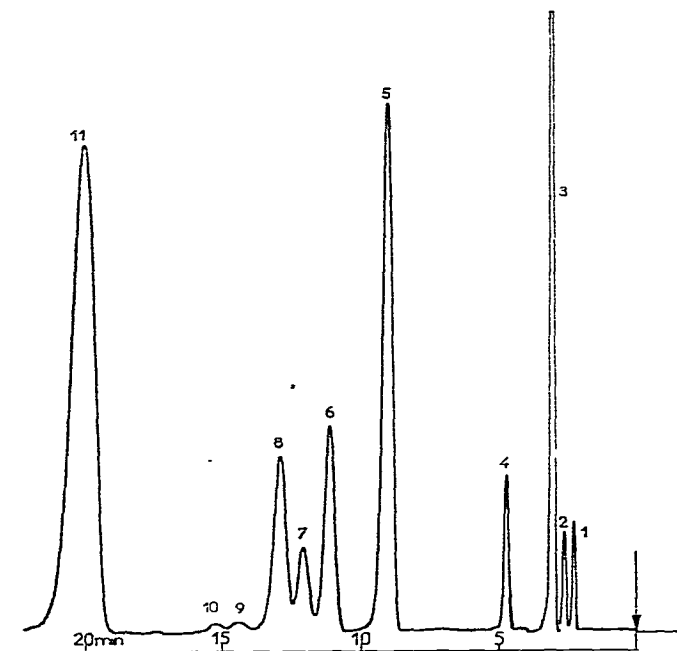


Fig. 10. Separation of some aromatic hydrocarbons. Peaks: 1 = benzene; 2 = toluene; 3 = styrene; 4 = naphthalene; 5 = biphenyl; 6 = anthracene; 7 = phenanthrene; 8, 9, 10 = unknown; 11 = 2-phenylnaphthalene. Column: 5 cm \times 4 mm I.D. Spherosil XOA 800, d_p 5.3 μ m. Mobile phase, dry *n*-hexane; flow-rate, 1 ml/min; ΔP , 12 bar; temperature, ambient.

ing the column length, with a concomitant increase in the specific surface area of the support, rather than increasing the flow-rate of the mobile phase, which has to be kept at the minimum of the Van Deemter curve.

APPLICATIONS

In order to show the capabilities of these two improved grades of Spherosil in adsorption LC, various separations were investigated in the isocratic elution mode using the simple apparatus previously described.

High column efficiencies, excellent separations, good baseline stability and symmetrical peak shapes were obtained, demonstrating the excellent characteristics of Spherosil and the capabilities of the technique. Some studies were performed on both Spherosil XOA 600 and XOA 800, in order to emphasize the advantages of high specific surface areas in low-pressure LC. Only columns of 5 or 10 cm in length were used in these applications, with minimal consumption of mobile phase.

Separation of pharmaceutical compounds

Separation of noscapine and promethazine (after ref. 17). Fig. 11A shows the separation of these two compounds in 1.5 min on a column of 5 cm in length packed with Spherosil XOA 600. Such a separation could be satisfactory for routine control, but an impurity is visible in front of the noscapine peak when using a 10-cm column

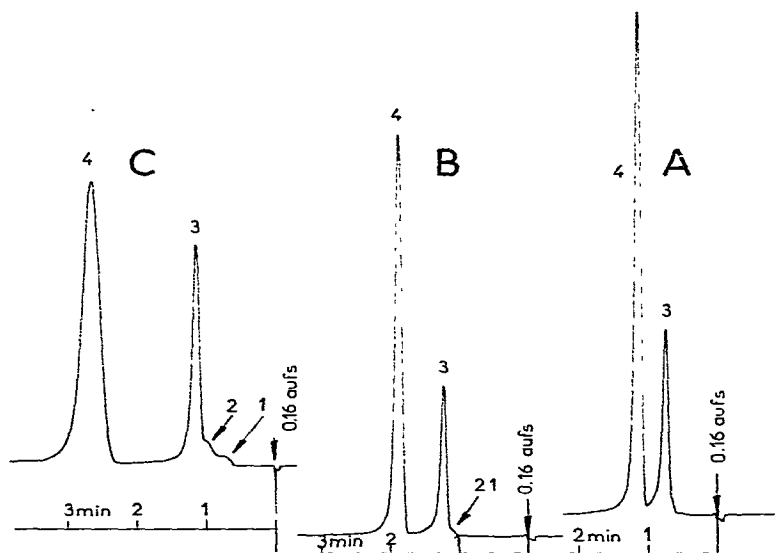


Fig. 11. Separation of noscapine and promethazine¹⁷. Peaks: 1, 2 = impurities; 3 = noscapine; 4 = promethazine. (A) Column: 5 cm \times 4 mm I.D., Spherosil XOA 600, d_p 6.5 μ m. Mobile phase, methanol-water-triethylamine (94.6:5.2:0.2); flow-rate, 0.8 ml/min; ΔP , 16 bar. (B) Column: 10 cm \times 4 mm I.D., Spherosil XOA 600, d_p 6.5 μ m. Mobile phase, as in A; flow-rate, 1 ml/min; ΔP , 33 bar. (C) Column: 10 cm \times 4 mm I.D., Spherosil XOA 800, d_p 5.3 μ m. Mobile phase, as in A; flow-rate, 0.9 ml/min; ΔP , 59 bar. Temperature: ambient in each instance.

packed with the same material (Fig. 11B). Finally, two impurities can be separated from noscapine with a 10-cm column packed with Spherosil XOA 800 (Fig. 11C). The excellent resolution between the major peaks and the reasonable pressure drop, despite the relatively high viscosity of the complex mobile phase, can be seen.

Separation of a mixture of phenobarbital, amidopyrine, caffeine and nicotinamide (after ref. 17). Figs. 12A and 12B show the separation of these compounds on a 10-cm column of Spherosil XOA 600 and XOA 800, respectively. The better resolution on XOA 800, especially between the pairs of peaks 1-2 and 3-4, indicates that there are some differences in surface energy between the two grades of Spherosil. On the contrary, keeping the analysis times equal would require a shorter, and consequently less resistant to flow, column of XOA 800 compared with XOA 600.

As far as possible, the greater potentialities of Spherosil XOA 800 compared with XOA 600 will be utilized either to improve separations or to reduce the column pressure drop.

From these two first examples, one can assume that the surface energies of the two grades of Spherosil are not only quantitatively but also qualitatively different.

Separation of a mixture of benzocaine, procaine and tetracaine chlorhydrates (after ref. 17). The correctness of the previous assumption is proved by this separation, which shows different peak elution orders on Spherosil XOA 600 and XOA 800 (Fig. 13). This effect is believed to be due to differences in the interaction forces between the silanol groups of the different types of silica and the eluted molecules.

Separation of a complex mixture of phenothiazines. Adsorption liquid chromatography on Spherosil of high specific surface area, combined with a mobile phase

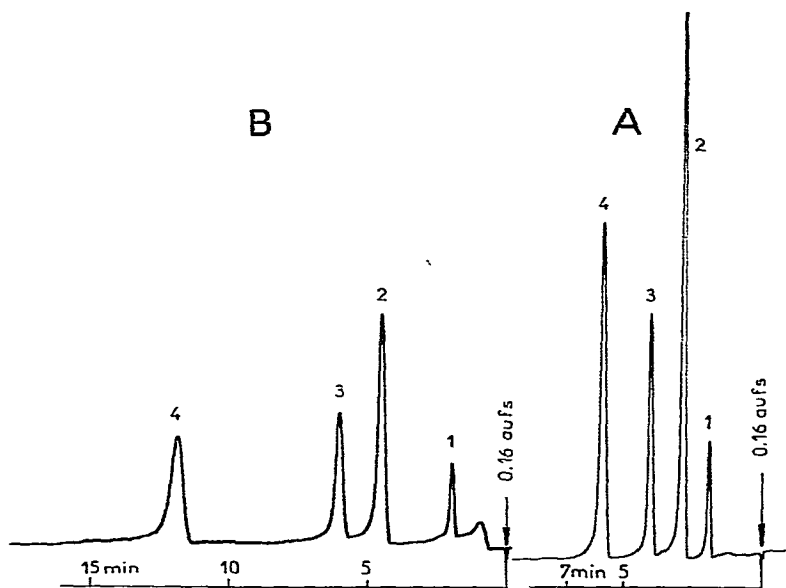


Fig. 12. Separation of a mixture of phenobarbital (peak 1), amidopyrine (2), caffeine (3) and nicotinamide (4)¹⁷. (A) Column: 10 cm \times 4 mm I.D., Spherosil XOA 600, \bar{d}_p 6.5 μ m. Mobile phase, isooctane-diisopropyl oxide-methanol-triethylamine-water (34.93:49.51:14.58:0.20:0.78); flow-rate, 1 ml/min; ΔP , 22 bar. (B) Column, 10 cm \times 4 mm I.D., Spherosil XOA 800, \bar{d}_p 5.3 μ m. Mobile phase, as in A; flow-rate, 1 ml/min; ΔP , 23 bar. Temperature: ambient in each instance.

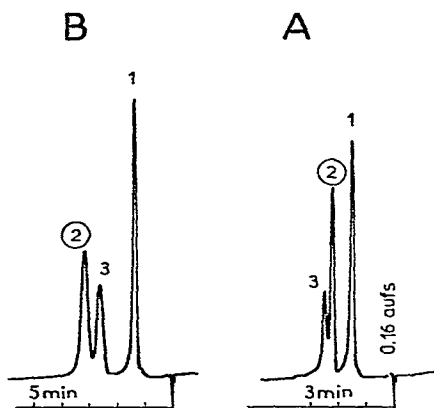


Fig. 13. Separation of benzocaine (peak 1), procaine chlorhydrate (peak 2) and tetracaine chlorhydrate (peak 3)¹⁷. (A) Column: 10 cm \times 4 mm I.D., Spherosil XOA 600, \bar{d}_p 6.5 μ m. Mobile phase, isooctane-diisopropyl oxide-methanol-triethylamine-water (39.92:49.55:9.63:0.20:0.70); flow-rate, 1 ml/min; ΔP , 25 bar. (B) Column: 10 cm \times 4 mm I.D., Spherosil XOA 800, \bar{d}_p 5.3 μ m. Mobile phase, as in A; flow-rate, 0.8 ml/min; ΔP , 34 bar.

with an optimal solvent composition, is a very powerful separation technique, as shown in Fig. 14. Nine phenothiazines were completely separated in less than 15 min on a 10-cm column packed with Spherosil XOA 600 at a mobile phase flow-rate of 1 ml/min, developing a pressure drop of only 20.5 bar. The peaks are narrow and symmetrical.

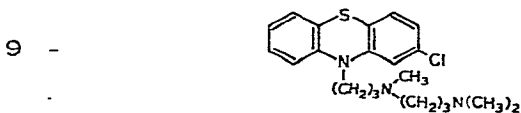
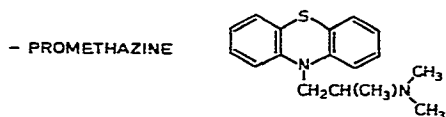
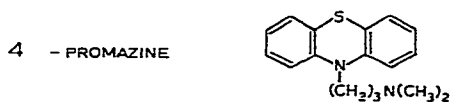
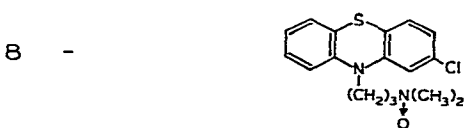
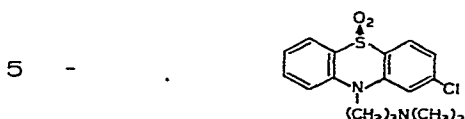
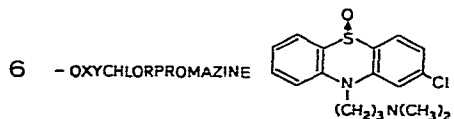
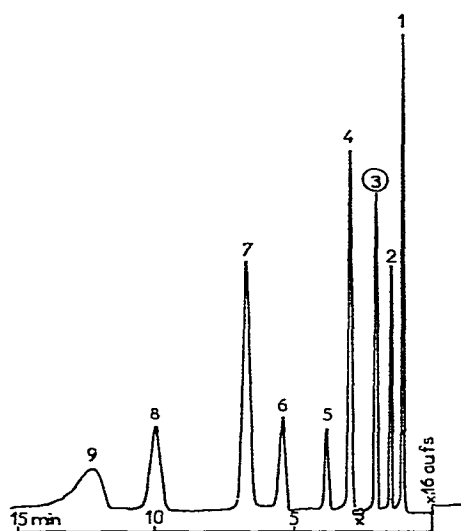


Fig. 14. Separation of phenothiazines. Column: 10 cm \times 4 mm I.D., Spherosil XOA 600, d_p 6.5 μ m. Mobile phase: 2,2,4-trimethylpentane-diisopropylalcohol-methanol-triethylamine-water (7.48:48.80:41.31:0.20:2.21); flow-rate, 1 ml/min; ΔP 20.5 bar; temperature, ambient.

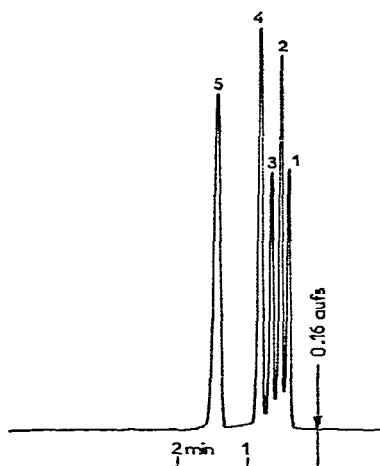


Fig. 15. Separation of phthalate esters. Peaks: 1 = octyl phthalate; 2 = butyl phthalate; 3 = allyl phthalate; 4 = ethyl phthalate; 5 = methyl phthalate. Column: 5 cm \times 4 mm I.D., Spherosil XOA 800, d_p 5.3 μ m. Mobile phase, *n*-hexane-isopropanol (90:10); flow-rate, 1 ml/min; ΔP , 13 bar; temperature, ambient.

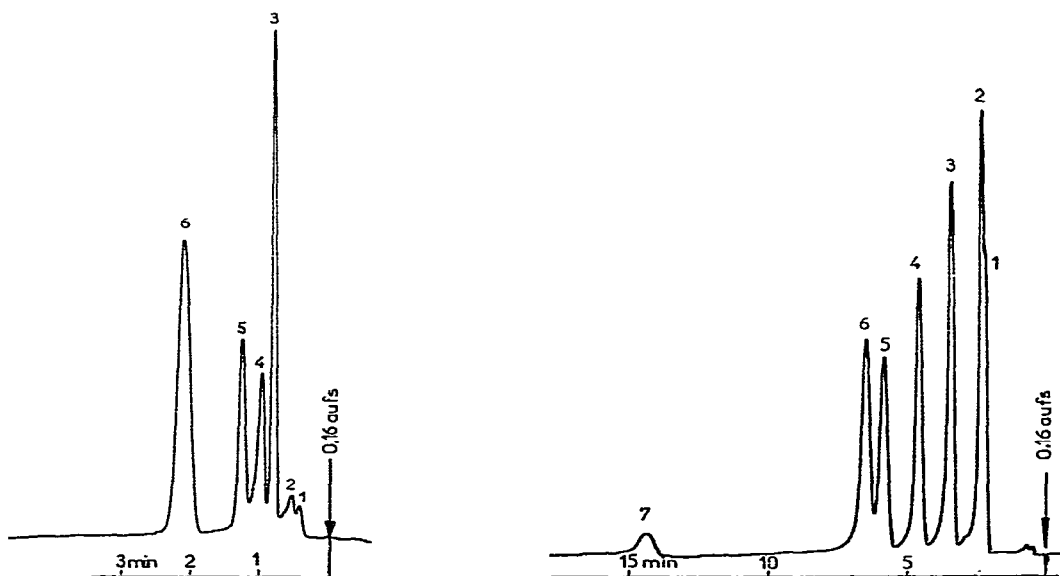


Fig. 16. Separation of phenolic derivatives. Peaks: 1, 2 = unknown; 3 = phenol; 4 = *o*-methoxyphenol; 5 = *p*-methoxyphenol; 6 = resorcinol. Column: 5 cm \times 4 mm I.D., Spherosil XOA 800, d_p 5.3 μ m. Mobile phase, *n*-hexane-isopropanol (90:10); flow-rate, 1 ml/min; ΔP , 13 bar; temperature, ambient.

Fig. 17. Separation of aromatic amines. Peaks: 1 = 2-nitro-4-methylaniline; 2 = *o*-toluidine; 3 = aniline; 4 = 5-nitro-2-methylaniline; 5 = 3-nitro-4-methylaniline; 6 = *m*-nitroaniline; 7 = *p*-nitroaniline. Column: 5 cm \times 4 mm I.D., Spherosil XOA 800, d_p 5.3 μ m. Mobile phase, *n*-hexane-isopropanol (90:10); flow-rate, 0.9 ml/min; ΔP , 15 bar; temperature, ambient; injection, 0.8 μ l of a 1% solution.

Separation of various components belonging to different chemical types

Fig. 15 illustrates an excellent separation of five phthalate esters, achieved in less than 2 min on a 5-cm column of Spherosil XOA 800 at 13 bar. Rather than this being a true exclusion phenomenon, one can assume that the polarizabilities of the different phthalate ester molecules are responsible for the order of elution, which can also be seen on other commercially available silicas with lower specific surface areas and larger pore diameters.

Some phenols (Fig. 16) were also separated on a 5-cm column of Spherosil XOA 800 at 13 bar. Obviously, with such moderate pressures the syringe injections do not require great operator skill.

Six aromatic amino and nitro derivatives (Fig. 17) were separated under the same conditions as in Fig. 16.

Despite the viscosity of some of the solvents used in these experiments, the column pressure drops never exceeded 50 bar.

CONCLUSION

The moderate pressure in high-performance adsorption liquid chromatography, below 50 bar in most instances, has been made possible by considering an often neglected parameter, the specific surface area of the support. At a constant total surface energy, a column filled with either Spherosil XOA 600 or XOA 800 will be shorter and have a lower resistance to flow than a column packed with silica with a lower specific surface area. Being shorter, such a column will be packed more easily and more efficiently, reaching up to 800 plates/cm.

This moderate-pressure HPLC technique with Spherosil columns is attractive and less expensive for routine control purposes.

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LIST OF SYMBOLS

A	Empirical constant of the Van Deemter equation
a	Constant of a given column
C	Empirical constant of the Van Deemter equation
C_{\max}	Maximum concentration of a peak
D_m	Diffusion coefficient
d_c	Column diameter
d_p	Particle diameter
H	HETP
h	Reduced plate height
K_{ad}	Adsorption coefficient
K_0	Specific column permeability
k'	Capacity factor
L	Column length

m	Amount of sample
N	Number of theoretical plates
ΔP	Column pressure drop
R_s	Resolution between two peaks
S	Specific surface area of Spherosil
t_R	Retention time
u	Linear velocity of mobile phase
V_0	Inter-particle liquid volume
V_p	Total pore volume
α	Relative retention between two peaks
β	Constant of a given column
γ	Knox HETP coefficient
ε_m	Inter-particle void fraction
ε_s	Internal void fraction of Spherosil particles
η	Viscosity of mobile phase
ρ_s	Density of silica (2.20 g/cm ³)
v	Reduced linear velocity

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