Operating characteristics of a preparative-scale radial compression chromatograph in isocratic and gradient elution

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ABSTRACT

The performance of a preparative-scale radial compression chromatographic unit was studied for a reversed-phase model separation with commercial packings. The effects of radial compression on column back-pressure, peak shape, efficiency and column stability were studied for isocratic and gradient elution. The application of radial compression was found to be beneficial, allowing the column to be operated with efficiencies comparable to those obtained with analytical-scale equipment and stably over long periods of time.

INTRODUCTION

The use of high-performance liquid chromatography (HPLC) is becoming increasingly important in preparative- and production-scale separations and purification of fine chemicals. The range of application of preparative HPLC has, in fact, been broadened considerably in recent years as a result of the availability of highly efficient chromatographic packing and advances made in the development of chromatographic equipment capable of operating efficiently and stably with small-particle packings.

In the past, a major hurdle in the scale-up of HPLC equipment was the severe loss of column efficiency that occurs when small particles are packed in large-diameter columns (>5 cm). Generally, poor separation efficiencies are obtained unless specialized equipment is used to pack the bed and maintain it stable. Such packing and stability problems have been discussed by several workers [1–5]. Typically, high-pres-

sure packing methods have to be employed in order to achieve an adequately uniform initial packing structure. Further, in order to preserve the initial efficiency of the column it is generally necessary to apply a compressive force to the bed in order to compensate for bed volume variations that may occur in operation.

The origin of the instability of large-diameter columns has been ascribed to different factors, including heat generation caused by frictional or viscous dissipation [1], chemical dissolution of the chromatographic medium by the solvent and/or sample and the collapse of unstable bridges of particles in the bed and their subsequent reorganization and settling [2]. In small-diameter columns, it is thought that the initial bed structure is stabilized by support from the column wall. In large-diameter columns, however, such instabilities are exacerbated and some means of either preventing the bed from collapsing or compensating for the effects of such a collapse are required.

Various techniques of bed compression have been developed to solve stability problems in large-diameter columns. An extensive review of

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the principles of operation and hardware was given by Verzele et al. [1]. In commercially available systems, compression is applied either axially [2,6] or radially [7]. Hybrid systems are also available in which both radial and axial compression components are implemented by the force applied by a conical spindle inserted in the bed [1].

Extensive information has been published on the operating performance of large-scale axial compression columns, particularly for systems with dynamic axial compression [2,5]. Such systems have been shown to provide chromatographic efficiencies, expressed by the reduced plate height, which are virtually independent of column diameter in the range 0.46–30 cm. Long-term column stability has also been demonstrated for these systems through the continuous application of axial compression to the bed. Comparatively little information, however, has been published on the performance of large-scale radial compression systems. In fact, although the radial compression technology has been available commercially for well over 15 years [7], no systematic investigation of the chromatographic performance has been reported for such systems. In this paper, we provide the results of study benchmarking the operating performance of a 4 in. (ca. 10 cm) diameter, 60 cm long radial compression preparative-scale chromatographic unit. A mixture of methyl and ethyl paraben was used as a test separation with commercial reversed-phase chromatographic media. Two particle sizes and configurations are used: 55–105-μm irregular and 30-μm spherical C_{18} silica. The chromatographic performance was characterized in terms of the HETP and peak shapes which were obtained for different radial compression levels.

EXPERIMENTAL

Equipment and methods

A Kiloprep 250 chromatographic system was used. The unit, obtained from Biotage (Charlottesville, VA, USA), consists of a skid-mounted solvent-delivery system and a radial compression module. The radial compression module uses the

radial compression technology developed by the Waters Chromatography Division of Millipore [7]. In this system, the chromatographic medium is contained in low-density polyethylene cartridges with external dimensions of diameter 11.9 cm and length 75 cm. The internal dimensions of the chromatographic bed are diameter 10 cm and length 60 cm. The bed is held between two sets of porous polyethylene frits which are fitted in a groove of the cartridge wall. Each set consists of a 3.2 mm thick containment frit with a 10 μm nominal pore diameter directly in contact with the chromatographic medium, and a second structural frit, 6.4 mm thick and with a nominal pore diameter of 40 μm, on top of the containment frit. Typically, the cartridge contains 2–3 kg of packing material (if silica based).

The cartridge is inserted in a flanged, carbon-steel housing rated at 2000 p.s.i.g. (ca. 14 MPa), within the confines of a rubber bladder that lines the interior of the metal casing. The bladder is used to apply radial compression with a hydraulic fluid. The cartridge is sealed at the top and bottom with stainless-steel connectors having the shape of a truncated cone. Two concentric rings of holes on the flat side of each connector provide flow distribution. After inserting the cartridge into the bottom connector, the top connector is lowered into place with an acme thread screw. A snug fit is obtained with the flat surface of each connector seated on the polyethylene frit on either side of the bed. A manual hydraulic pump is used to apply and remove radial compression fluid to the bladder surrounding the cartridge. Mobil DTE-24 hydraulic fluid was used.

The solvent-delivery system consists of a Bran and Lubbe double-diaphragm, dual-head, positive-displacement pump with pressure monitor and internal relief valve. The system operates at flow-rates up to 1000 ml/min and pressures up to 2000 p.s.i.g. (ca. 14 MPa). The flow-rate is set manually by adjusting the piston displacement of the solvent-delivery pump. Binary gradients are, however, generated by an electronic controller which ratios the fluids delivered to the main pump by two low-pressure priming pumps. A nitrogen-filled pulse damper is also included in the solvent-delivery system.

The unit was equipped with a Waters Model 481 preparative UV–Vis detector and the feed supply and gradient operation were controlled with a Waters Model 600E gradient controller. Millivolt signals from the detector were digitized with a microcomputer-based data acquisition system for graphical display and later mathematical manipulation. The column back-pressure and radial compression pressure were monitored with pressure gages.

Delivery of feed samples to the column was obtained through the gradient control system, by electronically switching the delivery pump inlet from the solvent supply tank to a feed supply tank. Reproducible feed injection in the range 100–2000 ml could be obtained. The solvents, distilled, deionized water and HPLC-grade methanol, were contained in stainless-steel, helium-sparged feed tanks. The feed sample was contained in a glass burette elevated to provide a sufficient hydrostatic head to the pump.

Two commercially packed cartridges obtained from Biotage were used. One was filled with 55–105-μm irregular particles of a bonded-phase octadecylsilica (Megabond; Waters, Milford, MA, USA) and the other with 30-μm spherical C_{18} silica particles (YMC, Wilmington, NC, USA). Both cartridges were slurry-packed in methanol by the manufacturer and had manufacturer-recommended radial compression pressures of 100 p.s.i.g. (0.7 MPa) and 200 p.s.i.g. (1.4 MPa), respectively.

Methyl and ethyl paraben were obtained from Sigma (St. Louis, MO, USA) and HPLC-grade methanol from Fisher Scientific (Pittsburgh, PA, USA). Isocratic experiments were carried out largely with methanol–water (50:50, v/v) as the mobile phase. The feed samples were prepared with the same mobile phase and contained 0.1–0.2 g/l each of methyl and ethyl paraben. Sample volumes between 100 and 2000 ml were injected. For these conditions, the separation occurred entirely under linear chromatographic conditions, as demonstrated by the fact that peak shapes and retention times were essentially independent of the volume of sample injected. Gradient elution experiments were carried out with a lower feed concentration (0.04 g/l) and with gradients from 30 to 60% methanol. The

elution profiles in either instance were obtained from the UV detector signal at 254 nm.

Evaluation of chromatographic performance

The chromatographic performance of the column was characterized in terms of the moments of the response peak for each component [8,9].

The two species were generally completely separated in the chromatograph, so that two experimental peaks with different retention times were obtained.

The first moment, μ, was calculated from

$$\mu = \frac{\int_0^\infty c(t)t \, dt}{\int_0^\infty c(t) \, dt} \qquad (1)$$

where $c(t)$ is the detector response trace. From linear chromatography theory, $\mu = t_0(1 + k')$, where $t_0 = L/v$ is the elution time for an unretained component which is excluded from the chromatographic medium and k' is the retention factor. For a symmetrical peak, the first moment coincides with the peak maximum. The HETP was obtained in terms of the second central moment of the response peak as follows:

$$\text{HETP} = \frac{\sigma^2 L}{\mu^2} = \frac{L}{\mu^2} \cdot \frac{\int_0^\infty c(t)(t - \mu)^2 \, dt}{\int_0^\infty c(t) \, dt} = \frac{L}{N} \qquad (2)$$

where σ is the standard deviation of the response peak and N the plate number. Nicoud and Perrut [10] have shown that this rigorous definition of the HETP provides a more realistic estimation of dispersion effects in chromatographic columns, since other methods, such as the peak half-width measurement, are insensitive to peak tailing. An additional advantage of the moment method is that, as the first moments and variances are additive for a linear system, extra-column effects can be easily accounted for by subtracting the external contributions. Hence the true first moment and variance of the chromatographic response peak are obtained from

$$\mu = \mu_{\text{app}} - \mu_{\text{extra}} \qquad (3)$$

$$\sigma^2 = \sigma^2_{\text{app}} - \sigma^2_{\text{extra}} \qquad (4)$$

where the subscripts app and extra refer to the moment values obtained with and without the chromatographic column, respectively. Because of the uncertainties associated with the calculation of moments higher than the second moment, the asymmetry of the peaks was expressed in terms of the peak asymmetry factor at 10% of the peak height, α_s [11].

RESULTS AND DISCUSSION

Pressure drop and radial compression

Fig. 1a and b show the column back-pressure as a function of flow-rate for different initial settings of the radial precompression pressure for the Megabond and the YMC silica packings, respectively. The precompression pressure was set for no flow conditions and column back-pressure values were recorded only after stable

readings had been obtained (about 30 min). The curves are fairly linear, but a significant decrease in the permeability of the bed is evident as the bed precompression level is increased. Such permeability variations appear to be more pronounced for the smaller spherical YMC packing, as evidenced by the greater variation of the slope of the curves. Further, for this packing radial precompression pressures of 500 p.s.i.g. (3.5 MPa) resulted in a permanent and apparently irreversible loss of column efficiency, indicating, perhaps, that for these conditions the particles were crushed and permanent cracks were opened in the bed structure. The irregular and larger Megabond particles appeared instead to form a stable bed at a 500 p.s.i.g. (3.5 MPa) precompression pressure.
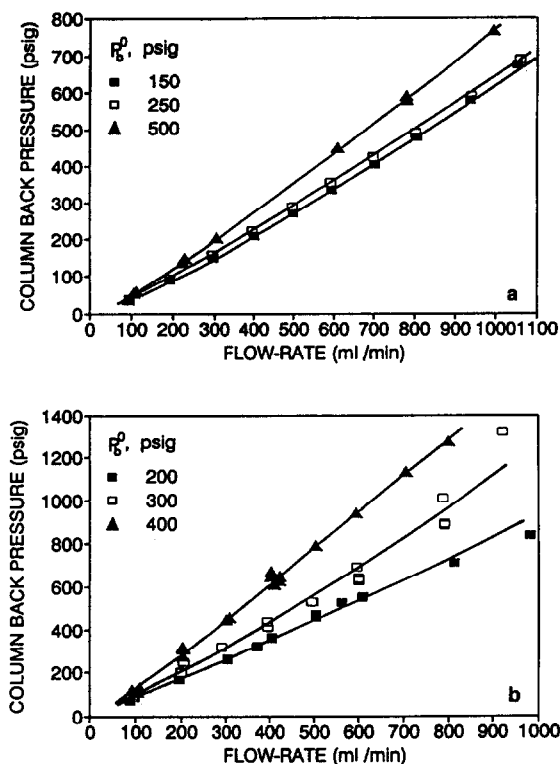
Fig. 2a and b show the measured difference

Fig. 1. Column back-pressure for different precompression settings as a function of flow-rate. Pressure values are given in relative gage pressure readings (1 p.s.i.g. ≈ 0.0069 MPa) for a methanol–water (50:50, v/v) mobile phase. (a) Megabond cartridge; (b) YMC silica cartridge.
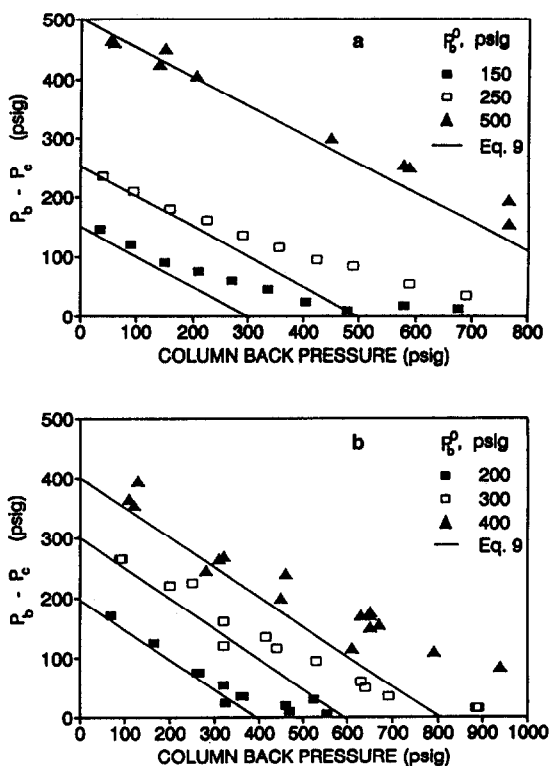

Fig. 2. Difference between bladder pressure and column back-pressure as a function of the column back-pressure. Pressure values are given in relative gage pressure readings (1 p.s.i.g. ≈ 0.0069 MPa) for a methanol–water (50:50, v/v) mobile phase. The lines are from eqn. 9. (a) Megabond cartridge; (b) YMC silica cartridge.

between the bladder pressure and the column back-pressure for different settings of the pre-compression pressure. It should be noted that in operation the net radial compression force on the bed varies from the entrance to the exit of the column as a result of the internal pressure distribution. As the pressure in the bladder is essentially uniform, the net radial compression force is maximum at the bed exit. The difference $P_b - P_c$ between bladder pressure and column back-pressure is therefore a measurement of the minimum radial compression force at the entrance. As can be seen in Fig. 2, this is inversely related to the column back-pressure. A simple model can be used to explain this behavior. If we assume that the packing can be regarded as a homogeneous elastic medium which undergoes small deformations, the initial compression of the cartridge corresponding to the initial setting of radial precompression with no flow, P_b^0, is given by

$$\Delta R^0 = \frac{R}{E} \cdot P_b^0 \tag{5}$$

where ΔR_0 is the change in radius, R the cartridge radius and E a bulk modulus of elasticity. When flow is established, a pressure gradient develops across the bed and the bladder pressure increases to a new value P_b. If we assume that the pressure profile inside the cartridge is linear, we have,

$$\Delta R = \frac{R}{E} [P_b - P_c(1 - z/L)] \tag{6}$$

Neglecting the compressibility of the hydraulic fluid in the bladder, the cartridge volume must remain constant. Hence we obtain

$$\frac{1}{L} \int_0^L \Delta R \, dz = \Delta R^0 \tag{7}$$

which leads to

$$P_b = P_b^0 + \frac{P_c}{2} \tag{8}$$

or

$$P_b - P_c = P_b^0 - \frac{P_c}{2} \tag{9}$$

The data in Fig. 2 appear to conform reasonably

well to this equation in their asymptotic behavior for small P_c values, indicating that the simple compression model developed is a reasonable approximation for these small deformation conditions. The approximation, of course, breaks down when ΔR approaches zero at the column entrance. When $\Delta R = 0$, in fact, the cartridge would be pushed toward the shell wall. For these conditions, as the cartridge has a low tension strength, the bladder pressure would become equal to the column back-pressure. The experimental data appear to approach this limit asymptotically for large P_c values. The utility of this correlation is that it allows one to estimate the minimum level of radial compression exerted on the chromatographic medium in operation. The pressure drop values in the abscissa of this graph are obtained from Fig. 1 or can be estimated from the Ergun equation if the permeability of the bed is known.

External dispersion

The system external hold-up volume and external dispersion were measured by connecting the column inlet and outlet lines together with a 10-cm long section of $\frac{1}{4}$-in. (1 in. = 2.54 cm) stainless-steel tubing and Swagelock fittings. Injections of methyl and ethyl paraben were then made, as described under Experimental, through the solvent-delivery system. A back-pressure in the range of 0–800 p.s.i.g. (0–5.5 MPa) was imposed with a needle valve placed on the outlet line. The first and second moments of the detector response peaks were calculated from the experimental data and the results are shown in Fig. 3. The first moment, μ_{extra}, is, as expected, inversely proportional to the flow-rate. A total external void volume of 63 ml, including the solvent-delivery system, tubing and detector, can be calculated from these results. The peak variance, σ^2_{extra}, also varies inversely with the flow-rate, but with a power greater than 1 (ca. 2.7). Such a correlation is purely empirical, but the high exponent found indicates that the mechanism of dispersion in the external volume is complicated and cannot be simply related to Taylor dispersion in laminar flow in a tube. The true moments of the chromatographic peaks obtained with the column were calculated from
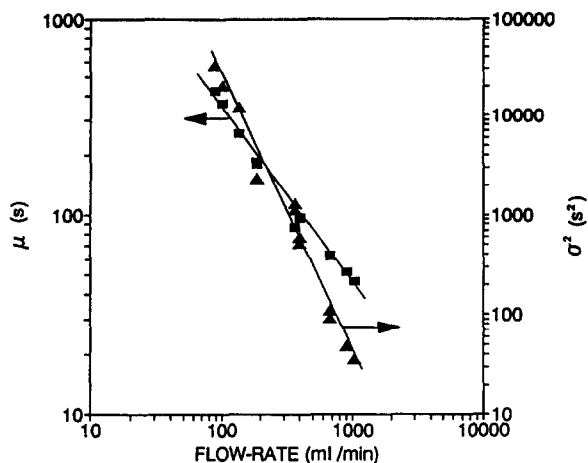
Fig. 3. External contribution to first moment and variance determined in a column by-pass mode.

the apparent values using those obtained from Fig. 3 at the different flow-rates.

Chromatographic performance

The chromatographic performance was initially assessed in a series of isocratic elution runs with a methanol–water (50:50, v/v) mobile phase. Fig. 4a, b and c show the effect of radial precompression for the Megabond cartridge. These three traces represent a chronological series of runs. After each run the flow was stopped and the precompression pressure reset. The flow was then restarted and the systems were allowed to settle before making the next feed injection. In the first run (Fig. 4a), the precompression pressure was set at 500 p.s.i.g. (3.5 MPa). Fairly sharp and nearly symmetrical peaks were obtained for the two injected components $(\alpha_s < 1.2)$. In the following run (Fig. 4b), however, when the precomposition pressure was reduced to 50 p.s.i.g. (0.35 MPa), several peaks were obtained when the same injection of two components was made. At least three pairs of peaks are visible in the resulting chromatogram, eluting in the ranges 630–800, 800–1200 and 1200–2500 s. Attempts were made to "deconvolute" the two peaks to determine the plate count of each peak. However, this proved inaccurate. Therefore, split peaks were not characterized any further, but they are obviously in-

dicative of a gross degradation of the column efficiency. In the third run (Fig. 4c), the radial precompression pressure was reset to 500 p.s.i.g. (3.5 MPa). The same injection again yielded two fairly sharp and symmetrical peaks $(\alpha_s < 1.2)$, essentially identical with those obtained in the first run of the series. The cycle appeared to be reversible and, when the precompression pressure was decreased again, a behavior similar to that shown in Fig. 4b was observed. Small
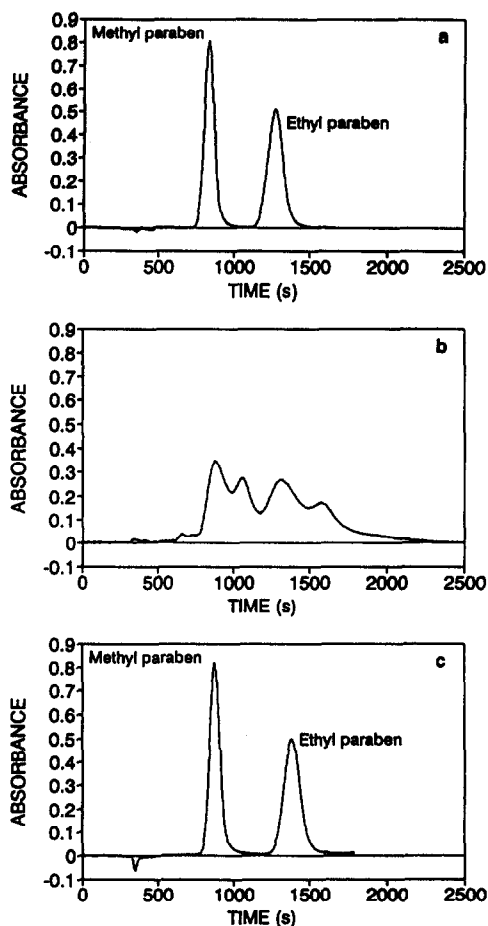

Fig. 4. Chronological series of isocratic elution runs for the separation of methyl and ethyl paraben mixtures with the Megabond cartridge. (a) 500 p.s.i.g. (3.5 MPa) precompression, 570 ml/min, 170-ml injection, $N = 422$ and $\alpha_s = 1.18$ for methyl paraben, $N = 430$ and $\alpha_s = 1.16$ for ethyl paraben. (b) 50 p.s.i.g. (0.35 MPa) precompression, 577 ml/min, 175-ml injection. (c) 500 p.s.i.g. (3.5 MPa) precompression, 610 ml/min, 210-ml injection, $N = 520$ and $\alpha_s = 1.19$ for methyl paraben, $N = 480$ and $\alpha_s = 1.18$ for ethyl paraben.

variations in the retention time were sometime observed, but these were probably caused by temperature fluctuations that occurred in the laboratory.

A second set of runs is shown in Fig. 5a, b and c, chronologically in the order in which they were carried out. The radial precompression pressure was 75 p.s.i.g. (0.52 MPa) for these runs. Each run lasted for 30 min, with an additional 45 min of steady mobile phase flow
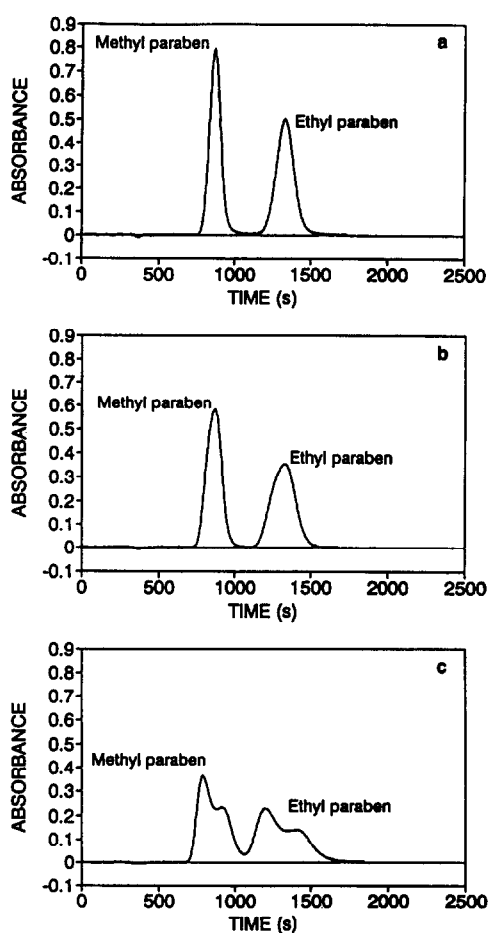

Fig. 5. Chronological series of isocratic elution runs for the separation of methyl and ethyl paraben mixtures with the Megabond cartridge. (a) 75 p.s.i.g. (0.52 MPa) precompression, 581 ml/min, 180-ml injection, $N = 505$ and $\alpha_s = 1.15$ for methyl paraben, $N = 494$ and $\alpha_s = 1.19$ for ethyl paraben. (b) 75 p.s.i.g. (0.52 MPa) precompression, 580 ml/min, 175-ml injection, $N = 260$ and $\alpha_s = 0.86$ for methyl paraben, $N = 251$ and $\alpha_s = 0.94$ for ethyl paraben. (c) 75 p.s.i.g. (0.52 MPa) pecompression, 585 ml/min, 182-ml injection.

before the next injection. A progressive degradation of the column efficiency is apparent from these runs. The two peaks are initially fairly sharp and symmetrical with α_s values less than 1.2. However, in time each peak broadens and becomes asymmetric with α_s values less than 1 and small plate counts (Fig. 5b). At a still later time (Fig. 5c), each peak splits into two partially merged peaks. As before, when after these runs the column precompression level was reset to a higher value in the range 150–500 p.s.i.g. (1.0–3.5 MPa), single sharp peaks were obtained. In general, we found that a stable column efficiency could be obtained in this precompression pressure range over a period of about 2 months. Precompression levels below this range, however, typically resulted in unstable or low-efficiency operation.

A smaller set of runs was performed for the spherical YMC packing. Similar results were obtained, but a higher precompression level was needed to obtain a stable operation. Fig. 6a, b and c show typical chromatograms obtained in chronological order. The column was initially operated with a precompression setting of 200 p.s.i.g. (1.4 MPa) with flow-rates of 500–600 ml/min. The plate counts obtained from the moments of these peaks were 429 and 430 for methyl and ethyl paraben. These values appear low as a result of the significant asymmetry of the peaks obtained with this cartridge. For a comparison, the plate counts obtained from the peak width at half-height are 976 and 1150, respectively. After approximately 4 days of operation under these conditions, the column performance became severely degraded (Fig. 6b) with the appearance of split peaks. In this case, a major peak with approximately the same retention time as in Fig. 6a is preceded by a smaller peak of the same component. As was seen with the Megabond cartridge, however, when the precomposition pressure was increased to 300 p.s.i.g. (2.1 MPa), the initial column efficiency was restored (Fig. 6c). The peaks were still fairly asymmetric, but the occurrence of split peaks was completely eliminated. It therefore appeared that increasing the precompression level could effectively compensate for a loss of efficiency.

Fig. 7. First moments obtained with Megabond-packed analytical-scale and preparative columns with different precompression levels. All measurements for a methanol–water (50:50, v/v) mobile phase.
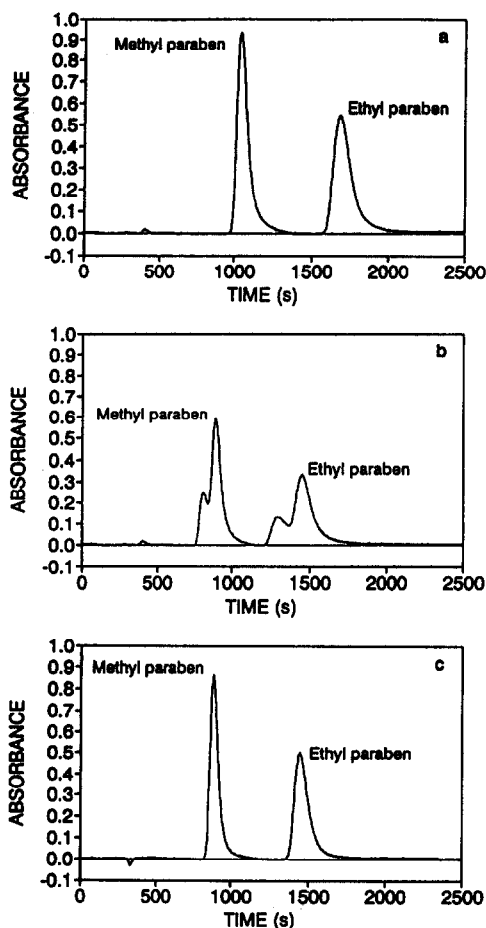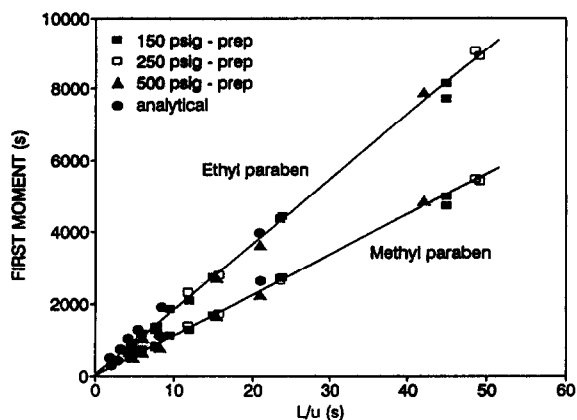
Fig. 6. Chronological series of isocratic elution runs for the separation of methyl and ethyl paraben mixtures with the YMC silica cartridge. (a) 200 p.s.i.g. (1.4 MPa) precompression, 500 ml/min, 180-ml injection, $N = 429$ and $\alpha_s = 1.60$ for methyl paraben, $N = 430$ and $\alpha_s = 1.95$ for ethyl paraben. (b) 200 p.s.i.g. (1.4 MPa) precompression, 600 ml/min, 180-ml injection. (c) 300 p.s.i.g. (2.1 MPa) precompression, 600 ml/min, 165-ml injection, $N = 405$ and $\alpha_s = 1.80$ for methyl paraben, $N = 405$ and $\alpha_s = 1.98$ for ethyl paraben.

Van Deemter curves

The calculated first moment and reduced HETP values ($h = \mathrm{HETP}/d_p$) for methyl and ethyl paraben are shown in Figs. 7 and 8a and b for the Megabond packing as a function of the reduced velocity $v' = v d_p /D$. The mobile phase was methanol–water (50:50, v/v). The values of μ and σ^2 used in these figures are those corrected for extra-column effects. Typical values of $\epsilon = 0.4$ and $D = 1 \cdot 10^{-5}$ cm^2/s were used for the

calculation of v'. The experiments were carried out at different levels of the precompression pressure, but only in the range of pressures
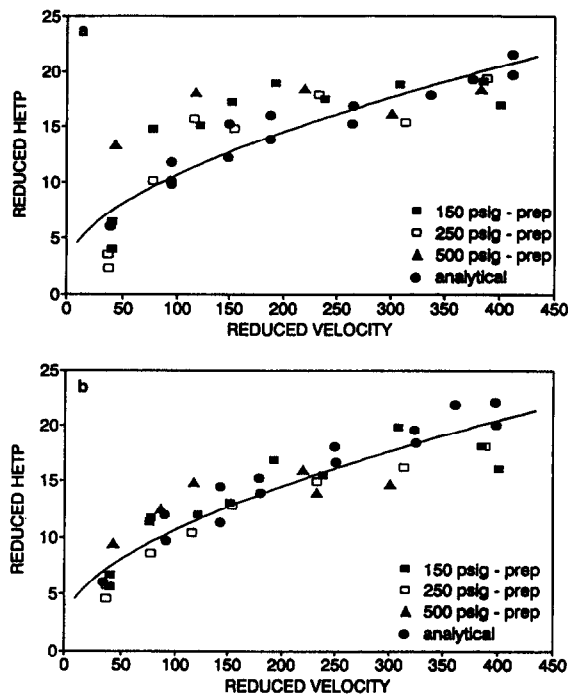

Fig. 8. Reduced HETP vs. mobile phase superficial velocity for Megabond-packed analytical-scale and preparative columns with different precompression levels. All measurements for a methanol–water (50:50, v/v) mobile phase. (a) Methyl paraben; (b) ethyl paraben.

where the column could be operated stably. For comparison, a series of runs were also made with an analytical column (25 cm × 0.46 cm I.D.) which had been pressure packed with the same packing used in the preparative-scale cartridge. The equipment and procedures used to obtain these data are described elsewhere [12].

For each component the first moment is proportional to the column length divided by the mobile phase velocity (Fig. 7), consistent with the assumption that the equilibrium is linear. Consistent results are obtained for the analytical-scale column and the preparative-scale cartridge and little or no dependence on the radial pre-compression pressure is evident. The Van Deemter curves also show little or no dependence on the radial precompression setting, although the HETP appears to vary significantly with the mobile phase velocity. The results for both methyl and ethyl paraben appear to be consistent with the Knox equation [13]:

$$h = \frac{B}{v'} + Av'^{0.33} + Cv' \tag{10}$$

An exact fit of the Van Deemter curves could not be obtained with this equation; however, an approximate fit with values of $B = 2$, $A = 2$ and $C = 0.015$ is shown in Fig. 8. Deviations from the calculated curve are evident. At low reduced velocities, in particular, lower HETP values are obtained experimentally than are predicted by the equation, indicating a reasonably well packed column. The close agreement between the results obtained with the preparative-scale equipment and those obtained with the analytical-scale column indicates that the relatively large HETP values observed are largely determined by the nature of the stationary phase, rather than by the column hardware.

Gradient elution performance

The performance of the Kiloprep 250 unit was examined also under gradient elution conditions for the Megabond packing. A series of isocratic elution runs were first made for ethyl paraben at a flow-rate of 500 ml/min and a radial precompression setting of 350 p.s.i.g. (2.4 MPa). The results are summarized in Fig. 9. The
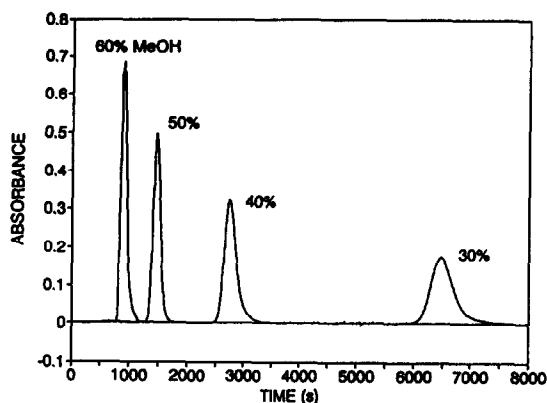

Fig. 9. Isocratic elution profiles for ethyl paraben with a Megabond-packed preparative column at 500 ml/min and 350 p.s.i.g. (2.4 MPa) precompression, with 920-ml injections.

retention factor for ethyl paraben was calculated from these runs using the equation

$$k' = \frac{\mu - \mu_0}{\mu_0} \tag{11}$$

where μ is the first moment of the chromatographic peak and μ_0 the first moment for a component which is excluded from the chromatographic medium; μ_0 was estimated to be 187 s at the flow-rate of the experiments, based on an estimated bed void fraction of 0.33. The resulting values of k' were well correlated by the equation

$$\ln k' = \ln \kappa - S\varphi = 6.02 - 8.18\varphi \tag{12}$$

where φ is the volume fraction of methanol in the mobile phase. This result is close to that obtained by Carta and Stringfield [12] for this packing in an analytical-scale column.

Gradient elution experiments were carried out for a flow-rate of 500 ml/min and three different gradient times of 2000, 3000 and 4000 s. The feed sample (740 ml) was prepared in the initial mobile phase (30% methanol in water). The gradient program was started immediately after injecting the feed sample. The results are shown in Fig. 10. The gradient profile obtained in a column by-pass mode for a gradient time of 4000 s is also shown. This profile was obtained by adding acetone as a tracer component to the methanol feed and recording the corresponding absorbance signal.
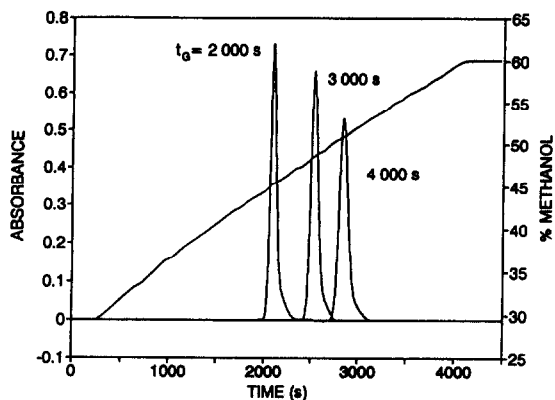
Fig. 10. Gradient elution profiles results for ethyl paraben with Megabond-packed preparative column at 500 ml/min and 350 p.s.i.g. (2.4 MPa) precompression with different gradient times. Gradient from 30 to 60% (v/v) methanol in water; 730-ml injections. The gradient profile for $t_G = 4000$ s obtained in a column by-pass mode is also shown.

The performance of the unit under gradient elution conditions was characterized in terms of the peak moments and standard deviation, again making allowances for extra-column effects. The results are summarized in Table I in comparison with calculated values obtained from the linear solvent strength (LSS) theory [14,15]. According to this theory, the retention time of a component in linear gradient elution is given by

$$t_R = t_0 \left[1 + \frac{1}{G} \cdot \ln (1 + Gk_0') \right] \qquad (13)$$

where $G = S\beta L/v$. The parameter $\beta = (\varphi_G - \varphi_0)/t_G$ is the gradient slope and k_0' is the value of the retention factor for the initial mobile phase composition. The standard deviation of a component peak is given by

$$\sigma = c \cdot \frac{\sqrt{HL}}{v} \cdot \left(1 + \frac{k_0'}{1 + Gk_0'} \right) \qquad (14)$$

where H is the HETP measured under isocratic conditions; c is a peak compression factor given by

$$c = \frac{(1 + p + p^2/3)^{1/2}}{1 + p} \qquad (15)$$

where

$$p = \frac{Gk_0'}{1 + k_0'} \qquad (16)$$

The retention times and standard deviations calculated from these equations are in good agreement with the experimental values shown in Table I, indicating that at this level of radial compression an efficient and reproducible operation is obtained under gradient elution conditions as well as in isocratic elution.

CONCLUSIONS

The chromatographic performance of the radial compression Kiloprep 250 unit has been examined in considerable detail for both isocratic and gradient elution operation. Radial compression is found to have some beneficial effects. By application of the proper level of bed precompression, voids could be eliminated and a high column efficiency, comparable to that obtained with analytical-scale equipment, could be obtained. Further, it appeared that stepping up the radial compression pressure could effectively restore the performance of a cartridge whose efficiency had deteriorated. In operation, we

TABLE I

GRADIENT ELUTION PERFORMANCE PARAMETERS

All measurements for ethyl paraben with a 30–60% methanol–water gradient at 500 ml/min.

t_G (s)	G	$\mu_{(exp)}$ (s)	$t_{R(calc)}$ (s)	$\sigma_{(exp)}$ (s)	$\sigma_{(calc)}$ (s)
2000	0.229	2021	1985	49.2	49.9
3000	0.153	2467	2454	59.1	55.9
4000	0.115	2783	2824	61.2	69.4

observed that because of the pressure drop across the column the net radial compressive force on the cartridge varies with flow-rate and from the column entrance to the exit. In spite of such variations, within a fairly ample range of column precompression pressures that yielded a stable operation, there appeared to be no significant effect of the actual level of precompression on the chromatographic efficiency.

SYMBOLS

c detector response
d_p particle diameter
D diffusion coefficient
E modulus of elasticity
h reduced HETP
H height equivalent to a theoretical plate (HETP)
k' retention factor
L column length
N plate count
P_c column back-pressure
P_b radial compression bladder pressure
R cartridge radius
S parameter in eqn. 12
t time
t_0 elution time for an unretained component
t_G gradient time
t_R elution time for a retained component
v mobile phase velocity
v' reduced velocity

Greek letters

α_s peak asymmetry factor at 10% of peak height
β gradient slope
μ first moment of pulse response
κ parameter in eqn. 12
σ standard deviation of pulse response
φ volume fraction of organic modifier

φ_0 initial value of φ
φ_G final value of φ
ϵ bed void fraction

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