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Improved Chromatographic Resolution from Pressure-Induced Changes in Liquid–Solid Distribution Ratios

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Summary

A high-pressure liquid chromatograph is described which will deliver eluent under pressures up to 60 000 lb/in.^{2} This instrument is compared to other common liquid chromatographic methods using Methyl and Ethyl Orange on silica. High pressures had a marked effect upon each distribution ratio and improved the separation.

INTRODUCTION

Considerable work has been reported recently in high-speed chromatography utilizing pressures in the range of 1000 to 5000 lb/in.^{2} (*1–9*). High pressure has allowed the use of small particles which otherwise would be too restrictive to eluent flow. Efficiencies have thereby been improved and analysis times decreased.

We undertook the study of particles in the size range of 10 μ and lower at very high pressures with the idea of being able to use long columns and still attain reasonable flow rates. A high-pressure chromatograph was designed which would allow inlet pressures up to 60 000 lb/in.^{2} to be utilized to obtain the desired flow rate.

In order to determine if significant improvement was being accomplished with high-pressure liquid chromatography (HPLC), a moder-

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ately difficult, classical chromatographic separation was chosen. In our laboratory, much work has been done on the two azo dyes, Methyl and Ethyl Orange. It was known that when a mixture was chromatographed on a ¼-in. i.d. × 12-in. length silica gel column using conventional techniques, both dyes had very badly tailed peaks which showed little resolution. Thin-layer chromatography, on the other hand, completely resolved the compounds.

The object of this communication is to present a brief comparison of HPLC to the other techniques, and more importantly, to show that with HPLC there was a significant change in the adsorbate distribution ratio as a function of pressure.

EXPERIMENTAL

Methyl and Ethyl Orange were obtained from Eastman Organic Chemicals (Rochester, N.Y.), and were recrystallized twice from distilled water. Davison 950 (100 μ diameter) and Syloid 63 (10 μ diameter) silica gels were obtained from W. R. Grace (Baltimore, Md.). Thin-layer plates of each silica gel were prepared with 25% Celite (Johns-Manville, New York, N.Y.) as binder, according to the method of Cerney (*10*).

The conventional chromatographic system consisted of a column ¼-in. i.d. × 12-in. length with Beckman fittings (Beckman Instruments, Inc., Fullerton, Calif.) as inlet and exit connections (*11*). Flow was provided by a Sigmamotor, AL-2-E (Sigmamotor Inc., Middleport, N.Y.). A modified Bausch and Lomb Spectronic 20 with a 50-μl flow cell was used as the detector, the output of which was displayed on a Sargent SRL recorder (E. H. Sargent and Co., Chicago, Ill.).

The column of the high-pressure chromatograph was a precision-drilled ¼-in. i.d. × 1¼-in. o.d. × 12-in. tube constructed from 17-4PH stainless steel. When flow was restricted at the outlet so as to operate the entire column at high pressures, a metering valve was attached to the exit, which added 0.135 ml dead volume after the column.

The injection port was a specially designed sampling valve capable of injecting a 32-μl sample while the eluent was being pumped at high pressures. The injector was located at the bottom of the column so that liquid flow in the upward direction would facilitate rapid elution of any air bubbles present. Although the system was capable of injecting under high pressure, injection was usually done while the eluent liquid was not under pressure to enhance the life of the "O"-rings in the sampling valve. As shown by Felton (7), stopped-flow injection has very little effect on the efficiency of the process. It should be noted that the 32-μl sample size and ¼-in. i.d. column represented compromises between the small sample size and narrow columns required for analysis and the larger sample and column size used for intermediate preparative work.

A 1000-ml glass reservoir was provided for the eluent. An infrared lamp was used for solvent degassing, and sufficient tubing was used to insure that the pumped liquid reached ambient temperature. The degassed liquid was filtered and drawn into a liquid pump, Model SC 10-600-50 (SC Hydraulic Engineering Corporation, Los Angeles, Calif.). Air pressure was used to drive a large pneumatic piston which, in turn, drove a smaller hydraulic piston capable of delivering liquid up to 60 000 lb/in.^{2} The refill stroke took less than 0.5 sec, and pulses seemed to be damped out in the remaining system. All auxiliary valves and tubing were constructed of 316 stainless steel.

The best procedure for packing the 12-in. column was to place it on top of a 36-in. column section and introduce a thick slurry of silica gel. Because micron-sized particles compress to a considerable extent, the system was then pumped at 40 000 lb/in.^{2} for 1 hr in order to insure a totally compressed packing. A similar procedure was followed when packing the 36-in. column, except that it was placed on top of the 12-in. section.

The distribution coefficient, K, was calculated from chromatograms using the equation:

$$V_r = V_i(K + 1) \tag{1}$$

where V_r is the retention volume and V_i is the interstitial volume of 4.82 ml. That value, based upon an estimate of packed spheres, was half of the total (empty column) volume.

RESULTS

Thin-layer chromatography, using water as the eluent, completely separated Methyl and Ethyl Orange only on the Syloid plates. This was attributed to the larger difference in K's between the compounds on the Syloid-binder plate.

Results for conventional column chromatography on Davison 950 are shown in Fig. 1. Resolution between the compounds was very poor. This is contrasted to the peak resolution obtained with HPLC with

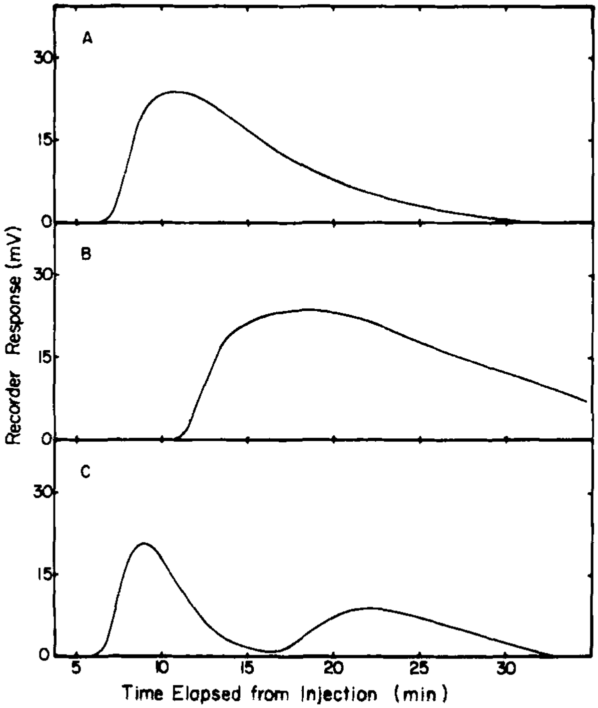

FIG. 1. Mixture of Methyl and Ethyl Orange chromatographed on silica gel. (A) Conventional system, flow rate of 0.720 ± 0.005 ml/min. (B) Conventional system, flow rate of 0.460 ± 0.005 ml/min. (C) HPLC system, flow rate of 1.050 ± 0.005 ml/min.

Syloid gel shown in Fig. 1C. Here, not only was resolution increased, but separation time was decreased considerably. Further investigation showed that the reduced plate heights for the conventional and HPLC systems were about the same. Therefore, the reason for the improved resolution seemed to be the favorable *K* values. The new *K* values might have been due to changes in the surface of the Syloid, but they also might have been influenced by the pressure.

If, indeed, *K* was independent of pressure, a longer column should show increased separation. However, even when 1-ml samples were injected into the 36-in. column, no peaks were detectable. A possible explanation is that *K* changed with pressure, so that a "*K* gradient" occurred along the column. As a result, Methyl and Ethyl Orange gradually bled off the column, giving exceptionally wide peaks which were undiscernable from mild baseline variation. On the shorter column, there was still a K gradient, but it was over a smaller length and pressure drop, so that the peaks were not spread as widely.

Figure 2 shows K as a function of eluent flow rate and pressure drop along the column. As can be seen, there was a definite dependence of K upon pressure.

In an attempt to reduce the ambiguity as to the exact cause of the fluctuations of K, the entire column was operated at a constant pressure drop with different inlet pressures. This was accomplished by utilizing an exit valve to attain the same flow rate, hence the same column pressure drop, for each inlet pressure with the remaining pressure drop occurring across the valve. Figure 3 indicates clearly that K depended upon inlet pressure. Although only 3 runs were made, note that the scatter from a straight line was considerably less in this set of experiments than in those illustrated by Figure 2.

Another result which dramatically illustrates the dependence of K upon pressure is shown in Fig. 4. As Ethyl Orange was eluting off the column with a head pressure of 45 000 lb/in.^{2}, a column pressure drop of 22 500 lb/in.^{2}, and a flow rate of 0.60 ml/min, the exit valve was

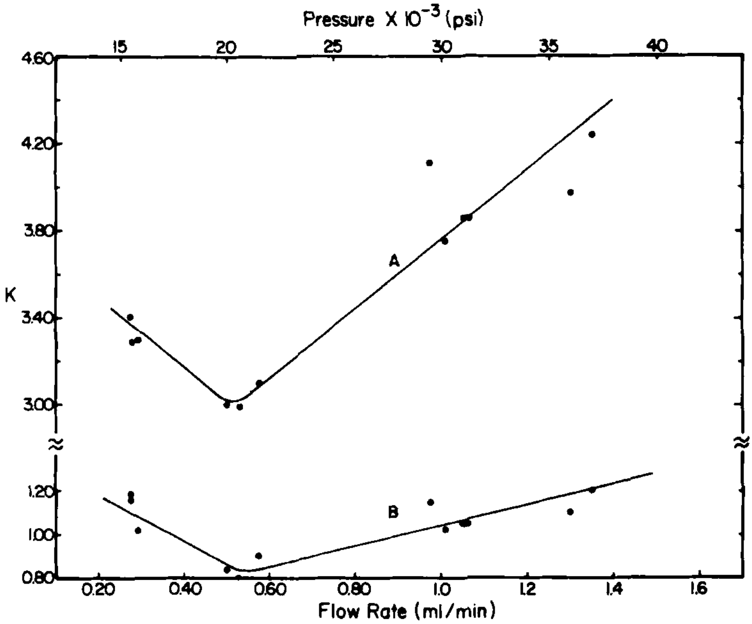

FIG. 2. Effect of pressure upon the distribution coefficient, K. (A) Ethyl Orange. (B) Methyl Orange.

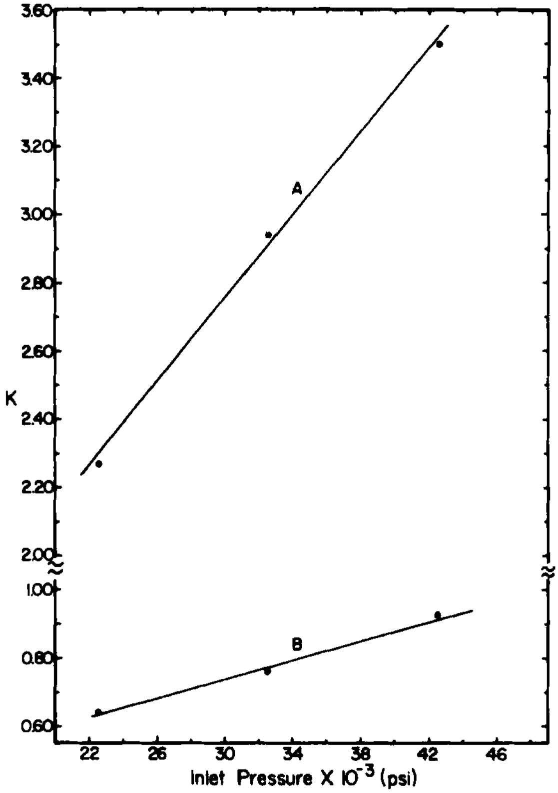

FIG. 3. Dependence of *K* upon inlet pressure with constant flow rate of 0.630 ± .005 ml/min and constant pressure drop of 22 500 ± 750 lb/in.^{2} (A) Ethyl Orange. (B) Methyl Orange.

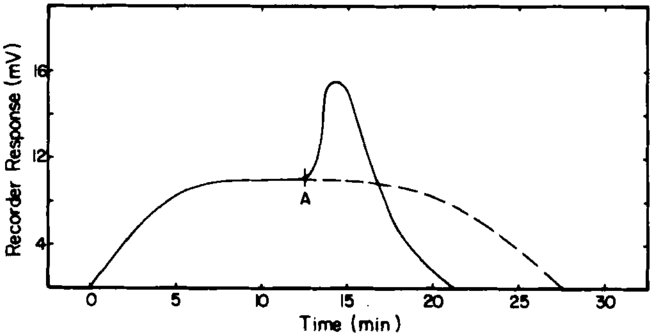

FIG. 4. Behavior of eluent concentration as function of pressure. Solid line is the actual elution profile. Dotted line is the predicted, idealized elution profile. Point A marks the opening of the exit valve.

opened. This allowed the entire pressure drop of 45 000 lb/in.^{2} to be taken up by the column and correspondingly increased the flow to 1.30 ml/min. A decrease in K must have occurred in order to account for the rapid rise of solute concentration in the eluent.

DISCUSSION

HPLC allows the use of very fine particles whereby not only resolution but also speed of analysis is increased. The existence of pressure-controlled adsorption equilibrium in liquid chromatography had been predicted by Giddings from extrapolation of his findings in dense-gas chromatography (*12*). Experimental verification of this phenomenon now confirms the introduction of a significant variable for effecting improved separations.

The scatter in the data in Fig. 2 may reflect the pressure control of ±750 lb/in.^{2}, or it may have been caused by slight temperature fluctuations. Although the massive column was a good heat sink and no noticeable temperature variations occurred in the experiments with 10-μ diameter Syloid, pumping at 60 000 lb/in.^{2} through 4-μ diameter Syloid particles caused a gradual temperature rise of 35°C at the exit of the column during a 2-hr interval.

But in spite of the scatter in Fig. 2, the minimum appears to be real. Perhaps micelle formation, which is known to give paradoxical changes in partial molar volumes with pressure (*13*), is partly responsible for the first decreasing and then increasing K values. Further experiments are being performed to gain an understanding of the system.

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