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INTRA-COLUMN INJECTION SYSTEM FOR LIQUID CHROMATOGRAPHY

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SUMMARY

An intra-column injection system is described whereby the contents of a sampling valve are discharged into the packing of a column and carried through the column by an ancillary curtain flow of solvent. The device has two advantages. Firstly, it permits columns, deteriorated by the formation of a void between the top of the packing and the frit to provide a satisfactory performance and in some cases, to recover their original efficiency. Secondly, it reduces column deterioration resulting from pressure pulse effects and packing shrinkage. The advantages of the device have been demonstrated by its use with poorly packed columns and columns that have seriously deteriorated over a period of time.

INTRODUCTION

One of the critical operations in a liquid chromatographic analysis is the introduction of the sample onto the column. The subject of sample injection has been of interest to a number of workers in the field and in particular Kirkland and co-workers[1,2] gave a very elegant demonstration of the effect of different methods of injection in 1972 and 1977. They concluded that the best method of injection was by means of a hypodermic syringe that penetrates a few millimeters into the center of the column packing. This, of course, could only be accomplished with the use of a septum injection system. If a sampling valve was used, then he suggested that the connecting tube from the valve be terminated in the center of the top column frit. In this way, the sample was discharged directly on the center of the frit and would be carried on to the column by a curtain flow of solvent surrounding the center tube. Alternatively the connecting tube could pass through a hole in the frit and the sample discharged onto the top of the packing. Contemporary columns are packed with 3- and 5-μm packing and consequently, high inlet pressures preclude the use of the syringe method of injection and, to date, injection of the sample below the surface of packing by means of a sample valve has not been reported. Furthermore, the compromise procedure of injecting the sample onto the center of the frit, as suggested by Kirkland and co-workers, would only function on a perfectly packed column where there was no void or loose packing at the top of the column. Although most columns start with the

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packing in close contact with the frit, with use, the packing almost invariably settles due to solvent changes and as, more recently noted[3], by pressure pulses resulting from continual actuation of the sample valve during repetative column injection procedures. Ferraris and Lauret[4] and Kelsey and Loscombe[5] also investigated the use of column injection on, or just through the frit in conjunction with secondary curtain flow, but these authors did not consider injection directly into the packing by allowing the tube to penetrate a few millimeters below the frit. Their system, therefore, could not cope with voids formed between the top of the column packing and the frit. Penetration of the packing by the tube from the sample valve, together with a supplementary curtain flow, could simulate the ideal system suggested by Kirkland for use with a hypodermic syringe. This paper describes such a procedure which has been termed intra-column injection and demonstrates its advantages when used with both poorly packed columns and columns that have deteriorated with use.

INJECTOR DESIGN

The basic design of the injection device, U.S. patent pending, is shown in Fig. 1A. Two models were made one to fit an 8 mm I.D., $^1/_2$ in. O.D. column and a second to fit a 4.6 mm I.D., $^1/_4$ in. O.D. column. The device consists of a center tube leading from the valve through a frit that has a central hole drilled through it, and penetrating about 8 mm below the frit and into the packing. The center tube should either have a small diameter or be packed with glass beads (about 35 μm in diameter) that have been sintered at 700°C to stabilize the bead bed. Either the small diameter tube or the packed tube will minimize dispersion of the sample as it passes from the valve to the column. The curtain flow enters the top of the column system between the frit and the column terminal and thus passes by the center tube and thence through the column.

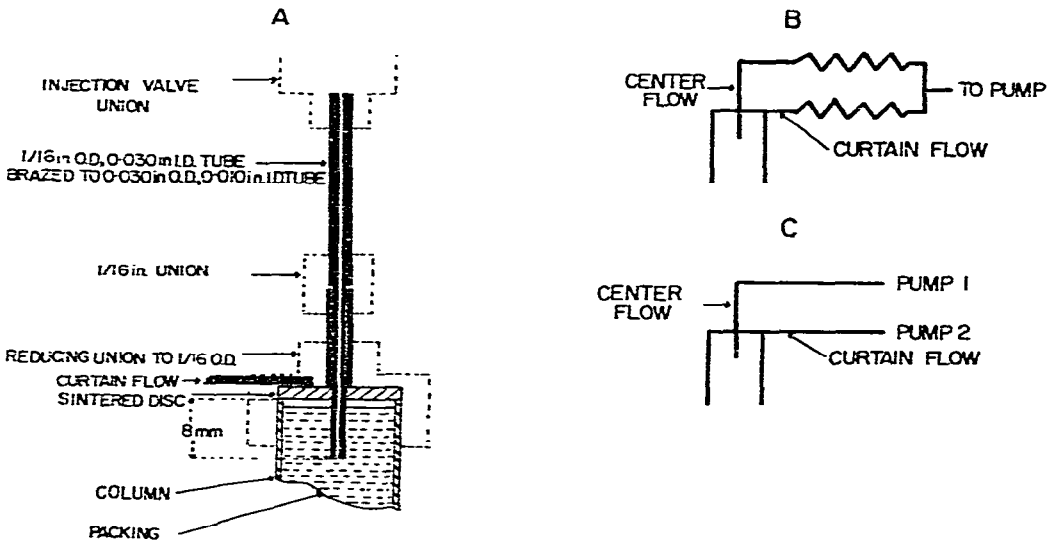

Fig. 1. Diagram of the intra-column injection system.

The top of the column that contains the drilled frit is an ordinary reducing union to 1/16 in. O.D. A short length of 0.030 in. I.D. 1/16 in. O.D. is connected to the column top which in turn is connected to a 1/16-in. connector. The center tube 0.030 in. O.D., 0.010 in. I.D. has a short length of 1/16 in. O.D., 0.30 in. I.D. tube brazed to one end. The 0.030 in. O.D. length of tube is threaded through the short length of the tube connected to the reducing union, through the hole in the frit and allowed to penetrate about 8 mm below the bed and into the packing. If the tube is not packed with sintered glass beads prior to assembly a length of stainless steel wire (0.09 in. O.D.) is passed down the center of the tube so that it is just terminated at the end. Thus, when the tube penetrates the packing, the packing is prevented from being forced into the tube. After tightening the top part of the 1/16-in. connector union onto the 1/16-in. tube that is brazed to the end of the center tube, the center wire is removed and the end of the tube then connected to the sample valve. The relative flows between the center tube and the curtain flow can be controlled either by using two hydraulic resistances as shown in Fig. 1B or by use of separate pumps as shown in Fig. 1C. The former is more practical and it permits gradient elution to be employed, but the latter allows a simple adjustment between center flow and curtain flow should it be desired. The injection device was used in conjunction with a Perkin-Elmer programmable pump Model 3B and a Perkin-Elmer Model LC 85 variable wavelength UV detector operated at 254 nm. The sample valve was a Rheodyne Model 7125.

EXPERIMENTAL

Optimum center flow/curtain flow ratio

The effect of the ratio of center flow to curtain flow was examined using two pumps in the manner shown in Fig. 1C, so that each flow could be individually adjusted. The column used was 10 cm × 4.6 mm I.D. packed with 5-μm ODS 2 and had an history of extensive use with consequent significant column deterioration. Irrespective of the relative center flow/curtain flow ratio, the total flow-rate was maintained constant at 1 ml/min. The test sample was a five component mixture of benzene, toluene, ethylbenzene, isopropylbenzene and *tert.*-butylbenzene as a 0.25% (v/v) solution in the mobile phase mixture. The sample volume was 1 μl and the separation obtained with a conventional method of injection employing the same Rheodyne valve is shown in Fig. 2. It is seen that the column had indeed seriously deteriorated, producing double peaks. The intra-column injector was then fitted to the column and the same sample injected over a range of center flow/curtain flow ratios and the results are also shown in Fig. 2. It is seen that at center flow/curtain flow ratios of 0.9/0.1 and 0.8/0.2 further deterioration in column performance was produced. However, it is also seen that at a lower flow ratio a sudden and remarkable change occurred. It would appear that at a ratio of 0.7/0.3 the column has virtually recovered all of its orginal performance. The column performance continued to improve as the ratio was reduced until at a ratio of about 0.4/0.6 the column performance began to deteriorate again. A plot of the efficiency of the second peak against curtain flow/total flow ratio is shown in Fig. 3. It is seen that the maximum recovered efficiency is about 5000 which occurs at a ratio of 0.6/0.4. However, the exact ratio was not critical, as any value between 0.6/0.4 and 0.3/0.7 provided a satisfactory performance.

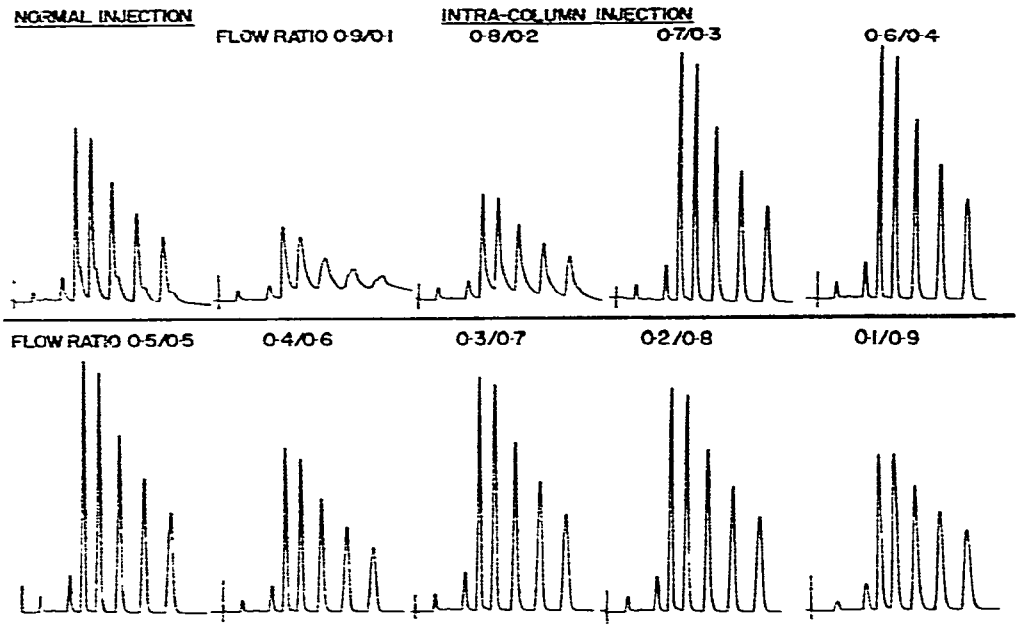

Fig. 2. Intra-column injection: the effect of the ratio of center flow to curtain flow.

Three additional columns were examined, a new column that gave a satisfactory performance, a column that had seriously deteriorated giving broad peaks and a column that had such a large void volume, that the center tube of the intra-column injection system hardly reached the surface of the packing. The first and last columns were examined employing two pumps as before, whereas the second column was employed with a flow splitting device as shown in Fig. 1B; the results obtained are shown in Fig. 4. It is seen that the column that was initially satisfactory continued to function in an equally efficient manner when fitted with the intra-column injector and when operated at a center flow/curtain flow ratio of 0.5/0.5. The efficiencies given by the column measured with a normal injection system and intra-column injection were

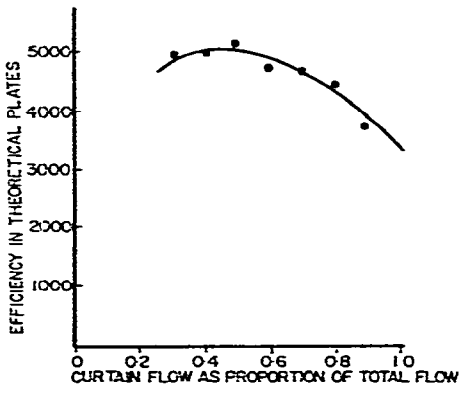

Fig. 3. Graph of efficiency against curtain flow.

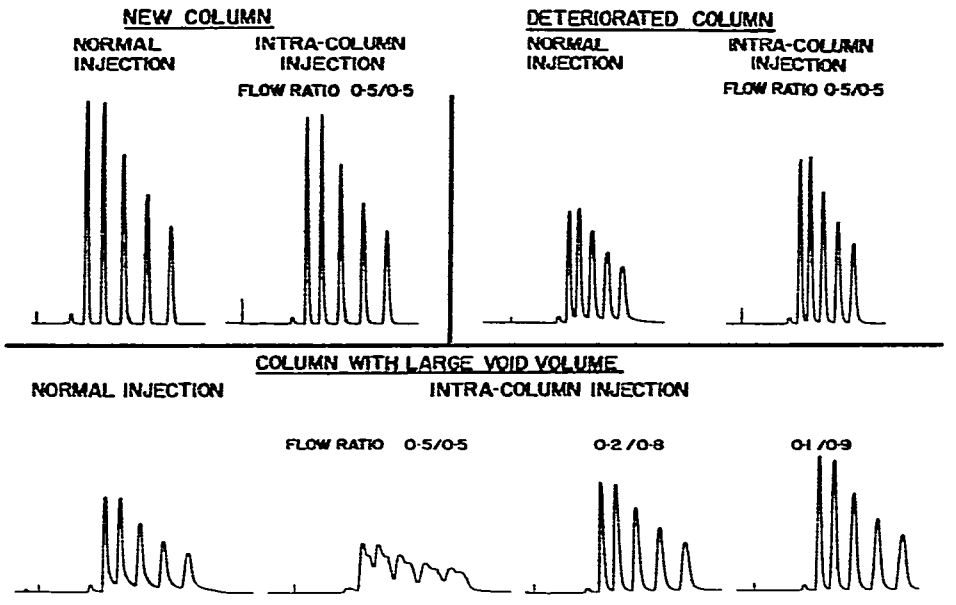

Fig. 4. Effect of intra-column injection on different columns.

8900 theoretical plates and 8700 theoretical plates respectively for the last solute eluted at a k' value of 1.9 the difference being within the error of measurement. This is not true, however, for the column with the large void, *e.g.*, at a flow ratio of 0.5/0.5, the column performance was completely unacceptable. However, at a ratio of 0.1/0.9, a reasonable, if not exceptional, performance was obtained, but one that is certainly better than that observed when a conventional injection system was employed. Finally, the improvement in the performance of the deteriorated column when using flow ratio of approximate 0.5/0.5 is demonstrated, employing a flow splitting device instead of two pumps.

The examination of reject columns

Three columns, 25, 24 and 9 cm × 8 mm I.D. packed with Partisil 20, 5 and 3 respectively were examined using a standard injection procedure and the intra-column injector system. The mobile phase employed was a 5% (v/v) solution of ethyl acetate in *n*-hexane. The sample consisted of a 5% (v/v) solution of equal quantities of *p*-xylene and benzyl acetate dissolved in the mobile phase; the columns were selected as failures from an experimental production batch. Their poor performance is clearly seen in the chromatograms shown in the upper part of Fig. 5. The first peak of each pair is for *p*-xylene and the second for benzyl acetate. All columns produced tailing peaks and the 3-μm column exhibited double peaks. The intra-column injection system was then fitted to each column in turn and operated under identical conditions. The results are shown by the peaks in the lower part of Fig. 5. The improvement is quite dramatic particularly for the column packed with the 3-μm material. It should be pointed out that all the columns examined were originally fitted with sample distribution plates, but these were removed when the columns were fitted

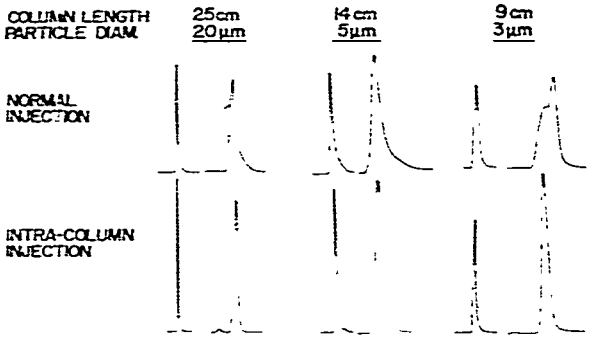

Fig. 5. Chromatograms demonstrating the effect of intra-column injection.

with the intra-column injection system. The injection system was inserted twice in the column packed with 20-μm material and four times in the column packed with 5-μm material. The efficiency was measured after each insertion. As no loss of efficiency was observed after each insertion it can be conducted that the insertion of the injection system did not appear to cause any column deterioration.

The effect of flow-rate on column performance when used with the intra-column injection system

It was necessary to determine whether the injection device would function over a range of flow-rates. A freshly packed column exhibiting poor efficiency, but giving symmetrical peaks was selected. The column was 25 × 8 mm I.D. and packed with Partisil 20. The mobile phase was 5% (v/v) ethyl acetate in *n*-hexane and the same sample of *p*-xylene and benzyl acetate was used as that previously. The column was

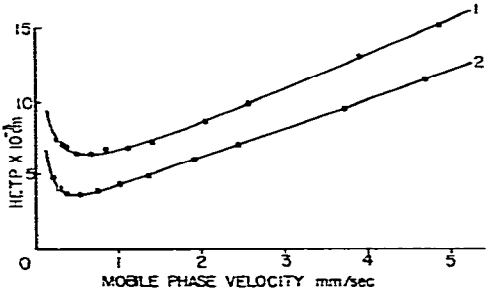

Fig. 6. HETP curves for a column operated with different injection systems. Column: 25 cm × 8 mm I.D., Partisil 20.1, Normal injection; 2, intra-column injection (1 cm penetration).

	1	*2*
Index of determination	0.9998	0.9999
A (cm)	$3.5 \cdot 10^{-3}$	$1.8 \cdot 10^{-3}$
B (cm^2/sec)	$9.0 \cdot 10^{-5}$	$4.7 \cdot 10^{-5}$
C (sec)	$2.4 \cdot 10^{-2}$	$2.1 \cdot 10^{-2}$
Std. error	$2.2 \cdot 10^{-5}$	$5.8 \cdot 10^{-6}$
HETP (min.)	3.5 d_p	2.1 d_p (d_p = particle diameter)

A, B and C are the constants in the van Deemter equation.

first fitted with a standard injection system and an HETP (height equivalent to a theoretical plate) curve determined over a linear mobile phase velocity range of 0.2–4.8 mm/sec (as determined from the retention time of *p*-xylene). The results obtained are shown in the upper curve of Fig. 6 and the quantitative fit of the data to the Van Deemter equation is included in the figure. The standard injection system was then replaced by the intra-column injection system and the HETP curve again determined over the same range of linear mobile phase velocities. The results are shown as the lower curve in Fig. 6. It seems that at the optimum velocity, intra-column injection has almost doubled the efficiency. It is also seen that by far the major difference between the two values of H (min) lies in the multipath term, A, and that this is reduced very significantly by the change in injection system. Thus the significant deleterious effects shown by the upper curve must be solely associated with the very top of the packing (less than 8 mm deep). Consequently, as the intra-column injection system penetrates the packing, the effect of any void or loose packing at the top of the column is circumvented. It should also be noted that the minimum HETP obtained using the intra-column injection system was 2.1 particle diameters.

CONCLUSIONS

Intra-column injection has distinct advantages over other methods of injection. Poor column performance from newly packed columns is often due to small voids or loose packing that forms between the top of the packing and the frit. Furthermore, voids or loose packing at the top of the column are also the major cause of column deterioration. The settling effect of the packing is almost unavoidable even if steps are taken to remove the pressure pulses that strike the packing during normal injection and which are one of the prime causes of column deterioration. However, with intra-column injection the sample introduction takes place about 8 mm below the top of the packing and thus a sample never experiences the irregular distribution caused by voids or loose packing. The system operates satisfactorily over a wide range of flow-rates and can permit reasonable efficiencies to be regained from the columns that have deteriorated, as well as improving the performance of poorly packed columns. All the columns examined were fitted with sample distribution plates and these failed to prevent column deterioration. It would seem that the distribution of the sample, over the top of the column might be an acceptable procedure for new, well packed columns however, long term, if voids are formed, sample distribution does not prevent column deterioration. It would appear that for analytical columns at least, the sample should be placed into the packing and sample distribution systems avoided where possible.

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