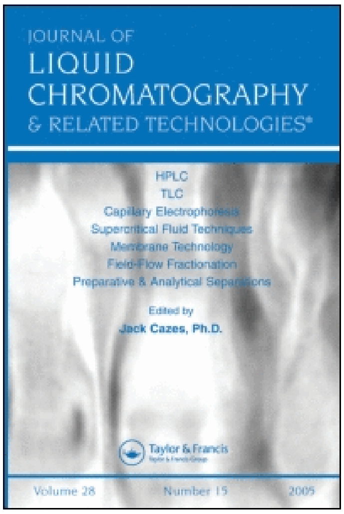

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MICRO-HPLC 2: PRODUCING LINEAR GRADIENTS WITH THE "LINEAR-GENERATOR" MADE FROM CONNECTED EXPONENTIAL-DILUTERS

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ABSTRACT

A new approach is described for generating linear gradients in micro-HPLC. The "linear-generator" replaces a single-chamber exponential-diluter with several smaller exponential-diluters connected in series and having the same total volume. This linear-generator provides the advantages of the exponential-diluter: (a) Only one high-pressure, precision, low-flow pump is required, thus reducing costs (an inexpensive low pressure pump is used to flush the chamber between gradients). (b) Gradient formation is simple and reliable and nearly independent of flow. In addition, the linear-generator has several advantages over the exponential-diluter: (c) Nearly linear (vs. convex) gradients are formed. (d) The full 0-100% gradient range can be covered (vs. nearly linear segments over only part of the gradient. (e) Gradients have a gentle on-set that quickly attains the final 100% concentration (vs. an abrupt onset and a very slow aproach to the final composition with the exponential-diluter.)

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INTRODUCTION

Micro-HPLC with columns below 0.4 mm i.d. provides a number of advantages e. g. conserving sample, solvents, and packing; capitalizing on instrument miniaturization; and utilizing the strength of polymers, fused silica, glass, ceramics, and cements in narrow tubes at high pressures (1). Gradient elution also brings many advantages e. g. eluting a wide range of sample polarity, increasing sensitivity, eliminating method development, and decreasing tailing (2).

However, gradients with micro-HPLC do not have the versatility found with conventional HPLC. While a number of approaches have been used, (reviewed in reference 3), one of the first, simplist, and least expensive methods for combining gradient elution and micro-HPLC was the exponential-diluter (4, 5). With this, a small 400 ul glass mixing chamber (usable to 70 bar), filled with weak eluent, was quickly switched to a strong eluent. The concentration profile of strong eluent coming from this container vs. time is a concave exponential profile.

Problems with these exponential diluters are (1) linear segments can only be obtained over limited solvent ranges and not from 0 - 100%, (2) the onset of the gradient is abrupt, often displacing several peaks together, and (3) the attainment of the final composition is slow.

Advantages to these simple exponential gradient generators are they are simple and reliable. In addition they are inexpensive; requiring only one high-pressure, precision pump and an inexpensive, low-pressure, low-precision pump to "wash" strong eluent from the diluter between runs. Although some recent instruments are available that permit direct gradient generation (e.g. reference 6), they are expensive. Other conventional HPLC instruments must use indirect approachs to produce micro-HPLC flows and gradients, such as splitting off part of a conventional gradient.

In our efforts to combine the advantages of gradients with micro-HPLC, we have investigated two new aproaches that maintain the advantages of the exponential-diluter, but permit linear or near-linear gradients to be generated. One such approach, reported elsewhere (7), is the "breakthrough-gradient" using the discontinuous interface between two eluents in a packed "gradient-generator" column. Symetrical gradients with a gentle onset, linear central portion, and rapid onset of the final composition with gradient volumes from 40-550 ul can be obtained. In addition, if the gradient-generator column is packed with porous particles, mobile phase mass transfer can be used to reproducibly change the volume of the gradient over a wide range simply by changing the flow rate through the column in the flush step. The flush step is used to quickly move the interface between the weak and strong eluent to the head of the micro-HPLC column. If solid particles are used in the gradient-generator column, the gradient volume is independent of the flush flow (no mobile phase mass-transfer spreading).

Ten years ago (7) while working with R.P.W. Scott's incremental gradient elution approach to normal phase LC, (8), we found that the exponential gradient generated from a single 80 ml mixing chamber could be turned into a near linear gradient if eight 10 ml mixing chambers are used in series. More recently (9), we showed that a more efficient system for approximating a series of connected exponential-diluters, was simply to use a long and narrow empty LC column (25 X 0.46 cm) with four small (7/8 X 3/16 in.) magnetic stir bars over a magnetic mixer. The long narrow configuration produced multiple mixing segments. In this case the "linear-mixer" was shown to remove the short-term (1-2 cycles/min) baseline meander noise observed with 190 nm UV detection.

This work relates further work with this "linear-generator" for making gradients in micro-HPLC. Reported here are modeling experiments with a number of configurations of linear-generators for producing micro-HPLC gradients. These modeling experiments used gradients from water (weak eluent) to water with ca. 1% acetone (strong eluent). Extensions of this work to real separations, as well as deducing the mathematical expression describing the gradient produced from a series of connected exponential diluters is given by Ishii et al. elsewhere (10).

EXPERIMENTAL

The apparatus is described in detail elsewhere (7). Briefly, it consisted of a weak-eluent pump (Waters #590 programmable pump, Milford, MA, USA) (this can be an inexpensive, low pressure pump), and a strong-eluent micro-flow pump (LDC microMetric pump, #920390, Riviera Beach, FL, USA). A 12-port high pressure valve-injector (#EQC12W, Valco Instruments, Houston, TX, USA) permitted rapidly switching from the "flush" mode (used to wash strong eluent from the linear-generator column) to the "run mode" in which the gradient was passed through the detector (UVIDEC 100, JASCO, Japan Sepctroscopic Co., Ltd., Tokyo, Japan) with a modified cell of 200 um thickness. No column was used in these gradient modeling experiments.

A TRANSPARENT HIGH PRESSURE MIXING CHAMBER. Gradients were generated in modified transparent (glass) high pressure (to 200 bar) Chrompack cartridges (#28117, Chrompack, Bridgewater, NJ, USA) in a cartridge holder (#28250, Chrompack) modified (11) by (a) removing the packing, (b) cutting two viewing windows in the cartridge holder, and (c) covering the glass cartridge with transparent heat-shrinkable Teflon tubing to prevent flying fragments should the glass rupture. The advantage of this transparent high pressure container is that it is possible to directly observe if bubbles are present, if the mixing bars are operating properly, how mixing is progressing (if colored liquid are used).

Mixing is achieved using a massage vibrator (Hitachi) with the mixing chamber held horizontally on the end with rubber bands, as used by Takeuchi et al. previously (4). In some cases a single magnetic mixer was used to mix a number of micro (0.8 X 0.2 cm) magnetic stirring bars in the Chrompack cartridge.

LINEAR-GENERATOR OF CONSTANT VOLUME AND VARIABLE NUMBER OF CHAMBERS. The following approach was used to create a transparent high pressure mixing chamber in which the volume can be kept constant but the number of chambers can be varied from 1 to 12. Movable partitions were created in a Chrompack cartridge by inserting a 2.4 cm length of Teflon tubing (0.3 cm o.d. X 0.05 cm i.d.) that was cut into 13 equal segments. Mixing for the 1-chamber configuration was achieved with a 6 cm mixing bar made of 0.15 cm o.d. stainless steel wire. Three wire segments having the same total length as the single wire mixer are used as mixing bars for a 3-chamber configuration with the Teflon partitions re-arranged. Note that the volume of the Chrompack chamber could be further decreased by increasing the length of the Teflon tubing and decreasing the length of the mixing bars or using instead, a glass bead in each chamber. Experimentally, it was found necessary to have Teflon tubing at both the inlet and outlet ends to prevent the mixing bars from generating particles by abrading the screens left in the end of the cartridges.

LINEAR-GENERATORS USING PINS-WITH-HEADS. In the limit, a very thin partition with a hole in the middle, can be approximated by a mixing bar with a partition fixed to the end. The separation of the chambers is achieved by having the partition nearly the same diameter as the inside of the container. This shape is approximated by a pin. Pins with 0.19 cm heads and 0.1 cm o.d. bodies were cut to a total length of 0.9 cm and 10 inserted in the Chrompack cartridge in which the inside diameter of 0.3 cm was reduced to ca. 0.2 cm so the pin heads more closely matched the i.d. of the cartridge. The inside diameter of the Chrompack cartridge was reduced by inserting a length of Teflon tubing (10 cm long X 0.3 cm o.d. X 0.2 cm i.d.), giving a final volume of 334 ul. When inserting Teflon tubing in the cartridges, it was often found necessary to stretch the tubing slightly or to try different segments from the same roll, since the nominal diameters vary slightly along the length.

The close fit of the pin head also prevents the shaft of one pin from becoming caught between the head and chamber wall of the next pin. The head must be loose enough to allow free vibration of the pins.

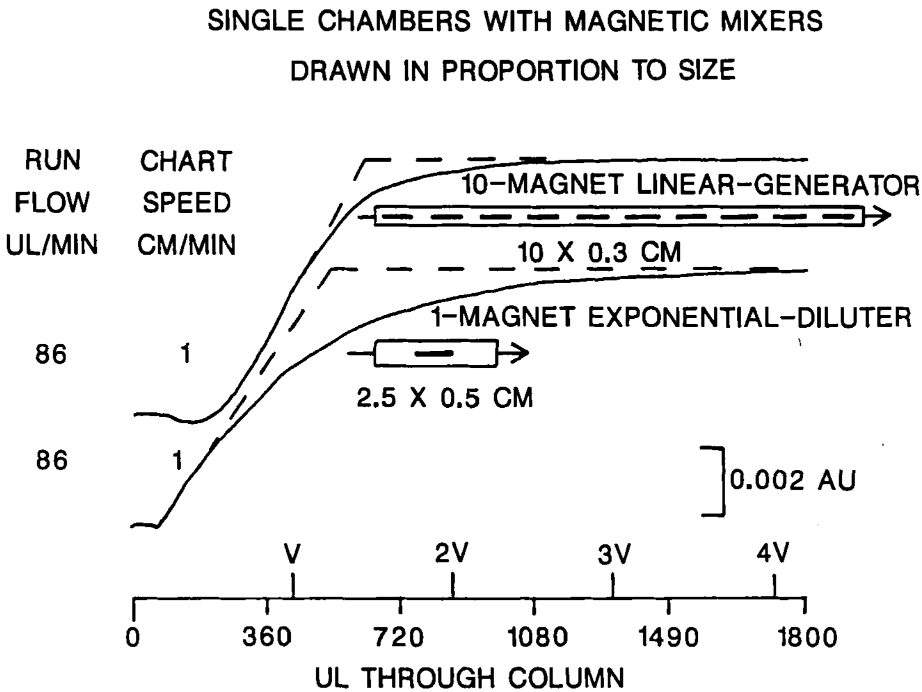

FIGURE 1. Comparison of the 400 ul 1-chamber single magnetic mixing bar exponential-diluter used previously (A) to the long narrow 430 ul 1-chamber 10 magnetic mixing bar linear-generator (B). Magnetic stir bar mixing was used.

RESULTS AND DISCUSSION

SINGLE-CHAMBER MIXERS: A SHORT CHAMBER WITH 1-MIXING BAR VS. A LONG CHAMBER WITH 10-MIXING BARS.

Fig. 1 illustrates the effect described in the introduction (8) in which short and long 1-chamber mixers of equal volume are compared. The shorter and wider (2.5 X 0.5 cm) exponential-diluter used previously (4) was compared to a much longer and narrower (10 X 0.3

cm) linear-mixer of the same volume using 10 small magnetic mixing bars (see Experimental). The linear-generator shows a less abrupt onset of the gradient, and a more linear gradient. In addition, there is a speedier attainment of the final composition by the linear-generator vs. the exponential-diluter (ca. 2.5 generator void volumes, V, vs. 4 V). However, the linear-generator shows a delayed onset of the gradient vs. the exponential-diluter (180 ul vs. 120 ul).

The delayed onset with linear-mixers can be dealt with using the methods described in the companion publications (7, 10). Namely, the initial portion of the gradient is vented to waste, and a high pressure valve is activated when the strong eluent reaches the end of the linear-generator.

This linear-generator design used small mixing magnets with poles attracting. Since the modified transparent Chrompack cartridge was used for the mixing chamber, it was possible to observe mixing, the presence of air bubbles, etc. The magnets were observed to create a zig-zag array, and all the magnets stayed connected and rotated as a chain. The isolation of one segment from the next was achieved by the narrow bore of the linear-generator (0.3 cm) vs. the wide bore of the magnet (0.2 cm). In order to determine the effect of more isolated chambers, the arrangement described next was used.

MULTI-CHAMBER MIXERS: EFFECT OF THE NUMBER OF CHAMBERS FOR A CONSTANT VOLUME MIXER. To investigate the effect of a linear-generator design in which the chambers were more isolated one from the other, and to experimentally investigate the effect of the number of chambers, the linear-generator with the Teflon partitions and 1, 3, 7, or 12 mixing bars was built (see Experimental).

Fig. 2 shows that the effect of going from 1 to 3 mixing chambers is much more significicnt than going from 3 to 7 chambers, etc. (This was confirmed later when the mathematical expression was derived, reference 10). For example, final attainment is ca. 3.5 V for the 1-chamber exponential-diluter vs. but 2 V for the 3-chamber linear-generator. Note also that the onset of the gradient is delayed as the number of chambers is increased.

A vibrator was substituted for the magnetic mixer used previously (4) and this brings several advantages. The vibrator operates more reliably than stirring multiple magnetic stirring bars, which sometimes stop moving. Also, the vibrator eliminates the constaint of having to use the available Teflon-coated magnets or ferro-magnetic irons (which often rust). The vibrator provided the advantages of: (a) any materials can be used as mixing elements, including glass, ceramics, polymers, metals, etc.; (b) any shape mixing element can be investigated, including bent bars, balls, pins,

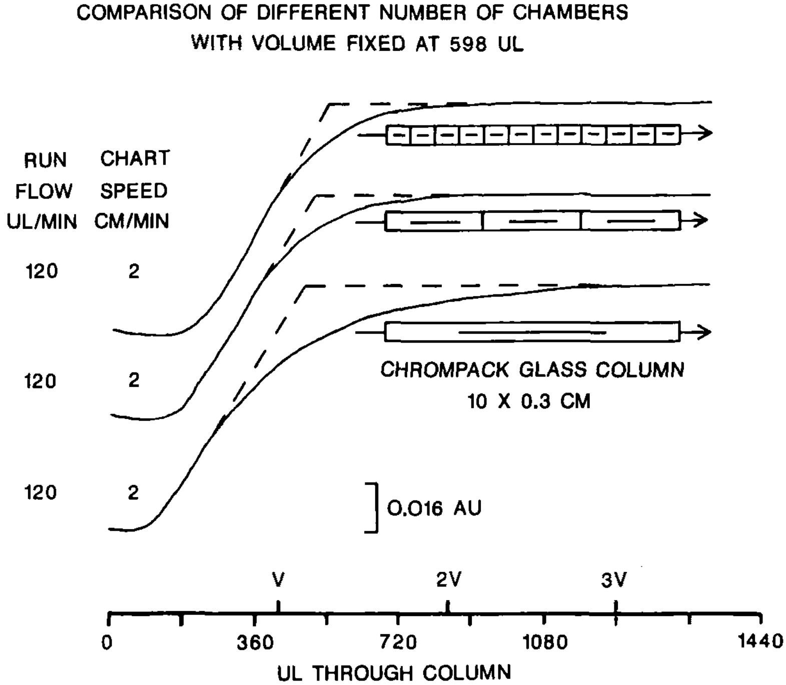

FIGURE 2. Effect of the number of chambers for a fixed gradient volume (598 ul) using a transparent, high pressure cartridge. Construction details are given in Experimental. Vibrator mixing was used.

hollow tubes, etc.; and (c) mixing bars never stop moving (as often happens with the magnetic system). Mixing could be observed to be extremely efficient, and the mixing bars evenly distributed themselves to form chambers of equal size when movable partitions were used.

EFFECT OF FLOW ON GRADIENT SHAPE. Flow rate is not expected to change the shape of gradients from exponential-diluters. With the exponential-diluter, the unexpected results were observed that lower flows (40 ul/min) give a slightly more exponentially shaped gradient than higher flows (240 ul/min). The equation for an exponential-diluter (Equation 1) indicates that for gradients that have progressed to the same volume through the mixer (mixer volume is V) (i.e. flow rate, v, multipled by time, t), the fraction of strong eluent in weak eluent, C/Co, should be the same. The cause of the observed dependence of C/Co on the flow may be imperfect mixing throughout the long (10 cm) 1-chamber mixer, so that a "sweeping out" of the more concentrated eluent on the outlet end occurs sooner than expected with high flows.

$$C/Co = 1 - e \exp(-vt/V) \qquad \text{Equation 1.}$$

In a similar experiment to determine the effect of flow on the gradient the 7-chamber linear-generator shows a more constant shape as flow was changed from 36 ul/min to 240 ul/min.

<u>SINGLE-CHAMBER MIXERS: LINEAR GRADIENTS USING PINS-WITH HEADS.</u> The multi-chamber linear-generator described above was difficult to construct and is not readily miniaturized further. Visualizing the partitions as narrower and narrower led to the hypothesis that

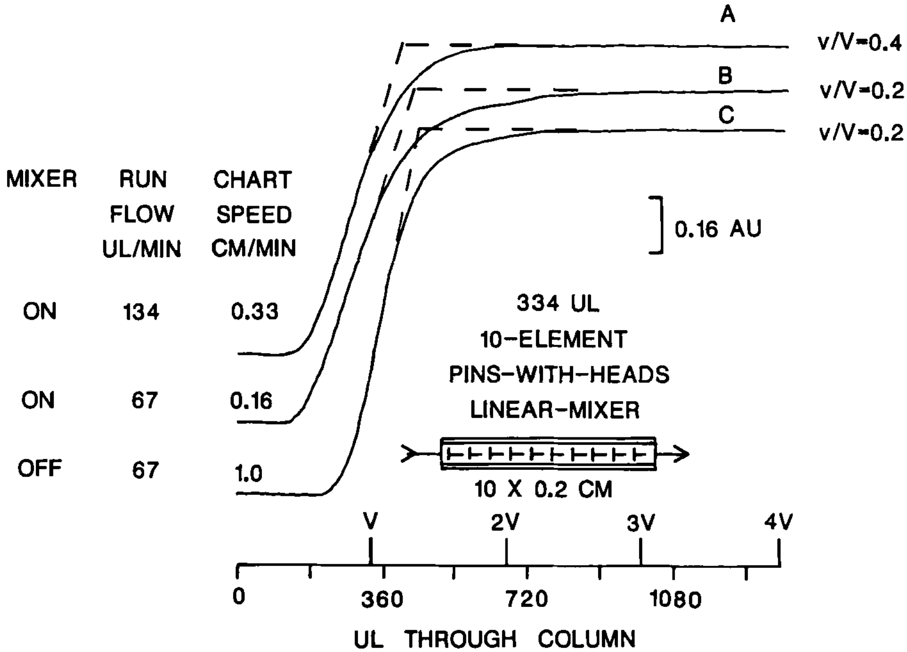

FIGURE 3. Near-linear gradients using the simpler construction of pins-with-heads to partially segment the 1-chamber linear-generator. Turning off the vibrator (C) vs. (B) shows a linear gradient.

fixing the partition, as a disk, to the end of the mixing bar might produce a linear gradient. Cut straight pins (see Experimental) inserted in a narrower Chrompack column gave the results shown in Fig. 3. Note that the gradients are nearly linear, with lower flows being slightly more exponential with longer to attain the final composition (2 V at 134 ul/min vs. 2.8 V at 67 ul/min). This may be the "sweeping out" effect at higher flows described above.

Comparing curves B and C shows the surprising results that with the vibrator mixer turned off, the gradient is still linear, but steeper, and with a longer delay (ca. 280 ul vs. 180 ul) with the attainment of final composition being similar. The pins-with-heads configuration may be acting simply as a passive turbulent mixer.

Note that the volumes of the gradients between 5 and 95% composition change is ca. 350 ul, 540 ul, and 360 ul, for conditions A, B, and C, respectively in Figure 3. These are volumes suitable for micro-HPLC. To use these gradients, the initial 180 - 250 ul dead volume would be vented at the fast flows, and the final gradient diverted to the analytcal column with a valve and the gradient used at slower flows (<20 ul/min), as explained in reference 7.

Fig. 4 shows the effect of connecting together two of these pins-with-heads mixers. Again, the gradients are nearly linear at the mid-flows of 62 and 124 ul/min (B and C). Turning off the vibrator mixer has an opposite effect to that observed with a single chamber, in that the gradient becomes less steep, with an earlier onset. At very low flows (21 ul/min) the gradient becomes almost exponential in shape. At such very low flows, the pins seem only to be inducing enough turbulence to make the eluent in the whole chamber homogeneous.

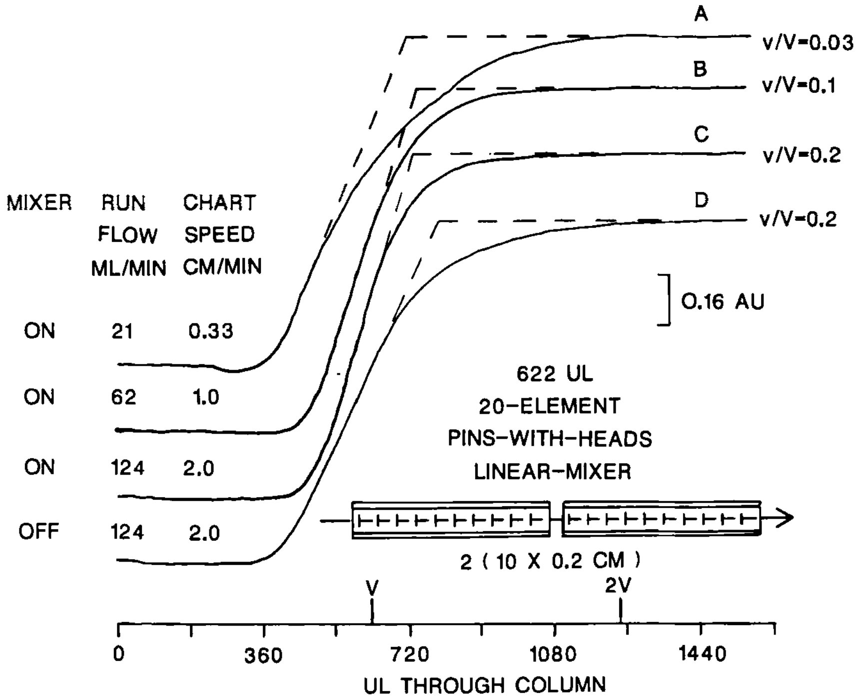

FIGURE 4. Near-linear gradients using two connected 1-chamber pins-with-heads linear-generators. Turning off the vibrator (D) vs. (C) shows a linear gradient.

For the pins-with-heads linear-generator, these results suggest that in order to overcome the tendency for this type of generator to produce exponential gradients at very low flows (<20 ul/min), smaller design is required. Instead of pins with 0.2 cm head, pins with 0.05 cm heads, or smaller, in a narrower tube may produce linear gradients even without mixing.

Some other connected mixer configurations were tried with some different results. When the Chrompack

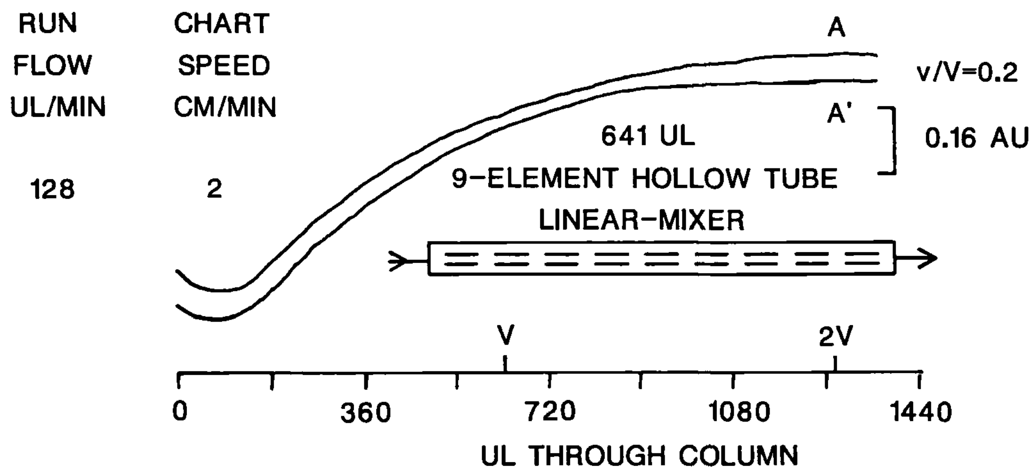

FIGURE 5. Single chamber cylinder-in-cylinder chamber producing an exponential gradient but with a more gentle onset of the gradient and faster attainment of the final composition vs. the 1-magnet exponential-diluter in Figure 1.

chamber was filled with 9 segments of hollow syringe tubing (0.083 in o.d. X 0.063 in i.d. X 1 cm long) and vibrated (Fig. 5). Compared to the 598 ul exponential-diluter in Fig. 1, this mixer, of similar volume (640 ul), still provided the exponential shape. However, this cylinder-in-cylinder configuration provides an alternative gradient generator that overcomes two of the problems with the classical exponential-diluter: the final composition is reached faster (ca. 2 V vs. 3V), and onset of the gradient is more gentle.

Another configuration in which the Chrompack column was 2/3 full of 1.5 mm solid glass beads and vibrated provided no improvement over the exponential-diluter.

CONCLUSIONS

The linear-generator approach of having a number of connected exponential-diluters in series was shown experimentally to give near linear gradients. A transparent, high pressure (200 bars) miniature vessel (598 ul) was designed that could be broken into any number of chambers, from 1 to 12, with the volume remaining constant. Linear-generators of 3 chambers were found adequate for near linear gradients, since the effect of increasing the number of chambers is greatest in going from 1 to 3 chambers, and much less in going to more chambers. This has since been confirmed mathematically (10). Other linear-generator designs that are simpler to construct were shown to produce near linear gradients. These included a single long chamber containing a series of miniature magnetic mixing bars, and a single chamber containing an array of pins-with-heads. Since the heads are nearly the diameter of the long narrow mixing tube, the heads act as the partitions between chambers. The pins-with-heads approach can be further miniaturized.

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REFERENCES

1. D. Ishii and T. Takeuchi, "Micro High-Performance Liquid Chromatography", p 87-112, in Reviews in Analytical Chemistry, T. S. West (ed.), 1982.

2. V.V. Berry, J. Chromatogr., 290 (1984) 143.

3. H. Schwartz and V. V. Berry, LC Magazine 2 (1985) 110.

4. T. Takeuchi and D. Ishii, J. Chromatogr. 53 (1982) 41.

5. T. Takeuchi and D. Ishii, JHRC and CC 6 (1983) 310.

6. H. Schwartz and R. Brownlee, Am. Lab., Oct., 16 (1984) 97.

7. V.V. Berry, D. Ishii, and T. Takeuchi, presented at 6th International Symposium on Capillary Chromatography, May 14-16, Riva del Garda, Italy.

8. V.V. Berry and J. Whittim, (unpublished results).

9. R.P.W. Scott and P. Kucera, J. Chromatogr. Sci. 11 (1973) 83.

10. D. Ishii, Y. Hashimoto, H. Asai, K. Watanabe, and T. Takeuchi, presented at 6th International Symposium on Capillary Chromatography, May 14-16, Riva del Garda, Italy.

11. V.V. Berry, Am. Lab., (to be published) Oct. (1985).