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SIMPLE DEVICE FOR SEQUENTIAL LINEAR GRADIENT ELUTION WITH A SINGLE, CONVENTIONAL, ONE-PISTON RECIPROCATING PUMP

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SUMMARY

A highly efficient proportioning device for versatile gradient elution in high-performance liquid chromatography is described, which can be used with an existing, conventional, one-piston reciprocating pump. The device consists of two constant-pressure solvent reservoirs, two air-traps, a three-way magnetic valve, a mixing chamber and an air-damper. Any desired gradient can be performed by setting the solvent composition–time profile on the signal generator that controls the three-way magnetic valve digitally. Solvents from two reservoirs are introduced into the three-way magnetic valve through each air-trap and are proportioned. The solvent from the valve is led to the mixing chamber, to which an air-damper is connected. The compositional fluctuations of the delivered solvent are minimized by the air-damper. Solvent profiles are produced with an error of less than 0.5% when compared to the designated compositions in the step mode.

INTRODUCTION

Gradient elution or solvent-programming chromatography is being used in an increasing number of applications. With the development of high-performance liquid chromatography (HPLC), a variety of sophisticated gradient elution devices have appeared[1–5]. In an analysis with the use of the gradient mode, the composition of the mobile phase is gradually changed in such a way that the solvent strength increases throughout the separation. Using a properly programmed and designed mobile phase gradient, compounds with both widely different and similar chemical structures can be separated in a relatively short period of time.

Gradient elution has been performed in two principal ways. In dual-pump systems, the gradient is formed on the high-pressure side of the pumps, each pump delivering a different solvent. The gradient composition is controlled by simultaneously varying the flow-rates of the two pumps so as to maintain a constant flow. In single-pump systems, the solvents are mixed and the gradient is formed on the low-pressure side of the pump. Since the solvents are mixed in appropriate ratio prior to entering the pump, this design has been believed to require a single high-pressure pump that operates at a constant filling flow-rate[6]. Recently, some sophisticated gradient systems with a single-piston pump have become commercially available but they cannot be added to existing chromatographs and are as expensive as one with a dual-pump system.

A number of characteristics are required of an ideal gradient device. The most important of them is the ability to perform any desired gradient profile. Reproducibility and accuracy are also required. In addition, there should be no compositional fluctuation when the gradient is formed, and no appreciable delay between the time when the gradient is formed and when it reaches the column. Finally, an ideal gradient device should be cheap and adaptable to existing solvent-delivery pumps.

The present work describes a simple gradient device for HPLC which was designed to meet the requirements mentioned above.

EXPERIMENTAL

Operating principle

The principle of the device is alternately to direct one of two solvents, A or B, to the inlet of a single reciprocating one-piston pump by using a three-way magnetic proportioning valve. When the valve is de-energized the pump is directed to solvent A, and when energized to solvent B. The proportion of solvents can be controlled by varying the ratio of the energized time to the "segment time", the time interval for proportioning. However, it is difficult to perform gradient elutions without compositional fluctuation of the solvent by using a single, conventional, one-piston reciprocating pump that operates in three intermittent strokes: filling, resting and delivering. Because of the interference beating, caused by the difference in time-cycle between energizing of the valve and filling of the pump, compositional fluctuations of the delivered solvent are inevitable. The heart of the device is an air-damper, which makes the flow from the valve to the mixing chamber nearly constant and minimizes the interference beating. That is, the solvent composition formed in the mixing chamber will be almost the same as that when a constant filling flow-rate pump is used. Using such a conventional one-piston pump as mentioned above, the delivered mobile phase composition will be the same as the programmed composition.

Device

The proportioning device comprises two constant-pressure solvent reservoirs, a proportioning three-way magnetic valve and a mixing chamber to which an air-damper is connected (Fig. 1). A Mariotte-type solvent reservoir is used, and the hydrostatic pressure at the valve nozzle is controlled by changing the height of the bottle. The three-way magnetic valve is a MTV-3 valve (Takasago Electric, Japan) which is controlled by a signal generator (Kimura Electric, Japan). The mixing chamber is made of glass and PTFE, and the inner volume is designed to be variable from 0.2 to 0.6 ml. The small stirring bar in the chamber is driven by three magnetic induction coils, which are situated around the chamber. An injection syringe is connected through a PTFE tube to the mixing chamber and is used as an air-damper. It is intended to smooth out the intermittent filling of the mixing chamber with the solvent, which is caused by the intermittent action of the pump.

Gradient programmes are written digitally on the signal generator, which has two functions: a memory for storing operation programmes, and operation of the

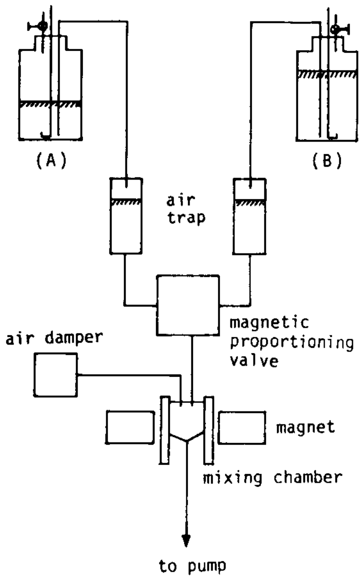

Fig. 1. Schematic diagram of the gradient device for a single-plunger pump. The magnetic proportioning valve and magnet are energized by a signal generator (not shown).

three-way valve. Five modes of programmes can be entered in the memory. A mode consists of ten sections, and every section has three spaces for the mixing ratio of solvent B, the time from the start and segment time. A series of written sections forms a gradient programme. Programmes in which the mixing ratio changes gradually on the time axis are called "linear gradient modes", and those in which the ratio stays at some given concentration are called "programmed step-gradient modes" to differentiate them from the conventional step-gradient, which is performed by only changing the solvents.

Performance

The performance of the device was tested by coupling it to either a SF-0396 pump (155/6 rpm; Milton Roy, Philadelphia, PA, U.S.A.) or another Milton Roy mini-pump (500/6 rpm). The outlet of the pump was connected directly to a UVI-DEC-100-II detector (JASCO, Japan). The connecting tubing to the detector was stainless steel (40 cm × 0.5 mm I.D.). Compositional changes of the delivered mobile phase were monitored at 260 nm by 0.15% acetone and distilled water.

RESULTS

The effect of the air-damper on compositional fluctuations of the delivered mobile phase was observed in four linear gradient modes of 100/10, 100/20, 100/40 and 100/80%/min at a flow-rate of 1.5 ml/min. The pump was at 500/6 rpm and the segment time was 15 sec. The air-damper volume was 1.0 ml and the mixing chamber volume was 0.4 ml (Fig. 2). Periodic movement of the boundary surface of solvent in the PTFE tube between the mixing chamber and the air-damper was visually

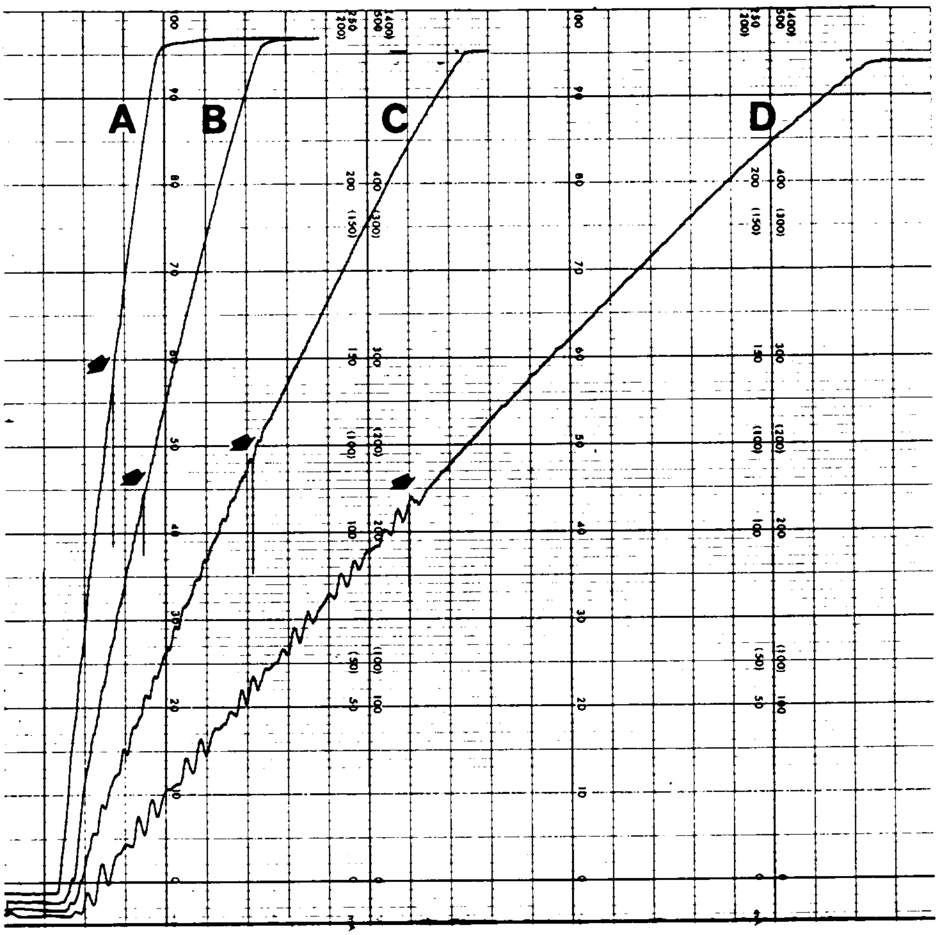

Fig. 2. Effect of air-damper on compositional fluctuations in linear gradients. Linear gradient (%/min): A, 100/10; B, 100/20; C, 100/40; D, 100/80. Flow-rate: 1.5 ml/min. Pump: 500/6 rpm. Segment time: 15 sec. Damper volume: 1.0 ml. Mixing chamber: 0.4 ml. Arrows indicate the time when the air-damper was on.

observed during gradient generation. Fluctuation of solvent composition was observed by the detector response. These effects were minimized when the air-damper was connected to the mixing chamber. The minimizing effect on composition fluctuation was most noticeable during slower changes of solvent composition, as shown in Fig. 2D. The air-damper also minimized fluctuations in the step mode.

Other factors that influenced the compositional fluctuation were examined. The segment time, *i.e.*, the time for proportioning, was studied to determine the conditions that minimized the compositional fluctuation with varying flow-rate, air-damper volume and mixing ratio. Eight segment times of 11, 10, 9, 8, 7, 6, 5 and 4 sec were examined in the programmed step-gradient mode of 60% at flow-rates of 0.5–1.5 ml/min with the pumps at 155/6 and 500/6 rpm (Fig. 3). The amplitude of

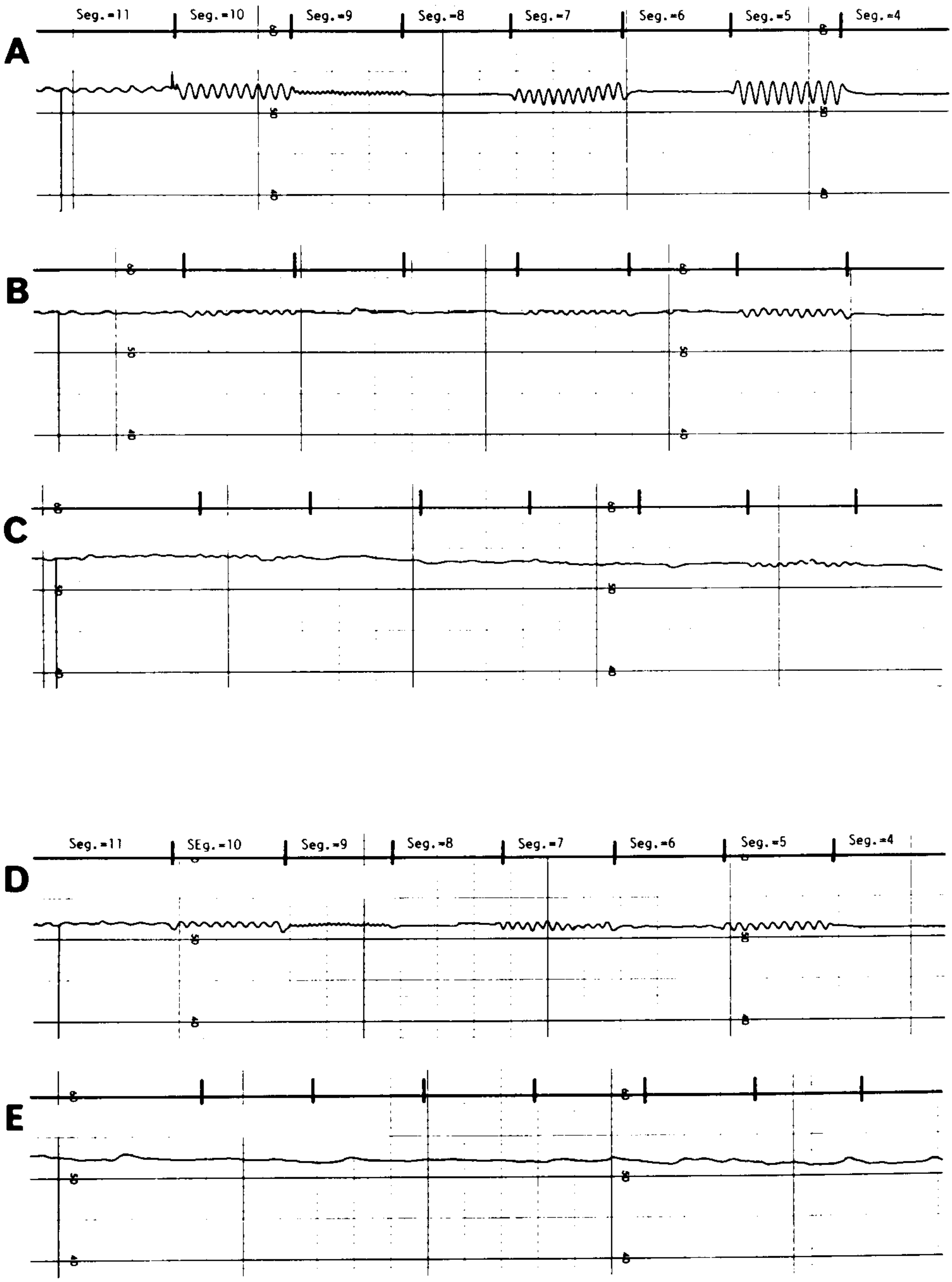

Fig. 3. Effects of segment time, flow-rate and damper volume on the compositional fluctuations in a step gradient. Step gradient: 60%. Segment times: 4, 5, 6, 7, 8, 9, 10 and 11 sec. Flow-rate: A, D, 1.5; B, 1.0; C, 0.5 ml/min. Pump: 155/6 rpm. Damper volume: A, B, C, 0.5; D, E, 2.0 ml.

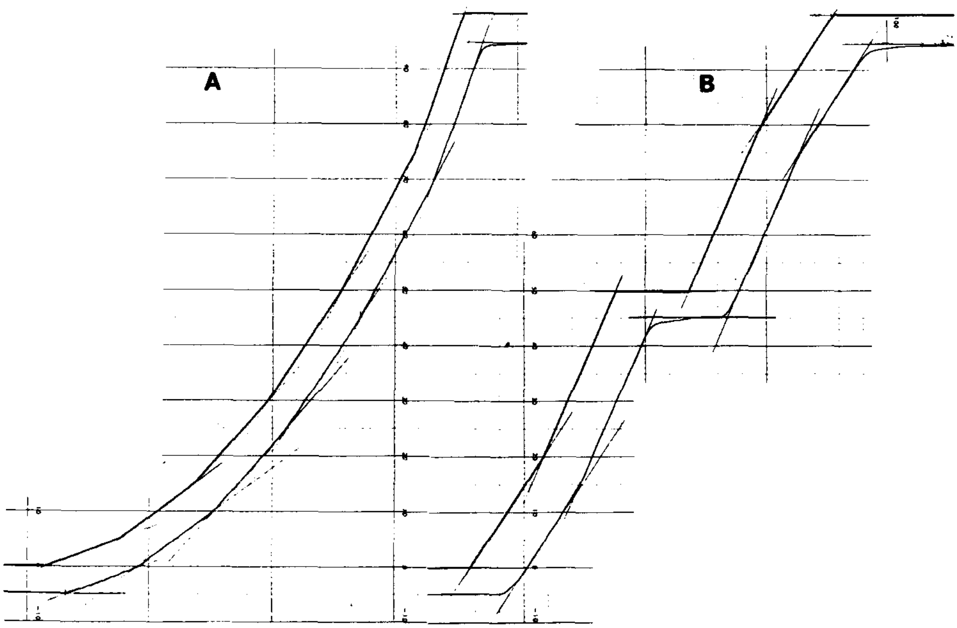

Fig. 4. Efficiency of the device in linear gradient formation. For gradient programme see the text. Flow-rate: A, 1.0; B, 0.5 ml/min. Pump: 155/6 rpm. Damper volume: 0.5 ml. Mixing chamber: 0.3 ml. Segment time: 8 sec.

fluctuation was affected by the segment time, flow-rate and the volumes of the air-damper and mixing chamber. Minimum fluctuations were found at a segment time of 8 sec for the delivery pump at 155/6 rpm, and 12 sec for the delivery pump at 500/6 rpm. Fluctuations were minimized when the volumes of air-damper and mixing chamber were adjusted according to flow-rate.

The effect of hydrostatic pressure at the inlet nozzle of the proportioning valve was established by varying the vertical distance between the bottom of the solvent container and the nozzle. Hydrostatic pressure at the proportioning valve did not affect fluctuations as much as the inherent error in the designed mixing ratio. In order to produce accurate and reproducible gradients, constant pressure and flow-resistance compensation were required. This was attained by using a Mariotte-type solvent reservoir and changing the height of the bottle as needed.

Selecting suitable conditions, as mentioned above, linear and step gradients were successfully performed. The programmes in Figs. 4A, B, E and F were written on the signal generator and stored in the modes I, II, III and IV. These programmes were as follows:

Mode	Section									
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
I (Fig. 4A)										
Time (min)	12	24	36	48	60	75				
Conc. (%)	5	15	30	50	75	99.99				
Seg. (sec)	8	8	8	8	8	8				
II (Fig. 4B)										
Time (min)	12	24	36	48	60					
Conc. (%)	20	50	50	80	99.99					
Seg. (sec)	8	8	8	8	8					
III (Fig. 5E)										
Time (min)	0:30	8	8:30	16	16:30	24	24:30	32	32:30	45
Conc. (%)	20	20	40	40	60	60	80	80	99.99	99.99
Seg. (sec)	8	8	8	8	8	8	8	8	8	8
IV (Fig. 5F)										
Time (min)	0:30	6	6:30	12	12:30	18	18:30	24	24:30	35
Conc. (%)	4	4	8	8	12	12	16	16	20	20
Seg. (sec)	8	8	8	8	8	8	8	8	8	8

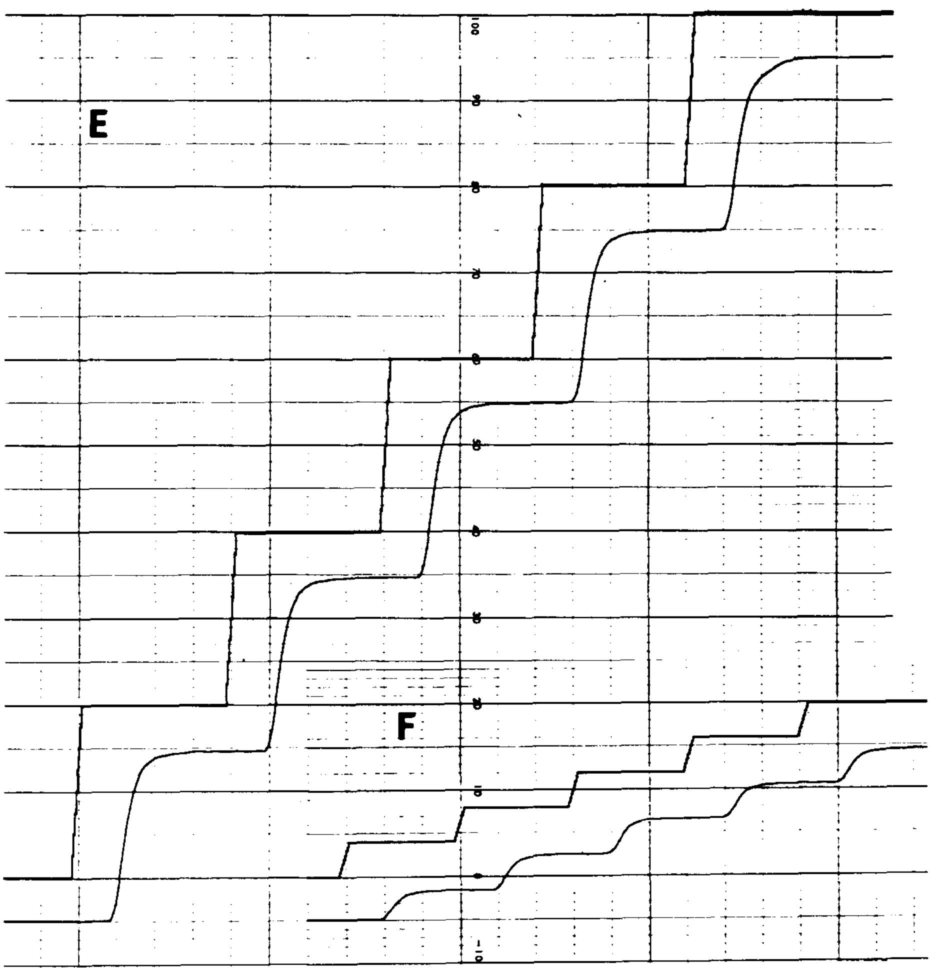

Fig. 5. Efficiency of the device in step-gradient formation. For gradient programme see the text.Flow-rate: 0.5 ml/min. Pump: 155/6 rpm. Damper volume: 0.5 ml. Mixing chamber: 0.3 ml. Segment time: 8 sec. Hydrostatic pressure: E(A), 18; E(B), 19; F(A), 18; F(B), 22 cm. Hydrostatic pressures are expressed by the vertical distance between the bottom of the solvent container and the valve nozzle.

Programmed gradient formation in the linear mode and step mode was accurate and reproducible. Using a segment time of 8 sec for the pump at 155/6 rpm, a 0 to 100% linear compositional change was well correlated with the optical tracing (Fig. 4A, B). The errors were less than 0.5% when compared to the designated compositions of 4, 8, 12, 16, 20, 40, 60 and 80% in the step mode (Fig. 5E, F).

CONCLUSIONS

Fluctuation of the delivered solvent composition was caused by the difference in operating phase between the filling stroke of the pump and the segment time; it was minimized by the use of the air-damper. It was possible to adjust the volumes of the air-damper and mixing chamber according to the reciprocating ratio of the pump and the flow-rate.

Differences in the delivered solvent composition from those selected were caused by the effective flow-rate of solvent at the inlet nozzle of the proportioning valve, and were controlled by adjusting the solvent pressure at the nozzle.

The experimental proportioning device equipped with an air-damper enabled it to perform a properly programmed mobile phase gradient with the use of a conventional, single-plunger pump. This device does not require a constant-filling flow-rate pump to produce gradients in a single-pump system.

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