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SINGLE-PUMP SOLVENT PROGRAMMER FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING SYNCHRONIZED VALVE SWITCHING

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SUMMARY

The performance of several commercial high-performance liquid chromatography solvent programmers has been tested for isocratic and gradient elution. Significant differences have been observed between the imposed and the actually delivered composition of the solvent mixture. The deviations can be traced to the principal limitations of the presently available single- or dual-pump programmers. Such shortcomings have been overcome in a single programmer that can be incorporated in any reciprocating piston pump. The design uses a three-way valve switching periodically between two solvents. Because the valve switching is synchronized with each refill stroke of the pump, highly stable, reproducible and accurate binary compositions can be delivered in both isocratic and gradient elution. The principle is easily extended to ternary systems.

INTRODUCTION

Of the different programming techniques[1] that may be conceived in order to overcome the general elution problem in high-performance liquid chromatography (HPLC), variation of the mobile phase composition is technically the most complicated. However, in comparison with less complicated techniques, such as flow programming and temperature programming, it is also much more powerful. Except for routine analysis it is more practical than column switching. For this reason all leading manufacturers of liquid chromatographs offer facilities for solvent programming as an accessory to the basic instrument. Such programmers are used not only for a gradual variation of the mobile phase composition (gradient elution), but also for the constant delivery of binary mixtures used in isocratic elution. This stresses the need for a versatile solvent programmer that meets the requirements of either application. These requirements may be formulated as follows:

(1) convenient and rapid change of the mobile phase composition;

(2) small and accurately known delay time between imposing a certain composition and its delivery to the chromatographic column;

(3) complete coverage of binary mixtures from 0 to 100%;

(4) reproducible and constant delivery to avoid variations in the chromato-

graphic separation and baseline fluctuations arising from unequal detector response to the parent solvents;

(5) accurate knowledge of the delivered composition to facilitate the exchange of chromatographic procedures and theoretical studies[2];

(6) versatility in gradient elution: stepwise or continuous; linear or non-linear; variable steepness;

(7) close agreement between the imposed and the delivered gradient;

(8) easy extension to ternary mixtures;

(9) low cost.

Now, obviously, these requirements are partly contradictory. For example, installation of a mixing chamber improves the constancy of the delivered composition but lengthens the delay time and deteriorates the slope of the delivered gradient. A real solvent programmer will necessarily present a compromise based on practical considerations. In this paper a programmer is described that meets the above criteria better than any device presently available. In order to validate this statement, a comparison has been made with commercial programmers. Unfortunately, manufacturers' brochures seldom specify the performance of a programmer with respect to the criteria formulated, except the last one. Frequently, even its operating principle is not clearly described. Therefore, eight currently available solvent programmers have been tested in the course of this study

Although the discussion will be restricted to the generation of binary mixtures, the arguments advanced are easily extended to mixtures of multiple solvents.

EXPERIMENTAL RESULTS

The solvent programmer proposed consists of a Waters M-6000A dual-headed reciprocating-piston pump, an Angar Scientific Model 250 three-way switching valve connected to the pump inlet, and a Hewlett-Packard Model 8005A pulse generator. If the double-pulse mode is selected, the pulse generator provides for each single trigger pulse two pulses with adjustable duration and interval.

Synchronization between the valve switching and the movement of the pump pistons is realized as shown in Fig. 1. To the gear box of the pump an additional toothed wheel is added, extending out of the pump housing and identical with that which controls the rotation of the main shaft. Thus, one revolution of this wheel corresponds to a complete pump cycle at all flow-rates. A piece of white reflecting paper, corresponding to the start of the refill period of one pump chamber, activates an optical encoder that triggers the pulse generator. The pulse generator responds instantaneously by producing a pulse that switches the valve from solvent A to solvent B. After an imposed time, the valve switches back to solvent A. At the start of the refill period of the other pump chamber the pulse generator sends a second pulse to the valve, because the variable interval time has been selected to correspond exactly to the time between the starting points of the refill strokes of the two pump pistons. Again, the valve is switched from solvent A to B and, after the duration of the pulse, is switched back to solvent A. The ratio of the pulse duration to the total refill period of a pump chamber will be called the *duty cycle* and this determines the composition of the delivered binary mixture. Gradient elution can be realized by slowly varying the duty cycle (*i.e.*, the pulse duration) during the chromatographic run.
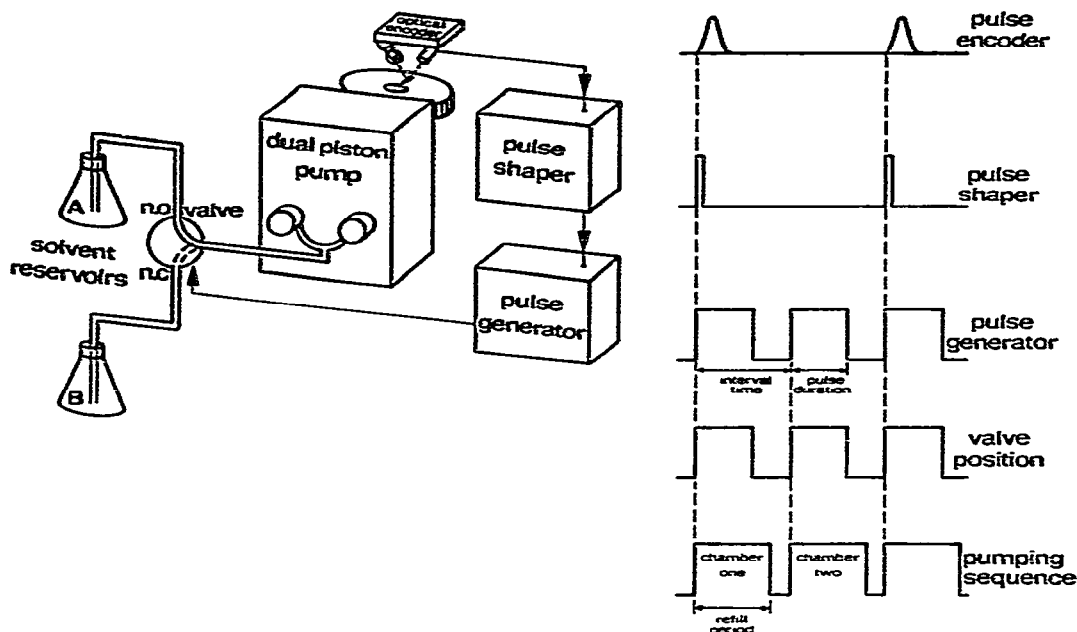
Fig. 1. Schematic arrangement of the proposed solvent programmer (left) and the synchronized valve switching (right).

The performance of the programmer has been tested by connecting the pump outlet to a conductivity cell positioned before the chromatographic column. The cell is made from stainless steel according to Tesarik and Kaleb[3] and has a dead volume of less than 1 μl. The cell response is measured with a Radiometer Model CDM 3 conductivity meter and calibrated against binary mixtures of known compositions. The same cell was used to test the following commercial solvent programmers, which were kindly placed at our disposal by users or distributors of the instruments: DuPont model 850, Hewlett-Packard Model 1084 B, Perkin-Elmer System 3, Pye Unicam LC-XP gradient programmer with LC3 XP pump, Spectra-Physics SP 8000, Tracor Model 980 A with Model 950 pump, Varian Model 5000 and Waters Model 660 with two M6000-A pumps.

Each instrument was subjected to a 1-day test programme consisting of the following experiments:

(a) stepwise change of the eluent from pure solvent A to pure solvent B;

(b) runs with several isocratic mixtures covering the full range between either solvent at a flow-rate of 2 ml/min;

(c) runs with an imposed linear gradient running from A to B in 15 min[4] and a flow-rate of 2 ml/min;

(d) in all instances solvent A was water and solvent B was 0.01 M potassium bromide in water to avoid additional problems arising from mixing two widely different solvents.

We should emphasize the limitations of this test programme. Owing to the limited time available the instruments were used under the conditions at which they were placed at our disposal. Obviously, all results refer to a single specimen. Conse-

quently, the data do not reflect optimal results obtained after careful adjustment. In particular, the results do not reflect the manufacturers' specifications and are our full responsibility. Therefore, the data will be discussed in terms of their general significance and, accordingly, the instruments cited above in alphabetical order will be indicated by letter in the following discussion. However, in order to interpret the results, the basic characteristics of each system must be known; these are summarized in Table I.

TABLE I

CHARACTERISTICS OF SOLVENT PROGRAMMERS

Designation	Pump	Chamber volume (µl)	Mixing chamber		Valve
			Type	Volume (ml)	
A	Single	90	Static	—	2-way
B	Single	400	Dynamic	2 × 1.9	2-way
C	Single	50	Dynamic	1.1	2-way
D	Single	100	Not specified		3-way
E	Single	100	Static	0.8	3-way
G	Dual	100	Capillary		—
H	Dual	100	Static	1	—
K	Dual	Variable	Dynamic	0.5	—
S*	Single	100	Static	1	3-way

* System described in this paper.

Operating principle and performance of existing solvent programmers

The first requirement for a solvent programmer is the easy and rapid change of the delivered composition. This rules out the ingenious but by now historic systems utilizing multiple reservoir–dilution vessels[5]. Except for routine analysis the multiple reservoir–automatic multiport valves[6] fail in convenience and versatility. The requirements of reproducible and accurate mobile phase composition cannot be met by dual syringe-type pumps[7]. In agreement with these conclusions, all modern commercial solvent programmers use reciprocating piston pumps.

The most recent discussion of such systems was given by Martin and Guiochon[8]. It must be noted that reciprocating piston pumps by their nature yield a ripple in the delivered flow, which may be reduced by various means such as pulse dampers, multiple piston heads[9] or feedback flow control systems[10,11]. In this way the delivered flow is smoothed, but the incoming flow is necessarily not constant. The non-square-wave flow profile during the refill period of the piston may create problems in single-pump programmers, as will be discussed below.

Solvent programmers using reciprocating piston pumps can be broadly distinguished in two categories:

(a) Dual-pump systems, in which each solvent is delivered continuously and independently by a separate pump. The two flows are mixed at the high-pressure side in a mixing chamber. The overall flow-rate of the mobile phase remains constant, but its composition is controlled by the ratio of the flow-rates of the two pumps (or sometimes by the stroke lengths of each of the pistons in a single dual-headed pump).

(b) Single-pump systems and valves regulating the solvent flows at the low-
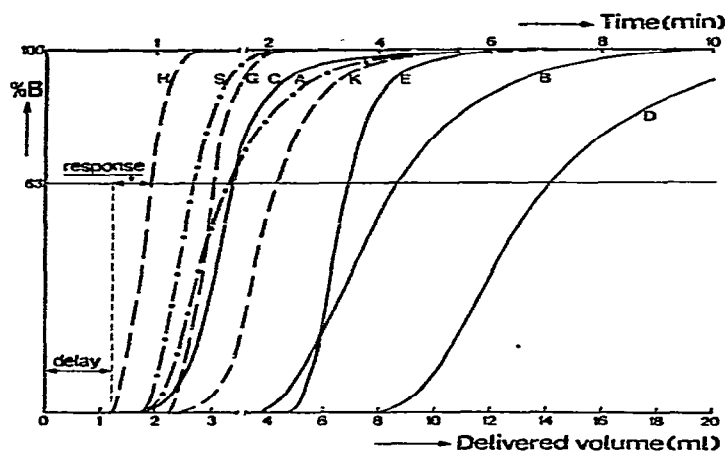
Fig. 2. Response of solvent programmers to a stepwise change of one solvent (water) to another (0.01 *M* potassium bromide in water). System designation as in Table I. Note change in volume and time scale for systems E, B and D. See also Table II. – – – –, Dual-pump systems; ———, non-synchronized single-pump systems; – . – . –, synchronized single-pump systems. See Note added in proof.

pressure side. Conceivably, this could be done continuously with variable needle valves, but the available valves do not provide automatic control and have too large dead volumes for the low flow-rates customary in HPLC. Instead, switching valves are used that deliver the solvents alternately and sequentially by periodically switching from solvent A to solvent B. The composition of the mobile phase is then determined by the times the valve is open to either solvent.

Each system has advantages and disadvantages. One obvious disadvantage of the dual-pump system is the cost of the second pump. The chromatographically more important differences will be discussed on the basis of the results of the test programme described above and presented in Figs. 2–5.
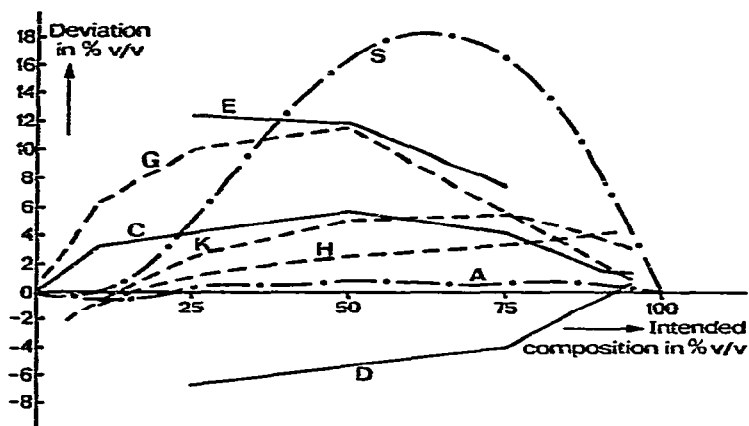

Fig. 3. Accuracy of delivered binary composition. The deviation in % (v/v) of the final binary composition is plotted for various intended compositions between pure solvent A and pure solvent B. System designation as in Table I. – – – –, Dual-pump systems; ———, non-synchronized single-pump systems; – . – . –, synchronized single-pump systems.
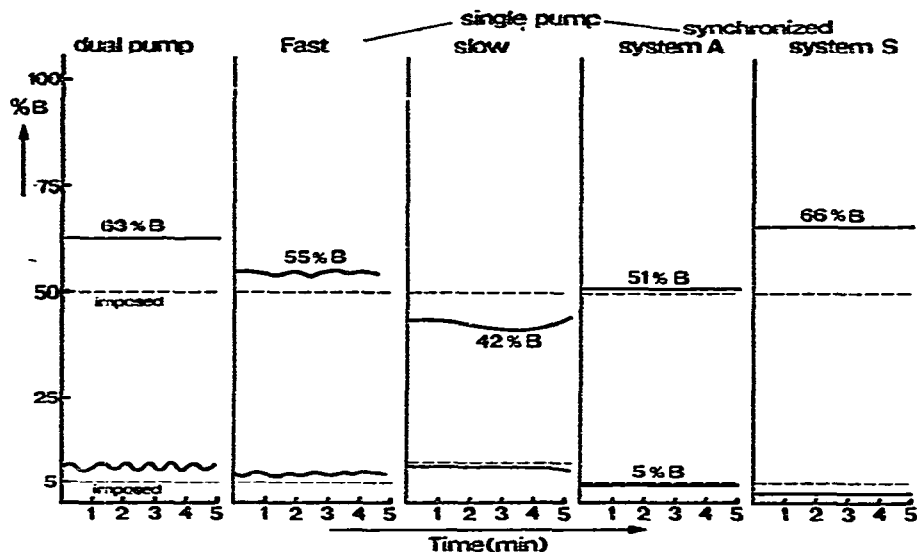
Fig. 4. Constancy of delivered binary compositions for typical pump systems. Four-minute recorder traces of final compositions are presented for a flow-rate of 2 ml/min at a low (5%) and a medium (50%) binary composition.
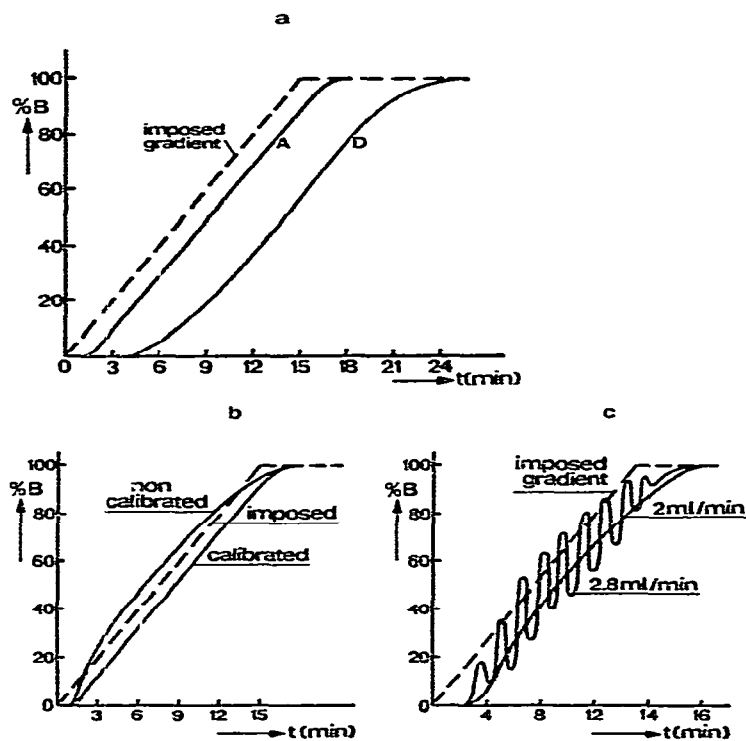

Fig. 5. Typical gradient elution curves obtained for an imposed gradient running from pure water to pure 0.01 M potassium bromide solution in 15 min (dashed line). (a) Single-pump systems using synchronization (system A) or large mixing chambers (system D). (b) Typical dual-pump systems without and with calibrated pump flows. Non-synchronized single-pump system with small mixing chambers at two different flow-rates: (1) 2.8 ml/min; (2) 2.0 ml/min.

Fig. 2 shows the response to a stepwise change from one solvent to the other and illustrates the wide variation in delay time and response time between the different solvent programmers. Here the delay time is defined as the time between the applied step function and the first noticeable change in the solvent composition delivered at the column inlet. The (quasi-first-order) response time is defined as the period between this latter moment and the point where the solvent reaches 63% of its final composition.

Actually, the time scale in Fig. 2 is a derived quantity equal to the ratio of the more fundamental delay and response volume to the flow-rate (2 ml/min in Fig. 2). The delay volume is the volume between the junction where the two solvents are combined and the top of the column. It is made up of mixing chambers, pump and valve chambers (for single-pump systems), pulse dampers and connecting tubing. For the programmers investigated the delay volume varies from 1.3 to 8.2 ml (Table II). Only those parts that involve actual mixing of the solvents contribute to the slow approach of the imposed composition. In Table II, the 63% response volumes are seen to vary from a low 0.6 ml to a highest value of 6 ml. Consequently, with a typical flow-rate of 2 ml/min, the changeover from one mobile phase composition to another takes from 1.4 to 15 min.

TABLE II

RESPONSE DATA FOR SOLVENT PROGRAMMERS

System	Delay volume* (ml)	Response volume** (ml)	99% response time*** (min)
A	1.7	1.6	3.8
B	3.6	5.1	9
C	1.8	1.6	3.7
D	8.2	6.0	14
E	4.8	2.1	6
G	2.3	0.7	2.2
H	1.3	0.6	1.3
K	2.5	1.7	3.9
S	1.7	1.0	2.1

* Defined as the volume between the imposed step change and the first noticeable composition change (Fig. 2).
** Defined as the volume needed to reach 63% of the final composition minus the delay volume (Fig. 2).
*** Derived from Fig. 2 for a flow-rate of 2 ml/min.

All three dual-pump systems (G, H and K) perform reasonably well, as do some single-pump systems (A and C). The poor performance of systems B and D is due to the incorporation of a large mixing chamber; for system D this is necessary to overcome limitations to be discussed below. Naturally, such a large mixing chamber lengthens both the delay time and the response time and, in fact, systems B and D provide useful gradients when actually instructed to produce a stepwise change.

It is seen that the single-pump programmer described in the present study (system S) performs better than all other systems, except for one dual-pump system.

Eventually, all solvent programmers accurately deliver a pure solvent. It is more interesting to compare the accuracy of binary mixtures. Fig. 3 presents the

deviation from the imposed composition for binary compositions varying from pure water (A) to a solution of potassium bromide in water (B). Naturally, all curves indicate zero error in the extreme compositions of pure A or pure B, but in between the deviation can be as large as 10%. For example, when set to produce a 50:50% (v/v) mixture, system G actually delivers a composition of 60:40% (v/v), whereas the output of 51:49% (v/v) by system A closely reflects the imposed value. The reason for the deviations differs for the two pump categories.

In dual-pump systems the deviations are reproducible and reflect a systematic error in the flow-rates of the two pumps. Consequently, the results for systems G, H and K may be improved by calibrating and adjusting the pumps. Unfortunately, in the three systems investigated such adjustments cannot easily be made by the user, even if he is aware of the error .

For single-pump systems the situation is more complicated, because for systems C, D and E the error is, in principle, not reproducible but may vary from run to run. Basically, the error in the delivered composition arises from the non-square-wave profile of the inlet flow to the pump during the refill stroke of the reciprocating piston. Therefore, the mixing ratio is accurate only if it corresponds to the ratio of whole numbers of pump chambers. Generally, of course, the valve will switch at an arbitrary position during a refill period and then the ratio of the delivered volumes does not correspond exactly to the duty cycle of the switching valve. Surprisingly, a large mixing chamber, incorporated in system D to overcome this error, fails to do so.

For the single-pump systems A and S the error is reproducible, because the valve switching is synchronized with the refill period of the piston pump. Again, the non-uniform inlet flow will produce large errors (system S), unless the duty cycle of the switching valve is corrected for this error (system A). This will be discussed more fully below.

Whereas the accuracy of the delivered composition is important for theoretical studies and for the interlaboratory exchange of chromatographic procedures, the short-term stability of the delivered composition directly influences the precision of quantitative analysis. Fig. 4 presents typical results for single- and dual-pump systems. In agreement with Fig. 3, the average compositions do not always agree with the intended composition. More important are the occasionally large oscillations observed in the delivered composition, because they will produce an unstable baseline in the chromatogram, if the detector responds differently to either solvent (as in the present test).

Dual-pump systems yield highly stable isocratic compositions only if the fraction of one solvent is more than 10% of the other. This is due to the well known fact that, at the flow-rate required to deliver a small fraction of the minor component, reciprocating pumps do not perform well. In fact, manufacturers of dual-pump systems explicitly advise against such extreme compositions. In contrast, most single-pump systems show significant fluctuations in the delivered composition over the entire range except the extreme ends. Again, the fluctuations arise from the non-uniform profile of the inlet flow. Rapid oscillations can be suppressed by incorporating a large mixing chamber as evidenced by slow non-synchronized single-pump systems in Fig. 4. However, such systems still show slowly drifting baselines and, of course, a long delay and response time (Fig. 2). An alternative and superior solution is to synchronize the valve switching with the movement of the pump piston. Indeed,

systems A and S, utilizing this principle, provide isocratic compositions stable to within 0.1%.

Fig. 5 presents typical results obtained for different pump systems set to produce a linear gradient running from solvent A to solvent B in 15 min at a flow-rate of 2 ml/min. They are easily explained from the data on isocratic elution in Figs. 2–4. Obviously, the delay time between the imposed gradient and the first noticeable change in solvent composition is closely connected to the delay time in Fig. 2. In the same way, a large response time in Fig. 2 will lead to a less than intended gradient slope in Fig. 5. In mathematical terms this is expressed by stating that the recorded change in composition is the convolution of the imposed gradient with the response to a step function shown in Fig. 1. Consequently, the decrease in the gradient slope is larger for steeper gradients. However, for all systems presently investigated the response is rapid enough to produce a reasonable 15-min gradient. Slow response systems (B and D in Fig. 2) yield a slightly concave gradient, which even allowing for the delay time lags behind the intended gradient.

On the other hand, all dual-pump systems initially produced convex gradients with the delivered composition exceeding and sometimes ahead of the imposed gradient (Fig. 5b). This behaviour is reproducible and is again due to inaccuracies in the flow-rates of the two pumps. If this is remedied, the result is greatly improved and even very steep gradients can be accurately produced. At the extreme ends of the gradient, where the fraction of one or the other solvent becomes very low, the typical fluctuations discussed in connection with Fig. 4 are again observed, and are more serious for slow gradients (>25 min).

Finally, Fig. 5c shows an example of gradients produced by a fast, non-synchronized single pump system run at two different flow-rates. Whereas at one flow-rate the result is acceptable, the gradient run at 2.8 ml/min shows considerable fluctuations in the delivered composition. The source of these fluctuation is the same as in the isocratic delivery (Fig. 4) and will be discussed below.

When considered in total, the results presented in Figs. 2–5 can be summarized as follows:

(a) Dual-pump systems perform well in terms of speed and constancy of the delivered binary composition, provided that extreme ratios (<10% A or B) are avoided.

(b) The flow-rates delivered by the two pumps in dual-pump systems must be carefully calibrated and adjusted in order to provide accurate binary compositions and correct gradients.

(c) Single-pump systems can be divided into two types. One type, illustrated by systems C, D and E, offers a compromise between speed and constancy of the delivered binary composition. Rapidly responding systems are prone to produce large variations in isocratic or gradient compositions at certain flow-rates. Better, but not perfect, constancy is obtained with slowly responding system incorporating a large mixing chamber.

(d) As will be shown in the next section, the above compromise is unnecessary and reflects a design error that can be easily corrected. This is realized in the commercial system A and also in the presently proposed system S, which yield both rapid and stable delivery. Our system S is superior in speed (Fig. 2) and comparable in constancy (Fig. 4), but not yet in accuracy (Fig. 3).

Design and performance of a synchronized solvent programmer

The solvent programmer developed in the present study is designed to yield optimal performance at minimal cost. This points to a single-pump system with a minimum dead volume between the switching valve and the chromatographic column. Therefore, a rapid (10-msec switching time) three-way valve is connected to the two solvent reservoirs on the one side and to a dual-headed reciprocating pump on the other side. No additional mixing chambers are added and only the noise filter incorporated in the pump is retained, which contributes a mixing volume of 0.6 ml.

However, when operated to yield an isocratic mixture more or less serious oscillations in the delivered binary composition are observed, even worse than those shown in Fig. 4 for a fast system. Such oscillations have also been observed in a similar system by Saunders[12] and explained by him as being due to the interaction between the frequencies of the switching valve and the pump piston. For the dual-headed pump used in this study the phenomenon is readily explained by a simplified example. Let us propose making a 50:50 binary mixture, which means that the valve is open to solvent A for half of its period and open to solvent B for the other half (50% entry cycle). Now, suppose that by chance the total switching cycle of the valve is equal to the complete cycle time of the pump and that, moreover, the valve switches at the point where a piston starts ist refill period. Ignoring the volume between the valve and the pump chambers, this means that solvents A and B will be directed alternately to either pump chamber. The pump will then deliver successive leaps of pure A and pure B, which, in the absence of a mixing chamber, will be transported to the chromatographic column virtually unchanged. In this extreme example, the oscillations in the delivered composition are maximal and permanent.

In practice, the situation is more complicated owing to the volume of the connecting tubes between the switching valve and the pump. Also, the oscillations will be less severe if the valve switching and the piston movement still have the same frequency, but are out of phase. Such small oscillations may then be effectively suppressed by incorporating a large mixing chamber. On the other hand, when the cycle times of the switching valve and the pump are unequal, then the oscillation pattern will vary in time and such slow variations are not removed by a mixing chamber. This is illustrated by the example of a slow system in Fig. 4.

As a remedy, Saunders[12] included a 2-ml mixing chamber after the pump and recommended avoiding cycle times for the valve that are within 25% of half-integer pump cycles (for dual-piston pumps). However, the latter condition is impractical, because the pump cycle varies with the flow-rate. Indeed, a simple analysis shows that there exists no unique valve cycle time that provides binary mixtures free from oscillations at all flow-rates between 0.5 and 5 ml/min. Saunders' recommendation agrees with our earlier observation that the oscillations observed in the output of single pump systems are flow dependent (Fig. 5c).

Indeed, one commercial system (E) has a provision to alter the valve switching cycle depending on the flow-rate; unfortunately, this is not mentioned clearly in the instrument manual. It occurred to us that the oscillations could be avoided if the valve cycle is exactly synchronized with the refill period of each *individual* piston stroke. Moreover, the system will be automatically adapted to all flow-rates if the valve switching is triggered by the moving piston itself. One possible realization of this principle is presented in Fig. 1 and explained above. With the present pulse

generator the optical encoder must be activated at the exact moment where one of the moving pistons starts its refill period. In a more sophisticated design the interval between the trigger pulse from the optical encoder and the primary pulse from the pulse generator to the valve will be adjustable. Then the location of the optical encoder is less critical because the start and the duration of the refill and delivery strokes of each piston are easily calibrated in terms of phase angle with respect to the position of the encoder.

At the start of a refill period the valve is opened to solvent B. After a pre-set phase angle dependent on the intended binary composition, the valve is switched back to solvent A for the remainder of the refill period. It remains open to A and the cycle is repeated for the other piston head. Obviously, this operation is feasible for single- and multiple-heated piston pumps. In the present pump the delivery strokes of the two pistons partly overlap to reduce pump pulsing, whereas the refill periods are separated by a small phase angle. This allows some latitude in the moment of opening the valve from solvent A to solvent B, so that only the exact timing of the switch from B to A is critical. However, this is not an essential requirement of the design. If the refill periods overlap, it only means that both switching moments are critical to obtain a correct binary composition.

An additional advantage of the design is the fact that mixing of solvents A and B is partly realized in the pump chamber. In the present pump extra mixing is provided by the small chamber where the high-pressure outlets of the two pump chambers meet, and by the pulse damper. No further mixing chambers have been added. The marked improvements resulting from synchronization are obvious from Fig. 6. Without synchronization large oscillations of the binary mixture around the intended composition (50:50) are observed. Even at ten-fold scale expansion oscillations with the synchronized system are minimal. Identical results have been obtained over the whole composition range and at all flow-rates.

In closer analysis, however, the delivered composition, although constant, is not equal to the intended composition (Fig. 3). This can be understood from the refill
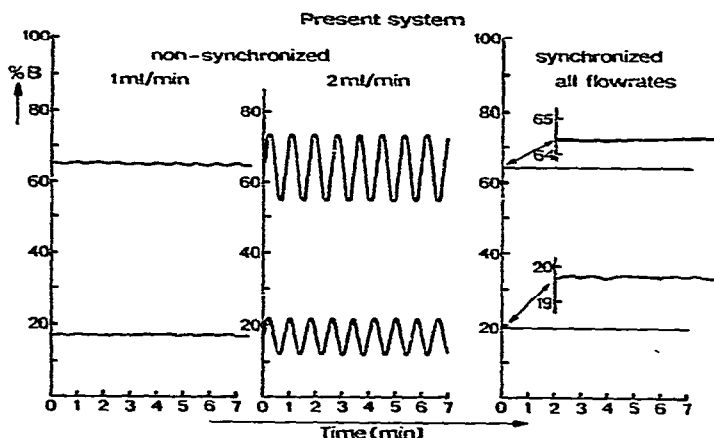

Fig. 6. Influence of synchronization on the constancy of the delivered binary composition. Without synchronization flow-dependent oscillations are observed, which are completely removed in the synchronized system. Note the expansion of the composition scale in the synchronized system.

profile of the piston pump. Let us first assume this to be square-wave, as assumed in Fig. 1. In that case the delivered composition is identical with the fraction of the refill period over which the valve is open to either solvent, irrespective of the change-over point from solvent B to A as long as synchronization is maintained. A slight uncertainty is introduced by the 10 msec it takes to change over from solvent B to solvent A. Now, the refill period of each 100-μl pump chamber is 1 sec for a flow-rate of 5 ml/min. Therefore, the finite switching time of the valve may introduce a deviation of 1 % from the intended composition, although it will not impair the constancy of the delivered composition. For a lower flow-rate of 0.5 ml/min the possible deviation is at most 0.1 % (v/v).

However, it should be noted that some reciprocating pumps employ a rapid (200 msec) refill in connection with a slow delivery to improve the constancy of the delivered flow. This puts higher demands on the switching time of the valve. The present valve is magnetically operated and its switching time is limited to 10 msec. Although more sophisticated valves are known to switch within 2 msec, the inertia of the solvent flow may become the limiting factor at extreme binary compositions (less than 10 % B in A). Indeed, system A in Figs. 2–5 using this type of pump extends the synchronized valve cycle time over four cycles of the single-headed piston pump. This makes the system slower than the presently proposed one (Fig. 2).

In reality, the refill profile of the piston is not a square-wave. The piston starts slowly, picks up speed and retards again before it reverses its direction to deliver the contents of the pump chamber at the high-pressure outlet. The resulting profile is shown in Fig. 7a and, if it were perfectly symmetrical, a 50:50 (v/v) binary composition would be accurately delivered when the valve is switched after half the refill period has elapsed. As can be seen from Fig. 7a, however, if we instruct the valve to switch after exactly 70% of the refill period has elapsed, then the delivered mixture contains more than 70% of the major component B. This is due to the retardation of the piston towards the end of the refill period.

This phenomenon explains the inaccuracies of the binary mixtures delivered by single pump programmers (Fig. 3). For non-synchronized systems (C, D and E) the switching point of the valve is arbitrary with respect to the piston movement and, consequently, the errors in Fig. 3 will be variable from run to run. For synchronized systems (A and S) the error will be reproducible, but in principle non-zero. However, it can be corrected by calibrating the delivered composition as a function of the changeover point of the valve.

It should be noted that such a calibration includes the influence of the finite valve switching time mentioned earlier. Fig. 7b presents the calibration graph for the present pump. In fact, the curves in Fig. 7b are the integral of the refill profiles shown in Fig. 7a. From these data the correct duty cycle of the valve can be easily derived for each intended binary composition. In the commercial system A the correction is realized by an intricate collection of optical encoder signals during the piston's refill period. As shown in Fig. 3 this results in accurately delivered binary mixtures. It is our intention to include the correction in a microprocessor program. In the near future the microprocessor will replace the pulse generator in Fig. 1, providing the opportunity to generate a wide variety of gradient elution curves. This is important as recent studies in our laboratory have shown that non-linear gradients are superior for many organic modifiers in reversed-phase HPLC[13].
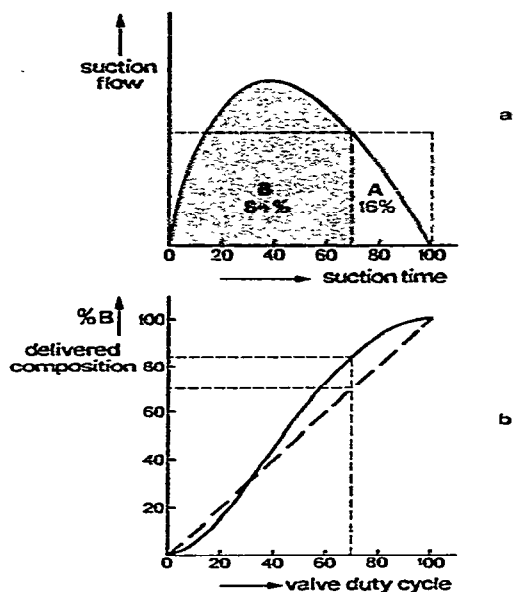
Fig. 7. Influence of a non-uniform refill profile on the accuracy of the delivered binary composition. (a) Hypothetical square-wave profile (dashed line) pump; both traces are normalized to the same area. The delivered binary composition is equal to the ratio of the areas on either side of the switching point, illustrated for a valve duty cycle of 0.7. (b) Calibration graph between delivered binary composition and duty cycle for the square-wave (dashed line) and the true refill profile (solid line).

The non-square-wave profile of the piston offers slight advantages in delivering mixtures containing very small amounts of one solvent in the other. Because the profile levels off at the extreme ends of the refill period, the duty cycle of the valve must be taken longer than proportional in order to obtain binary mixtures containing less than 20% of one solvent (Fig 7b). This relaxes the requirements for gradient elution covering the full range from 0 to 100%.

In testing the proposed system two problems were anticipated: solvent contraction and air bubbles arising form mixing in the pump chamber. Problems due to solvent contraction might have been avoided by including a mixing chamber between the valve and the pump, but this will upset the synchronization scheme. Fortunately, contraction appears to present no problems for any of the binary mixtures tested: mixtures of water on the one hand and methanol, acetonitrile or tetrahydrofuran on the other hand all yield perfectly constant compositions over the full range from 0 to 100% of modifier.

Air bubbles released in the pump chamber do constitute a problem, as they give rise to fluctuations in the output composition. Vigorous, ultrasonic stirring of the solvents was insufficient, but passing a helium flow through the solvent reservoirs[14] proved to be an effective remedy.

CONCLUSIONS

The performance of the present single-pump solvent programmer can be judged from the data in Figs. 2–4 (system S). The proposed system appears to combine

the favourable properties of current single- and dual-pump systems. It is cheap and versatile and can be adapted to all reciprocating pumps. As a result of the small mixing volume, the response is equally rapid to that of dual-pump systems and faster than any existing single-pump system (Fig. 2). The output is constant to better than 0.1 % (v/v) over the complete composition range (Figs. 3 and 6), a feature matched by only one commercial system (A). The synchronization principle is easily extended to generate accurate mixtures of more than two solvents. Ultimately, the finite switching time of the valve imposes limits on the precision to which compositions can be adjusted. For reasons explained above, the accuracy of the present programmer is not yet optimal (Fig. 3). It will be improved by microprocessor control correcting for the non-square-wave refill profile of the pump. This will be reported in a subsequent paper.

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NOTE ADDED IN PROOF

During the preparation of the manuscript, the operating principle of system B was not available to us. From our observations we considered it to be analogous to non-synchronized systems. Recently, the manufacturer has supplied information (*U.S. Patent* 4,128,476, December 1978) that system B does employ synchronized valve switching. Although we regret the incorrect designation in Fig. 2, it does not alter our conclusions and observations, which, we repeat, refer to a single unit, not specifically optimized by us.

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