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# COMPUTER-CONTROLLED SINGLE-PUMP SOLVENT PROGRAMMER FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A solvent programmer for high-performance liquid chromatography is described that consists of a dual-piston reciprocating pump equipped with displacement transducers, and two synchronously switching two-way valves connected to the solvent reservoirs. The system is controlled by a PDP 11/03 microcomputer. The software includes correction for solvent compressibility, generation of gradients of widely varying shape and delivery of binary mixtures between 0 and 100%.

INTRODUCTION

The mobile phase is usually a mixture of two or more solvents in high-performance liquid chromatography (HPLC) especially in the reversed-phase mode. If the composition changes during the chromatographic run, we speak of gradient elution chromatography. More commonly, the mobile phase composition varies between chromatographic runs, because different samples require different mobile phases for optimum separation. In either case it is desirable to make mixtures of arbitrary composition from the parent solvents. To this end a variety of solvent programmers has been developed that can be broadly divided into two categories: dual-pump systems with a mixing chamber at the high-pressure side, and single-pump systems with a switching valve at the low-pressure side. In a previous publication[1] the two designs were compared and it was demonstrated that single-pump programmers can provide optimum performance, provided that the distributing valve between the solvent reservoirs is switched synchronously with the stroke of the pump piston. Specifically, it was shown experimentally that a single-pump system using synchronized valve switching rapidly delivers a very stable mixture. On the other hand, the accuracy of the solvent composition was less than desired, with deviations of up to 18% between the imposed composition and the actual output. Also, the system was not tested for gradient elution, because it was manually operated.

In the single-pump system to be described presently the principle of synchronized valve switching is maintained. The pump is interfaced with a microcomputer to provide gradient elution facilities. The problem of obtaining high accuracy is addressed specifically, because other studies in this laboratory have shown that the

composition of the mobile phase is critically important in reversed-phase liquid chromatography (RPLC)[2,3]. Accurate solvent composition has also been shown to be essential for predictable gradient elution chromatography[4,5].

## PRINCIPLE OF OPERATION

A single-pump solvent programmer operates on the principle that the composition of the mixture is determined by the periods during which the distributing valve before the pump is open to either of the parent solvents. For example, if the valve is open to either of two solvents for equal periods of time, then the delivered mixture should contain equal fractions of the two solvents (50:50 composition). In our previous publication[1] we showed that this is indeed the case for the overall composition, avaraged over a large number of pumping cycles, but not necessarily for each stroke of the pump piston. In fact, if the switching sequence of the valve is unrelated to the pumpstroke, successive strokes may yield quite different mixtures leading to a periodically varying composition. To a certain extent, the problem can be overcome by incorporating a large mixing chamber after the reciprocating pump, but this makes the system rather sluggish.

It has been pointed out that constant delivery by successive pump strokes is easily accomplished when the valve switching is synchronized with the stroke of the moving piston. In our previous design this was realized with an optical encoder that sent a switching command after a predetermined lapse-time during each refill period of the pump. The delivered mixture was indeed very stable: better than 0.2 % of the actual composition. On the other hand, a certain ratio of the two time fractions in one refill period does not produce the same ratio in the binary composition. The reason for this inaccuracy can be explained as follows.

Understandably, pump manufacturers place great emphasis on the constant flow of the solvent during the delivery period of the piston. Dual pistons with partially overlapping delivery periods, as well as acceleration and retardation of the pistons, are used to provide nearly pulse-free delivery. Much less attention is given to the refill stroke of the piston, with the result that the inflow of the solvent is far from uniform during the refill period. As a result, partitioning of the refill period on a time basis does not yield the expected solvent composition. This is illustrated in Fig. 1, where an experimentally measured refill profile is presented, which is clearly not square-wave. Consequently, switching the distributing valve after 70 % of the total refill time has elapsed does not produce a 70:30 mixture, but in this case a 84:16 mixture. It should be noted that this difficulty is inherent to the synchronization principle. If the valve switching is not synchronized to the piston stroke, the switching moment changes from one stroke to another and the deviations average out to zero. This means that the ouput of non-synchronized systems fluctuates, but on the average accurately represents the value imposed by time partitioning.

The remedy for this problem seems trivial. Once the refill profile is known, it is easy to draw up a conversion table connecting the desired composition to the switching moment during the refill period. In the example of Fig. 1 a desired composition of 70:30 could be obtained by switching the valve after 59 % of the refill period has elapsed. This can easily be programmed into a computer provided that the refill profile of the pump remains unaltered.
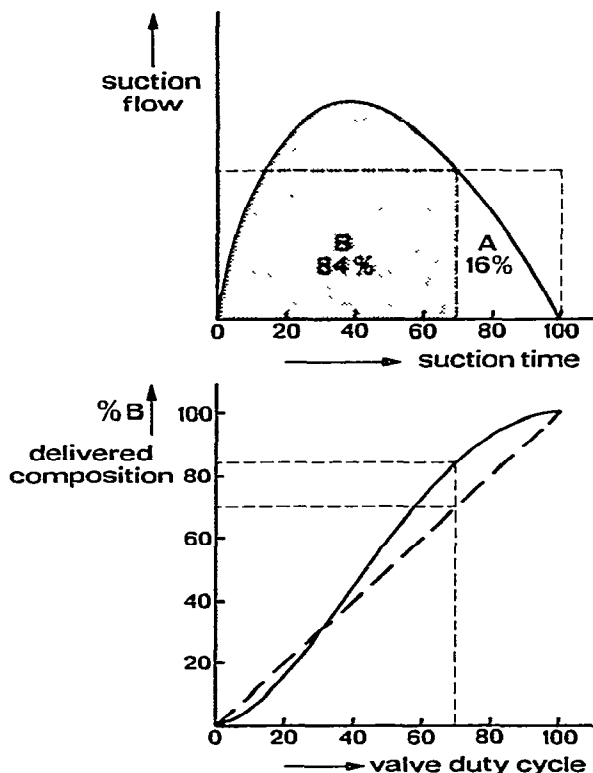
Fig. 1. Inaccuracy of synchronized valve switching based on time partitioning. The lower graph illustrates the non-linear relationship between the delivered binary composition and the fractional periods of the switching valve, if the inlet profile of the reciprocating pump piston is not square-wave as exemplified by the upper graph.

In practice, this condition is not always fulfilled. Certainly, the refill profile varies from one pump to another, requiring calibration for each individual specimen. Also, the refill profile is neither square-wave nor symmetrical, so that the conversion must probably be put in tabular form. More important is the fact that some reciprocating pumps incorporate a pressure feed-back device that again ensures constant-delivery flow independent of column resistance or solvent viscosity. As a result, however, the refill profile of such pumps varies in shape and duration with the pressure after the pump. This makes synchronized valve switching on a time basis quite impossible and, consequently, this principle would lose its general applicability.

It must be realized that the integrated refill profile at the top of Fig. 1 actually represents the non-linear movement of the piston in the pump chamber. Consequently, the switching command to the valve should not be based on the time elapsed since the turning moment of the piston, but on the displacement of the piston since its turning point. In that case, the correct solvent composition will always be delivered, independent of any variation in the speed of the piston either during its refill stroke or during an entire chromatogram. The only condition is that the position of the pump piston is monitored continuously, so that the total length of the piston stroke can be determined and switching commands to the valve can be issued at the appropriate

position. A possible solution will be presented in the next section. Here we note only that it appears to be rather difficult to impose switching commands close to the turning points of the moving piston. Therefore, if the valve is switched only once during each refill stroke, binary mixtures containing only a few percent of one component become rather irreproducible. The precision is significantly enhanced if the valve is switched twice, so that the minor component is bracketed in between the major component. In other words, a switching cycle A/B/A is recommended over a switching cycle A/B. The two switching points should be positioned symmetrically to the midpoint of the piston stroke. The A/B/A switching cycle offers the further advantages of requiring less supervision time from the computer and improved mixing inside the pump chamber. This removes the need for an additional mixing chamber after the pump, so that the solvent delivery system responds rapidly to changes in imposed composition.

CONSTRUCTION

The equipment consists of two Angar Scientific two-way switching valves (368-NO-24-30 Z), and a Pye Unicam LCX-3 dual-piston reciprocating pump equipped with two EMI Labs. SE 373115 displacement transducers. The latter convert the 9-mm stroke of a piston into a voltage varying by about 2 V. The signal from the transducer is sampled every millisecond by a 12-bit analogue-to-digital converter (ADC) and the digitized signal is processed by a DEC Model PDP 11/03 microcomputer supplied with 8 K memory. The configuration is shown in Fig. 2 and will be discussed with reference to Fig. 3, which shows schematically the movement of the two pistons.
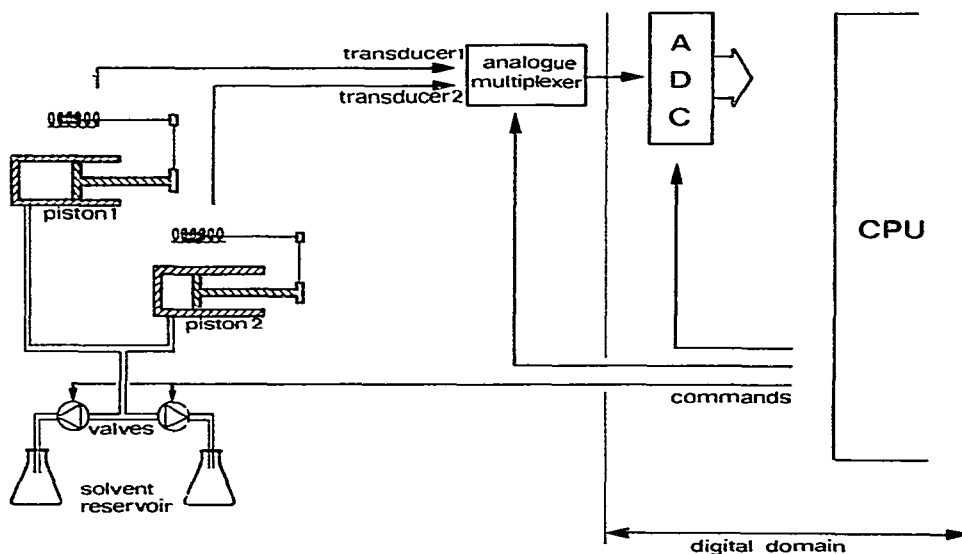


Fig. 2. Schematic picture of the microcomputer controlled single-pump solvent programmer using synchronized valve switching based on position partitioning. Two simultaneously switching two-way valves allow either of two solvents. A or B, to enter the chambers of a dual-piston pump. The position of each piston is detected by a displacement transducer, the digitized signal of which is processed by the microcomputer, indicated in the figure as Central Processing Unit (CPU), to derive the switching commands for the valves.

Let us consider piston 1 just before it ends its delivery stroke. The digitized signal from its displacement transducer is smoothed by a running eight-point averaging routine and the minimum value is stored in the memory. At the end of the refill period the process is repeated to detect the maximum position of piston 1. Because the pistons take more time to deliver the solvent than for refilling there is now a void period, where both pistons 1 and 2 are delivering. This period is used by the computer to calculate the positions of piston 1 where the valve will be ordered to switch from solvent A to B and back again. As discussed in the preceding section, these positions are located symmetrically around the midpoint. Their separation, of course, is determined by the desired composition of the minor component B present with the excess of A. Well before piston 2 reaches the end of its delivery stroke the computer is ready to accept the signals from the displacement transducer of piston 2, whereupon its minimum and maximum position are also stored in the computer memory. In the next void period the computer calculates the switching positions for piston 2.
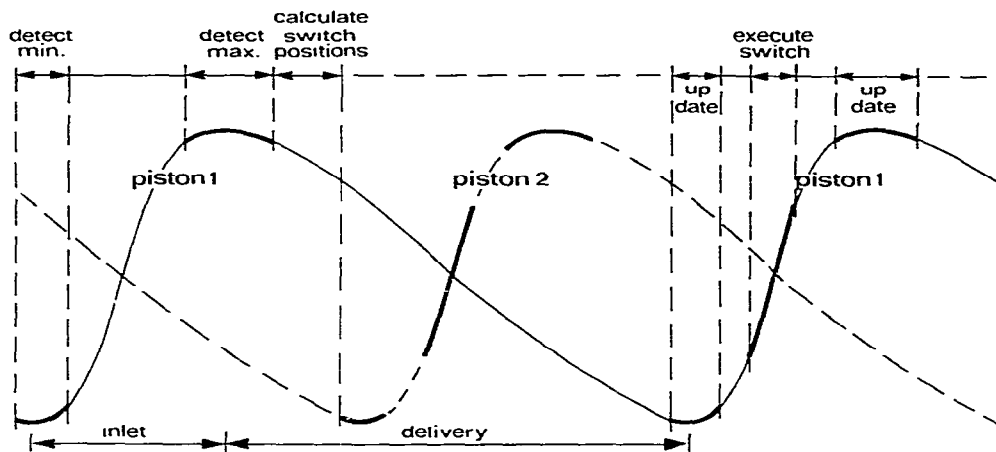


Fig. 3. Sequence of computer operations. The vertical scale represents the transducer signal, indicative of the piston position. Thick line segments represent periods during which the computer supervises the piston movement, thin line segments the void periods reserved for data processing. The sequence of computer operations in the horizontal scale at the top refers to piston 1 and is explained in the text.

Returning to piston 1, the first task of the computer is to update the minimum position of this piston at its turning from delivery to refill. After a brief void period the computer picks up the signal from the displacement transducer of piston 1 again to compare this with the first switching value calculated from the *previous* refill stroke. At the appropriate position a switching command is sent simultaneously to both two-way valves so that component B is allowed to enter the pump chamber. At the second command position the two-way valves are switched back to let solvent A enter the pump chamber again. Once again the computer is momentarily relieved of its supervising task. It then updates the value for the maximum position of piston 1, recalculates the switching positions, returns to piston 2, etc.

The continuous updating of the turning positions of the pistons corrects for drift in the transducer signal, but this drift is small enough to allow extrapolation

from one refill stroke of a piston to the next one. Possible differences between the two pistons are taken into account by using two displacement transducers.

As can be seen in Fig. 3 the total cycle time of a piston is divided into four equal parts. The first quarter is taken up by the refill stroke, the second quarter is free for calculating the switching positions and in the final two quarters these processes are repeated for the other piston. The actual duration of the periods obviously depends upon the imposed flow-rate of the solvent. The present pump is designed for flow-rates up to 10 ml/min, at which rate the piston cycle time is 1200 msec and each quarter takes 300 msec.

Because the transducer signal is sampled every millisecond, the switching points and hence the solvent composition can be determined to better than 1 %. This could be improved by increasing the sampling frequency from the present value of 1 kHz to a few kHz. Obviously, however, the sampling error is already quite insignificant at more common flow-rates of about 2 ml/min.

Similarly, at the highest flow-rate at least 300 msec are available for the computer to calculate the switching positions needed in the next refill stroke. As is clear from Fig. 3, the actual time is even longer if we take into account the two additional void times during each refill stroke. Clearly, 300 msec are abundant for the straightforward calculation of switching positions when the pump is operating in a constant delivery mode, say 30 % B and 70 % A.

In the case of gradient elution the desired solvent composition varies continuously according to an imposed time function. Linear gradients are the most widely used[6], but in order to test the system more fully, two subroutines that generate more complex gradient shapes have been written in assembler language. The first subroutine permits gradients with the general shape

$$\varphi(t) = \varphi(0) + St^n \tag{1}$$

where $\varphi(t)$ is the volume fraction of solvent B at time $t$ and $S$ the speed of the gradient. The shape of the gradient is determined by the exponent $n$, which can be selected between 1/9 (convex) through $n = 1$ (linear) and 9 (concave). The second subroutine generates gradients according to

$$\varphi(t) = (1/q) [1 - (1 - q)^{t/t_g}] \tag{2}$$

where $t_g$ is the total gradient time and $q$ has values from 0 (linear gradient) to 0.9 (convex gradient). The shape expressed by eqn. 2 has been proposed by Schoenmakers *et al.*[3].

Even at the highest flow-rates the calculation of either gradient did not interfere with the data processing of the transducer signals.

We conclude, therefore, that the proposed system is sufficiently versatile for practical HPLC.

RESULTS AND DISCUSSION

*Valve switching*

The system described differs in several respects from the design proposed in our previous publication[1]. The changes will be discussed first.

The changeover from time partitioning of the refill period to position partitioning of the piston displacement is essential for pumps using pressure feed-back. The feed-back option is designed to ensure constant solvent delivery against variable column pressure. This is realized by adjusting the piston speed at the start of the delivery stroke. As a result, the refill period of the other piston varies, depending on solvent viscosity, column deterioration, etc. Indeed, the calibration curve relating the binary composition to the switching moment is not only non-linear (Fig. 1), but also varies significantly from one solvent to another. By contrast, a similar calibration curve based on piston position is perfectly linear and independent of solvent or of column pressure.

The accurate determination of the total length of the piston stroke is an essential requirement in the present system. Initial attempts to detect the turning points of the pistons by analogue devices failed, because the derivative circuitry is very sensitive to noise in the transducer signal. The use of a microprocessor allows all software functions to be executed in the digital domain. A simple smoothing routine readily overcomes the noise problem and guarantees precise determination of the piston stroke length.

Changing the valve switching routine from A/B to A/B/A improves the mixing of the two solvents inside the pump chamber. It also decreases the period during which the transducer signal must be supervised to execute the valve switching commands. The resulting additional void time can be utilized for composition calculations or other tasks.

Either valve switching routine may be realized by a single three-way valve. However, moderately priced valves take non-negligible time to open or close. In fact, the two lag times are unequal, so that the amount of the interspaced component, B, differs somewhat from its intended value. For example, a typical three-way valve used by us switches in 8 msec to one solvent by applying a 24-V pulse, but takes 43 msec to switch to the other solvent due to a slow spring release. The difference of 35 msec is significant in relation to the total refill time varying between 300 and 3000 msec.

This problem was overcome by replacing the single three-way valve by two symmetrical two-way valves, both of the normally open type. At the start of the refill cycle valve A is open to solvent A and valve B is closed. At the first switching command valve A is closed after 8 msec, whereas valve B is opened only after 43 msec. There is thus a period of 35 msec during which both valves are closed. At the second switching command the situation is reversed: valve B closes rapidly and valve A opens more slowly, again leaving a period of 35 msec with both valves closed. This arrangement gave satisfactory results. A 50:50 mixture could be produced by either sequence A/B/A or B/A/B, *provided no chromatographic column is connected.*

*Compressibility correction*

With a chromatographic column present the delivered binary composition differs systematically from the imposed composition, as shown in Fig. 4a. Apparently, when operating against column pressure, the contribution of the interspaced minor component is larger than anticipated on the basis of the switching commands. The deviation is proportional to the concentration of the minor component, being larger for more compressible solvents, and increases with the pressure exerted by the pump during delivery.
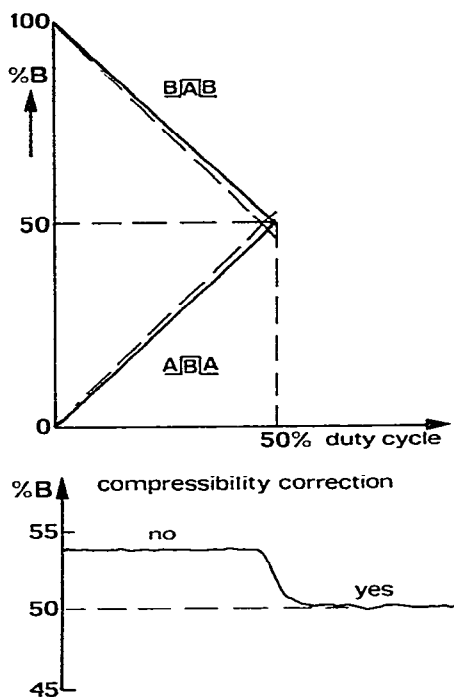
Fig. 4. Influence of the compressibility correction upon the delivered composition. a, Binary composition of a mixture of water (A) and 0.1 % acetone in water (B) as a function of the fractional separation between the two switching positions of the valves; the pump is operating against a pressure of 15 MPa. — — —, Without compressibility correction; ————, with compressibility correction after eqn. 4; the same straight lines are observed when the pump is operating at 100 kPa, *i.e.*, without a chromatographic column. b, Generation of a mixture of 50 % methanol and 50 % water against a pressure of 30 MPa, without and with correction for solvent compressibility.

The obvious explanation is that the true refill stroke experienced by the piston is less than derived from the turning positions of the piston. The influences of solvent compressibility and back pressure seem to indicate that the pump piston upon retracting from its delivery stroke does not start to refill the pump chamber immediately. Rather, the release of the pressure induces some back flow of solvent through the outlet valve of the pump chamber. Also, the decompression of the dead volume in the pump chamber and the connection tubes to the outlet and inlet valves will reduce the actual refill stroke length.

The latter effect can easily be expressed mathematically as

$$L^* = L - l \beta P \qquad (3)$$

where $L^*$ is the true refill displacement, $L$ is the stroke length derived from the turning points of the piston, $l$ is the equivalent length of the pump dead volume, $\beta$ is the compressibility of the liquid in the pump chamber and $P$ is the pressure. With this correction the separation between the two positions for the switching commands to the distributing valves becomes

$$D = \varphi L^* = \varphi L (1 - l \beta P/L) \qquad (4)$$

where $\varphi$ is the desired volume fraction of the minor component. Experimentally, the correction factor varies from about 1 % for pure water to 3 % for pure methanol at 15 MPa. This is in the ratio of the compressibilities of the two solvents. Quantitatively, however, the correction expressed by eqn. 4 can only be reconciled if $l/L$ is taken to be as large as 3. Although the dead volume of the pump is certainly less than the volume of the pump chamber (100 $\mu$l), it appears that eqn. 4 approximately corrects for both solvent compressibility and back-flush through the closing outlet valves (Fig. 4a).

The correction expressed by eqn. 4 can readily be incorporated in the computer program even for mixed solvents, provided that we know the compressibility of mixtures of variable composition. Unfortunately, such information is not available. However, crude interpolation between data for pure liquids appears to yield acceptable results. With the compressibility correction, the calibration curve becomes very accurate, as shown in Fig. 4a. This is also confirmed by the recorder trace of methanol–water (50:50) with and without the correction (Fig. 4b).

*Performance*

Fig. 5 shows a stepwise and linear variation of a binary mixture of water and methanol. Perfectly linear gradients have been obtained for flow-rates up to 4 ml/min. When the correction for compressibility is applied the accuracy of the delivered binary mixture is better than 1 % and over a period of several hours no drift in the composition is noticeable. When the pressure filter of 1.5 ml supplied with the pump is removed, the response of the system to a stepwise variation takes only 0.71 ml, which compares favourably to existing commercial solvent programmers[1].

On the other hand, removal of the pressure filter gave rise to small rapid fluctuations in the delivered binary composition, synchronous to the piston movement (Fig. 6a). The amplitude of the oscillations is proportional to the concentration of the
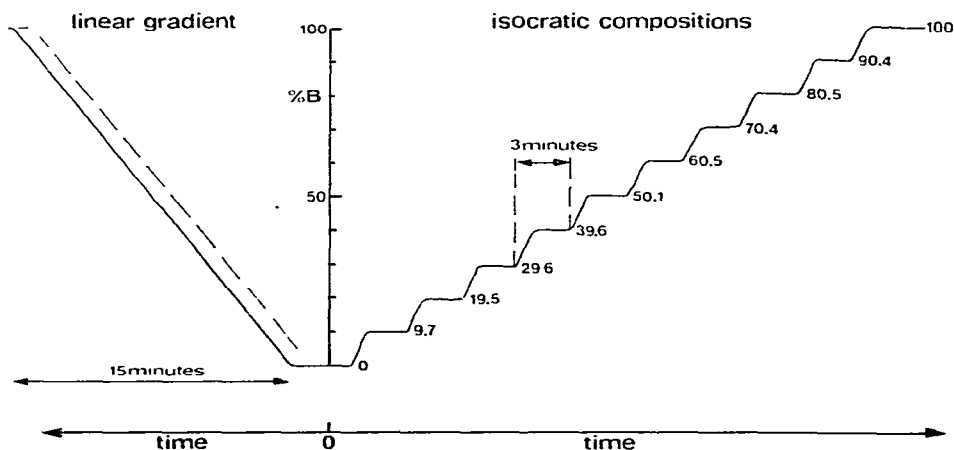


Fig. 5. Generation of linear gradient (left) and isocratic mixtures (right) against 15 MPa with compressibility correction. Flow-rate: 1 ml/min. The dashed line shows the imposed gradient and the solid curve represents the delivered gradient measured at the top of the chromatographic column. Isocratic compositions are varied in steps of 10 % and the numbers represent the experimentally obtained binary compositions.

minor component and rarely exceeds 0.1 %. Apparently, the effect is due to a slight mismatch of the two transducer units. As is clear from Fig. 6b, it is readily overcome by including the pressure filter, which acts as a mixing chamber, but obviously this lengthens the response time of the system.
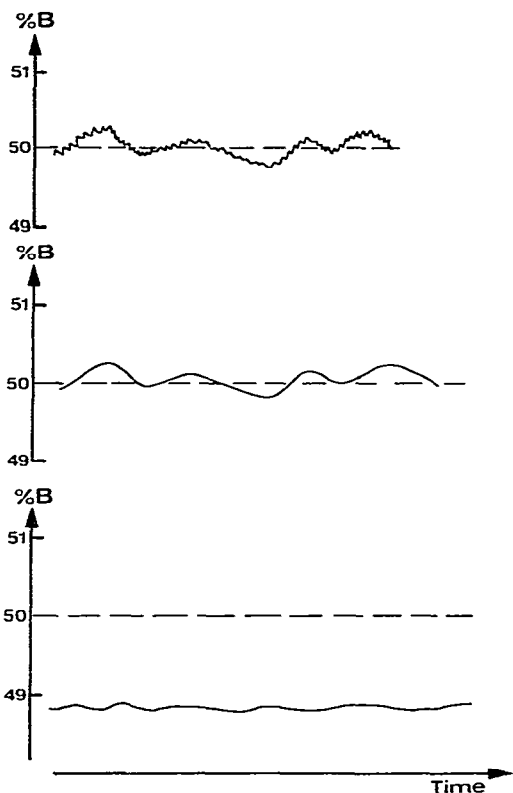


Fig. 6. Stability of the delivered composition intended to be 50 % methanol and 50 % water (worst case). a, Without pressure filter; b, with pressure filter added to the pump; c, with strong analogue filtering of the transducer signals.

Unfortunately, the displacement transducers used in our system produce a rather noisy signal. Consequently, the location of the turning points of the pistons and the switching commands becomes somewhat imprecise. Therefore, the stability of the delivered binary composition is less than aimed for. Under scale expansion, slow (1 min) variations with amplitude up to 0.3 % can be observed (Fig. 6b), which cannot be removed with the pressure filter. When the output of the transducer is passed through an analogue filter before it is fed into the ADC, the solvent composition becomes more stable (Fig. 6c), but also inaccurate because the analogue filter retards the detection of the turning points of the pistons too much. (For improved performance, see Note added in proof.)

Finally, the system was tested under true chromatographic conditions by subjecting a sample with six solutes to eight consecutive isocratic and gradient elution runs. Under isocratic conditions (acetonitrile–water, 35:65) the average standard

deviation of the solute retention times was 0.4 %. When a mixture of the mobile phase was pumped continuously no improvement in precision was observed. Under gradient elution conditions (15 min from pure water to pure methanol), the solute retention times showed a standard deviation of 0.4 % or less.

CONCLUSIONS

The equipment described has been used to demonstrate that a single-pump solvent programmer can be constructed that fulfils all current needs of HPLC. The programmer provides a rapid response and yields binary mixtures that are stable and accurate to within 1 %. With improved displacement transducers the specifications could be improved to better than 0.1 %. This is considered to be adequate for current chromatographic requirements.

It should be emphasized that the present design is largely dictated by the features of the dual-piston reciprocating pump available to us. Specifically, the pressure feed-back option necessitates the use of displacement transducers. For other pumps this need not be true. For example, pumps operating at constant motor speed could be instructed on the basis of time partitioning provided that the non-uniform inlet profile (Fig. 1) is taken into account by the computer software. Alternatively, the presently employed linear displacement transducer can be replaced by more sophisticated revolution encoders coupled directly to the rotating shaft that drives the pump pistons.

Whatever solution is adopted, the present investigation has shown that single-pump solvent programmers can be highly competitive for liquid chromatographic practice.

ACKNOWLEDGEMENTS

NOTE ADDED IN PROOF

After completion of the manuscript, the low frequency noise of the transducer signal has been significantly reduced by:
(i) Using separate power supplies for both displacement transducers.
(ii) Maximizing the transducer sensitivity with an increased impedance (20 kOhm).
As a result, the stability of the delivered binary composition has been improved from 0.3 % (Fig. 6b) to 0.1 %.

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