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MICROCOMPUTER-CONTROLLED COLUMN SWITCHING SYSTEM FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A microcomputer-controlled switching system is described that does not rely on a fixed timing sequence but uses the intelligence of the microcomputer to monitor the process in real time and make a logical decision, based on a programmed switching file stored in the memory. The system has been applied to the separation of dibenzo-*p*-dioxins from similar compounds such as PCBs and organochlorine pesticides.

INTRODUCTION

Column switching has been applied to gas chromatography (GC) for many years and the approach originally described by Deans[1] has been used for both packed and capillary column[2] separations. More recently, column switching has been applied to high-performance liquid chromatography (HPLC)[3–5] and is particularly useful for the quantification of trace components in complex matrices, *e.g.*, foodstuffs, body fluids, soil and water. The technique is usually applied to two columns in series that have different chromatographic characteristics owing to either different phase ratios or selectivity. A selected group of compounds partially resolved on the first column may be switched to the second column for further separation. In this way a higher separating power can be obtained compared with that of a single column in which separation is limited by band broadening for any given column packing. The time at which the switch is made is often critical. For example, when a specific peak (or peaks) of interest appears in an ill-resolved group or, as often happens, on the tail of a solvent peak, the switching must be accurately timed in order to avoid quantitative errors. Conventionally, the switching is done either manually by the operator or at pre-determined times by a sequential timer. The new generation of commercial computing integrators usually incorporates an external event relay interface to allow several different devices to be controlled in a fixed timing sequence during an analysis. Automatic column-switching systems demand very stable chromatographic conditions for accurate switching by timing alone. HPLC can be used for the analysis of samples without prior clean-up and extraction and in these instances the performance of the first column is often degraded. In this instance a fixed sequence is unsatisfactory.

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In this paper we describe a microcomputer-controlled switching system that does not rely on a fixed timing sequence but uses the capabilities of the microprocessor to monitor the chromatogram in real time and make a logical decision based on pre-determined parameters stored in the microcomputer memory.

EXPERIMENTAL

Fig. 1 shows a schematic diagram of the flow switching system and its links to the microcomputer.

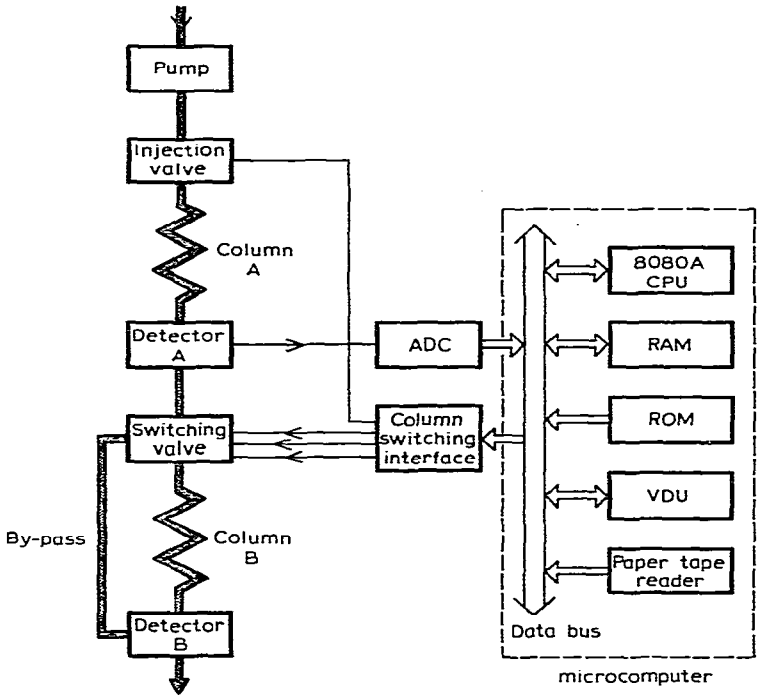


Fig. 1. Schematic diagram of liquid chromatograph and microcomputer.

Liquid chromatograph

Pump. Model 20 LC (Pye Unicam, Cambridge, Great Britain).

Injection valve. Designed and constructed in these laboratories with a sample volume of 5 μl.

Column A. 250 × 4.9 mm I.D. packed with 5-μm LiChrosorb Alox T (BDH, Poole, Great Britain) using the conventional slurry packing technique[6].

Column B. 250 × 4.6 mm I.D. packed with 5-μm LiChrosorb SI60 (BDH) using the conventional slurry packing technique[6].

Detector A. Pye Unicam LC3 UV detector. The detector cell operated satisfactorily at pressures up to 40 bar without modification. However, the manufacturers offer no guarantee that other cells will operate at this pressure.

Detector B. Cecil Instruments CE212 UV detector, the flow cell of which was required to work only at atmospheric pressure.

Switching valve. Siemens four-port, three-way valve (Model C74451-A1380-A3). This valve could be switched so that flow could be transferred from column A to either a by-pass or column B, leaving one outlet port unused in the latter instance. This valve was chosen because it can operate at high pressures (>300 bar) and has a small internal volume (15 μl). The injection valve and switching valve were operated pneumatically under microcomputer control.

By-pass. Stainless-steel tube, 400 × 0.25 mm I.D. Before entering the detector the exits from the by-pass tube and the column were joined by a low dead-volume stainless-steel Y-piece made in these laboratories.

Chemical standards

The polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated biphenyls (PCBs) were supplied by Analabs (North Haven, Conn., U.S.A.) and the organochlorine pesticides by Applied Science Labs. (State College, Pa., U.S.A.). Standard solutions were prepared in either the mobile phase or AnalaR-grade chloroform (BDH). The mobile phase, *n*-hexane (HPLC grade; Rathburn Chemicals, Walkerburn, Great Britain), was used without further purification. Care should be taken in handling these toxic compounds and in particular the PCDDs.

Microcomputer

Hardware. The microcomputer was constructed from a set of cards based on the Intel 8080A microprocessor (Philips, Science and Industry Group, Eindhoven, The Netherlands): 4K bytes of programmable read-only memory (PROM) were used for storing the system monitor and a number of commonly used subroutines, *e.g.*, floating point arithmetic and input–output procedures. Also 12K bytes of random access memory (RAM) were available for storing programs under development. The microcomputer was interfaced to conventional peripherals including a video display unit (VDU), paper-tape reader and teletype. In addition to those modules indicated in Fig. 1, a real-time clock was also used and elapsed time was displayed on a seven-segment display. Eight light-emitting diodes were also incorporated in the front panel to display the status of programmed variables such as the control byte, which will be described later.

An interface between the microcomputer and the pneumatically operated injection and column-switching valves was constructed to allow the solenoid valves (which operate the pneumatics) to be energized by solid-state relays which were operated by an undecoded 8-bit control byte output by the microcomputer. Hence, up to eight solenoids (or any other control parameter) were under software control from the microcomputer. In this particular chromatographic arrangement (as in Fig. 1), a maximum of four bits were used to switch the injection valve and to select one of the three outlet ports of the Siemens valve. The specific separations described in this paper utilized only two of the three outlet ports but a third column having different chromatographic properties from both columns A and B, *e.g.*, a chemically bonded phase, could be incorporated to carry out a further type of separation.

Analogue-to-digital converter (ADC). Chromatographic signals from the LC3 UV detector (detector A) were digitized using a voltage-to-frequency converter and 16-bit counter. The signal input was buffered using a low-noise, low-drift amplifier, which also provided switchable gains of 1, 10 and 100 (to suit different detector out-

puts). The voltage-to-frequency converter had a full-scale frequency of 1 MHz and a usable dynamic range of *ca.* 10^5, which is compatible with most chromatographic detectors. Additionally, noise is reduced by the integrating technique. Hence, this type of ADC is suitable for conventional chromatographic data acquisition and data processing[7], *e.g.*, integration of peak areas, as well as the application described here.

Software. Software development for microcomputers can be extremely time consuming and hence expensive if one is forced to work in a very low-level language, *e.g.*, directly in machine code. Our laboratories have extensive support for the 8080, including facilities for software development in either assembly language or a high-level language (a sub-set of Coral 66) using either the multi-access laboratory computer (ICL 1904S) or an Intel MDS microcomputer development system. For this work, most software development was carried out on the main laboratory computer and output in machine code on paper tape, which was then loaded into RAM using the high-speed paper tape reader. Programs could then be run, debugged, altered, etc., in an iterative manner using the system monitor resident in PROM.

The program operated in an interactive mode and presented the operator with options to override a pre-set series of parameters whenever necessary. These parameters were the time of sample injection, duration of sample injection pulse, the time window in which data was searched for a particular function (σ) of the chromatographic signal and the time taken for complete elution of components from the second column.

The function searched for, *e.g.*, valley detection, signal level, peak number or group separation, determined the initial valve switching position and specified the appropriate bit combination of the control byte at each stage of the operation.

The real-time clock operated at 10 Hz and latched the 16-bit count from the ADC into a register, re-set the counter and gated the data from the register on to the data bus as two 8-bit bytes on receipt of the device select signal. The counter continued incrementing until receipt of the next clock pulse, when it was re-set. The data acquired in this way were subsequently smoothed to prevent false detection of a switching function. The degree of smoothing necessary is dependent on the nature and level of the noise present in the data but in this work it is not as critical as in data processing where the peak shape is adversely affected by incorrect data smoothing. For detection of switching functions, we have found that data summation over a group of ten points, followed by a moving average filter over five points[8], is sufficient for most applications. However, the overall bandwidth of the filter (*ca.* 5 sec) could easily be adapted to different signal conditions such as those produced by very rapid peaks.

The program maintained a memory of the five most recently acquired smoothed points and this series was continually up-dated in a first-in, last-out buffer in RAM.

RESULTS AND DISCUSSION

Automated column switching based on pre-set times has been reported for capillary column GC[2] and HPLC[5]. If progressive degradation occurs in the first column due to the nature of the sample, then a fixed timing sequence needs to be continually up-dated in order to allow for this, otherwise significant loss of the component of interest may occur during its transfer to another separation process.

Some events in an automated column switching sequence are not critical, *e.g.*, injection and flow switching when the separation is ended, and for these events a fixed time (T) is adequate. However, if after separation on the first column one wishes to select one peak out of a complex group of peaks (heart cutting) and transfer it to another column for further separation, it is more accurate to make a real-time decision based on a well defined feature of the separation obtained in the first column. To do this, it is necessary to monitor the eluent from the first column by having a non-destructive detector in-line between the two columns. We found that the Pye Unicam LC3 flow cell could withstand pressures of up to 40 bar without leaking, which was typically more than the pressure existing between the two microparticulate columns at the flow-rates we were using. The signal from this detector is continuously monitored for a pre-defined function (ø), *e.g.*, a valley between two peaks, during a given time window (ΔT). Only if both the function and the time window are "true" will the switching occur, *i.e.*, when the logical operation, ø AND ΔT, is true. For a particular separation, a programmed switching sequence can be constructed that consists of both fixed-time events and variable-time events dependent on software interpretation. Table I shows a typical sequence that would be used for heart-cutting.

TABLE I

TYPICAL PROGRAMMED SWITCHING SEQUENCE

*Control byte**	*Function*	*Time/window*	*Description*
010	—	T_0	Standby configuration through column A and by-pass
011	—	T_1	Start injection } on column A
100	—	T_2	Finish injection } on column A
100	$ø_1$	$.1T_1$	Locate start of peak(s) and switch to column B according to function detection via detector A
010	$ø_2$	$.1T_2$	Locate end of peak(s) and switch remainder of sample through by-pass to detector B
100	—	T_3	When all components have eluted from column A, switch back to column B where the heart-cut peaks have been stored
010	—	T_4	End of sequence and back to standby

* Control byte: bit 0, injection valve on
bit 1, switching valve to by-pass
bit 2, switching valve to column B
} Action for specified bit high.
bits 3–7, unused.

Such a switching sequence is exemplified in Fig. 2, which shows the separation of a mixture of PCBs, DDE and DDT. Fig. 2a shows the separation obtained on an alumina column alone, and Fig. 2b, c and d show the consecutive heart cutting of different individual peaks, which were then transferred to the silica column where further separation was achieved. It can be seen that efficient heart cutting results since the composite chromatogram that would be produced by the sum of each individual heart-cutting operation is very similar to that of the chromatogram of the total sample through both columns in series (Fig. 2e). The first part of the chromatograms in Fig. 2b, c and d should be compared with that in Fig. 2a, where it will be seen

that the transferred peak is absent. Peak broadening in a column-switching system can be kept within acceptable limits provided that attention is paid to minimizing the dead volume in all column connections and valves[9]. This separation was obtained by defining σ_1 and σ_2 as valleys between peaks and these were identified in a simple manner by comparing consecutive filtered data points x, y and z such that they fulfilled the condition $x > y < z$. Alternatively, the first differential of the signal could have been calculated using a Savitzky and Golay[8] least-squares smoothing function and the valley identified when the differential passed through zero.

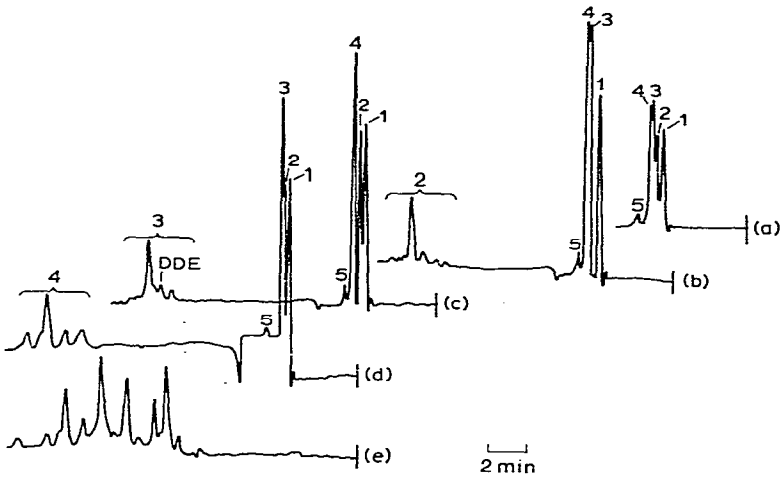


Fig. 2. Separation of a complex mixture of PCBs, *p,p'*-DDE and *p,p'*-DDT. Solvent, *n*-hexane; flow-rate, 1.5 ml/min; wavelength, 230 nm; attenuation detector A, 0.16 A.U. and detector B, 0.05 A.U. (a) Chromatogram from detector A after separation on alumina column. (b) Chromatogram from detector B. Peaks 1, 3, 4 and 5 through by-pass and peak 2 through silica column. (c) As (b) except peaks 1, 2, 4 and 5 through-by pass and peak 3 through silica column. (d) As (b) except peaks 1, 2, 3 and 5 through by-pass and peak 4 through silica column. (e) Chromatogram from detector B after the total sample undergoes separation through the alumina and silica columns in series. Peaks: 1, 2, 4 = mixed PCBs; 3 = PCBs + *p,p'*-DDE; 5 = *p,p'*-DDT.

Another common need is to switch a peak or group of peaks that is the first or last in a chromatogram. In this instance the location of a function related to a valley may not be applicable and switching is more convenient at a threshold signal or level above the baseline. An example of this is shown in Fig. 3, where both σ_1 and σ_2 were defined as the same threshold level. At S1, peak 1 is switched to the silica column and at S2 flow is switched back to the by-pass. The remainder of the peaks eluting from the alumina column are detected until at S3 the flow is switched back to the silica column where peak 1 has been stored, and this peak now undergoes further separation on the silica column.

Some chlorinated dibenzo-*p*-dioxins are highly toxic and a number of accidents have resulted in environmental pollution. The analysis of samples for these compounds is complicated by interfering compounds such as PCBs. We have recently reported the group separation of chlorinated dibenzo-*p*-dioxins from most other

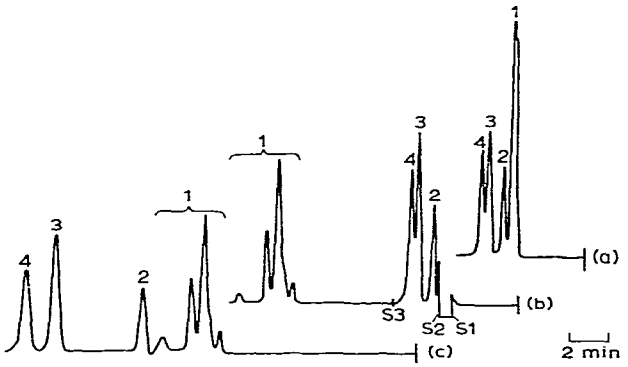
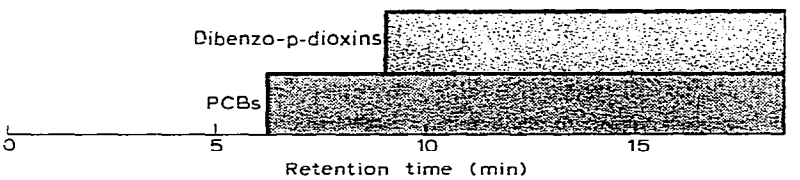
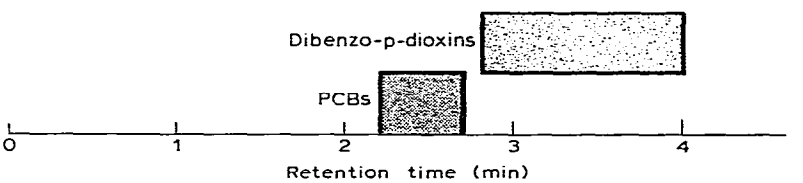


Fig. 3. Peak switching by threshold level detection. Conditions as in Fig. 2 except attenuation: detector A, 0.16 A.U.; detector B, 0.1 A.U. (a) Chromatogram from detector A after separation on alumina column. (b) Chromatogram from detector B after "cutting" peak 1 from alumina column and transferring it to silica column for further separation. (c) Chromatogram from detector B when total sample is separated on alumina and silica columns in series. Peaks: 1 = PCBs; 2 = *p,p'*-DDE; 3 = dibenzo-*p*-dioxin; 4 = *p,p'*-DDT.

chlorinated congeners by HPLC on a microparticulate alumina column[10]. Fig. 4 illustrates the group separations achieved on such a column compared with a very marked overlap achieved on a silica column. However, a silica column resolves chlorinated compounds according to the degree and position of substitution and having carried out a group separation on alumina the separation could be enhanced by additional separation on a silica column. Column switching is ideal for this type of application and Fig. 5 shows the separation of dibenzo-*p*-dioxins from PCBs, DDT and DDE using this technique. The chromatogram in Fig. 5a shows the group sep-



(a) Silica column



(b) Alumina column

Fig. 4. Retention characteristics of dibenzo-*p*-dioxins and PCBs on alumina and silica columns (based on results in ref. 10).

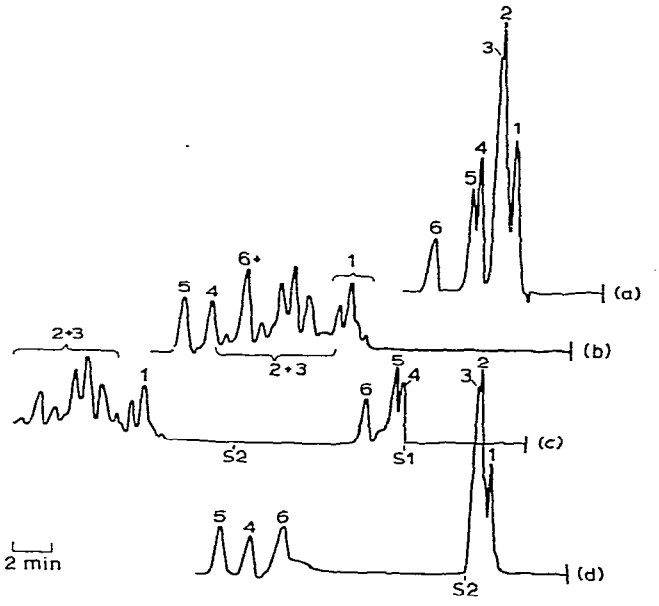


Fig. 5. Separation of dibenzo-*p*-dioxins from PCBs, *p,p'*-DDT and *p,p'*-DDE by column switching. Conditions as in Fig. 2 except attenuation: detectors A and B, 0.02 A.U. (a) Separation on alumina column only (detector A). (b) Separation on alumina and silica columns in series (detector B). (c) PCBs and *p,p'*-DDE selectively transferred to silica column for further separation (detector B). (d) Dibenzo-*p*-dioxins and *p,p'*-DDT selectively transferred to silica column for further separation (detector B). Peaks: 1, 2, 3 = PCBs + *p,p'*-DDE; 4 = dibenzo-*p*-dioxin; 5 = *p,p'*-DDT; 6 = octachlorodibenzo-*p*-dioxin.

aration on alumina and then there is the option of either selecting the PCBs group or the dibenzo-*p*-dioxin–DDT group for transfer to the silica column as shown by the chromatogram in Fig. 5c and d. Positions S1 and S2 indicate where switching occurred to the by-pass and second column (silica), respectively. In this instance the critical switching event between peaks 3 and 4 was identified by a valley function. The chromatogram in Fig. 5c shows how a PCB could be identified by its elution profile[11]. The dibenzo-*p*-dioxins and PCBs fall within the same retention range through both columns in series which follows the same pattern as through the silica column alone. However, from the chromatogram in Fig. 5d it is evident that by column switching the dibenzo-*p*-dioxins can be separated from the PCBs, DDE and DDT. We have previously shown[10] that the dibenzo-*p*-dioxins are well separated from other common organochlorine pesticides by the alumina column. The column-switching method described here is therefore a useful technique for screening environmental samples that are likely to have many interfering compounds present. For applications that require higher sensitivity or selectivity, the method could be used for group separation prior to concentration and additional analysis by another technique such as GC–MS.

The use of a microcomputer to control switched events in an intelligent manner should lead to more reproducible results and improved accuracy for difficult analyses in which column switching is used.

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