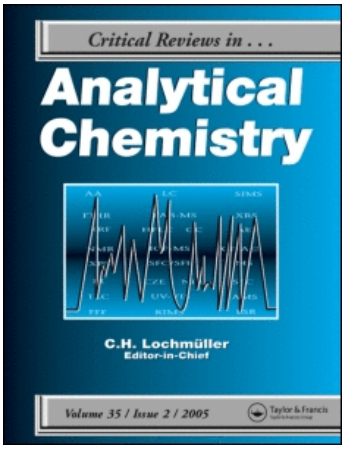


Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

Computerized Multiple-Input Chromatographic Analysis of Time-Varying Substance Flows

M. Koel[a]; M. Kaljurand[a]
[a] Department of Analytical Chemistry, Institute of Chemistry, Tallinn, Estonia

To cite this Article Koel, M. and Kaljurand, M.(1996) 'Computerized Multiple-Input Chromatographic Analysis of Time-Varying Substance Flows', Critical Reviews in Analytical Chemistry, 26: 2, 149 — 194
To link to this Article: DOI: 10.1080/10408349608050570
URL: http://dx.doi.org/10.1080/10408349608050570

Critical Reviews in Analytical Chemistry, 26(2&3):149–194 (1996)

Computerized Multiple-Input Chromatographic Analysis of Time-Varying Substance Flows

M. Koel and M. Kaljurand

Department of Analytical Chemistry, Institute of Chemistry, Akadeemia tee 15, Tallinn EE0026, Estonia

ABSTRACT: This article reviews the application of chromatography analysis to time-varying material flow in chemical reactions and dynamic extraction and desorption studies. Special focus is given to those cases where a variation appears due to various novel approaches to the sample input method. Use of random and regular multiple-input sequences in chromatography are discussed and clarifying examples are presented.

KEY WORDS: flow analysis, process monitoring, chromatography, correlation chromatography, instrumentation, computer methods.

I. INTRODUCTION

One promising application of chemometrics to analytical chemistry is a fundamental redesign of common analytical procedures and methods driven by developments in signal processing. Basically, one could develop completely new analytical methods using theoretical bases provided by the data-processing capabilities of computers. Compared with data processing, computerized chromatographic-input control has received little attention beyond routine sampling-valve actuation. In the interim, other analytical techniques have benefited greatly from a radical redesigning of signal generation, signal input, and data processing via computerization (e.g., FT-NMR). Most computer applications to chromatography have been a rather straightforward imitation and automation of an average "chromatographer's" labor. The built-in microprocessors simply set proper mobile-phase velocities, set column temperatures, detect peaks, and calculate peak areas. Although contemporary instruments are highly sophisticated in construction, few new ideas for performing the measurement tasks have been developed. Recent achievements in data processing using chemometrics methods have had little influence on commercial chromatographic equipment design. The current state of commercial art would suggest a human inability to interpret chromatographic results other than as a sequence of well-separated peaks. Were this true, all chromatography would involve the use of a sample in the form of a narrow concentration impulse in the entrance of the column. On the other hand, the practice and the achievements of modern spectrometry clearly suggest that new directions should exist for chromatography as well. The computer can help interpret much more sophisticated patterns using modern signal processing methods (e.g., signal transformations, the results of coding and system identification theories, factor analysis, or even pattern recognition). All these mathematical developments are central to contemporary chemometrics. Extensive software packages exist to be used

1040-8347/96/$.50

by an analyst. As a result, the historical, strict requirement for the traditional single impulse input is removed and new frontiers for measurement and measurement application are revealed.

Given the opportunities, one can predict an extension of the application of chromatography to new areas via novel approaches to the sample input procedure. To date, the most challenging of these extensions has been chromatographic analysis of time-varying substance flows. Such flows appear, for example, in chemical reactions, dynamic extraction or desorption studies, and are being explored presently by rapid-scan spectrometric instruments. Analysis of these flows implies the construction of time-dependent concentration curves of sample components. Such curves can be built using measured peak areas with interval Δt.

Frequently, the term *monitoring* is applied in connection with time-varying flows. This term suggests "instantaneous", on-line information about the sample. Techniques such as Kalman filtering are applied to on-line monitoring of the time-varying flows. In this review, we devote more attention to *the post-processing* of the recorded output. Emphasis is on reconstruction of the processes involving the sample (what happened? vs. what is happening?) and thus we prefer the term *analysis* instead of monitoring. This does not imply that only analysis is possible using the methods described; on the contrary, monitoring is in many cases possible as well.

Analysis itself is the following of the process by using several sampling procedures or inputs into the chromatographic column during the overall process running time. By its nature, it means dealing with multiple-input chromatographic methods. Computerized multiple-input chromatography can provide its own solutions that frequently do not surrender even to expensive spectroscopic methods. This is important because chromatography is a less-expensive and more rugged/field-reliable method than, say, mass spectrometry. Over the last decade, several research groups have been actively engaged in developing computerized, multiple-input chromatographic experiments. Happily, considerable improvement, expressed either as a decrease in detection limits or an increase in the selectivity of a method of analysis, has been shown.

The output of these kinds of methods is usually a data structure giving the instrumental response as a two-dimensional (2D) response surface that will later become a subject of application of statistical and mathematical methods. The aim of this paper is to give examples of the new possibilities opened up by using the computer to control the chromatograph (i.e., to demonstrate how 2D data for chemometric analysis can be produced using chromatography).

The first results of computerized, multiple-input chromatography are presented in Reference 1. In the last several years, this field has been actively explored, and new results have been obtained concerning theory, methodology, and application. These results are reviewed as well.

II. CHARACTERIZATION OF TIME-VARYING SUBSTANCE FLOWS FOR THE CHROMATOGRAPHIC ANALYSIS

When searching for new fields of application for chromatography, one must study the potential and fundamental parameters of chromatographic measurement systems. Signal processing is a field concerned with estimates of the inherent parameters of the instrument used and manipulations of analytical responses of a system and treatment of the output signal. The goal is to make the information content more accessible to the analyst. Responses of the measuring system to different types of input signals are a potential source of information for characterizing it. The historic narrow impulse, single-injection method is very well suited for studies of time-invariant samples. In what

follows, various types of input signals used in time varying process studies are considered.

A. Sampling and Detection of Time-Varying Substance Flows

Each step of an analysis requires some time to get the result; therefore, the analysis procedure is *discrete in time*. This is true even for seemingly "continuous" analytical instruments (e.g., monitoring a single spectral line or band with a spectrometer) because of the time constant of the instrument. This demonstrates the importance of the parameter called *analysis time*. Generally, analytical chemistry treats many of its objects of analysis as stationary in time (i.e., the concentrations of the components in the sample do not change during the measurement process). Then, there is no need to take into consideration the time course of the analysis time. However, if one wants to determine the dynamics of a chemical reaction, dynamic extraction, or desorption, the *sampling* (or measurement) *rate* directly related to analysis time must have a certain relationship to the *process rate* as well. Thus, when considering objects for study whose component concentrations change, the most important parameter of this kind of analysis is how many measurements per time unit an analytical instrument is able to process. This time unit determines the rate of changes in the flow of the sample of interest that can be studied by a given instrument. The relationship between the sampling interval, Δt (here called time resolution) and the functional form of a process is generally given by the sampling theorem that states that the value of the sampling frequency (i.e., $1/\Delta t$) must be twice that of the highest frequency, f_m, in the signal:

$$1/\Delta t > 2f_m$$

Calculations are possible only for some simple functions. For example, in the case of the Gaussian curve, 2 to 4 points per peak half-width are necessary to reconstruct the curve with a precision of about 0.01%.[2] If the statistical parameters of the noise on the signal are known, the precision of curve reconstruction can be set equal to the standard deviation of the noise to find the required sampling rate. The influence of a correct and an invalid sampling interval on the curve reconstruction process is shown in Figure 1.

Although the sampling theorem is the only firm theoretical background for sampling interval estimation, its practical value is nevertheless limited because the functional form of flow changes (process) are usually not known beforehand and the correct time resolution must be found by trial and error. It is also evident that with decreasing time resolution, the measurement cost increases because the number of measurements increases and, when the time resolution of a particular method is exhausted, a new measurement principle (possibly more expensive) should be implemented to study a particular signal of interest. As in traditional chromatography, the time resolution (sampling interval) of traditional chromatography is determined by the time of separation taking place in the column. Depending on the mode, this time varies from 1 to 10 min or longer for gas chromatography (GC) and high-performance liquid chromatography (HPLC). Using special sampling devices in GC and narrow-bore capillary columns, Gaspar[3] and Schutjes et al.[4] demonstrated that the separation of simple mixtures lasted for a few seconds.[3,4] The same magnitude of extremely fast separation was demonstrated by van Es et al.[5] This seems to be an ultimate speed of this kind of analysis. Other practical aspects of high-speed chromatography are discussed in Reference 6.

A second limiting factor is the detection limits for a particular application. Different chromatographic detectors can detect substances starting from 10^{-6} g and higher in the case of a thermal conductivity detector, or the counted number of molecules, as proven

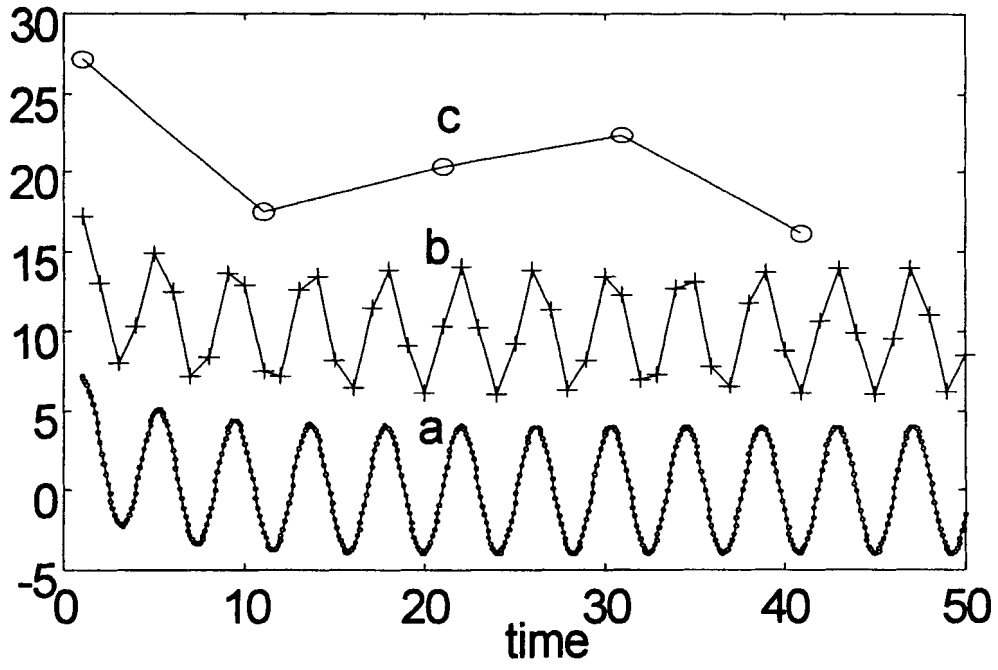


FIGURE 1. Effect of time resolution of sampling on reconstruction of sampled function demonstrated on simulated curve. Points on the curve indicate the moment of sampling: (a) continuous function, (b) adequate time resolution, (c) incorrect time resolution.

in capillary electrophoresis with a laser fluorescent detector;[7,8] however, such very specific, sensitive detectors are usually very expensive as well. This best-detection-possible value combined with the actual detector noise level sets the detection limit of the instrument. The two most important parameters driving instrument choice in the case of process analysis are possible sampling interval and detection limit.

Another possible approach to make use of chromatography for the analysis of rapidly changing sample flows is to inject multiple samples during the analysis in such a way that the new samples are introduced to the column before the complete elution of previous samples. This approach has been of interest since the very beginnings of chromatography. The attractive characteristics of this approach are the possibility of using standard commercial equipment and commercially available automatic sampling devices. In this type of setup, the chromatographic bands of the different samples are certainly overlapped, but when the input sequence is carefully chosen there exist computational techniques for the deconvolution of the concentration curves of sample components from the detector signal.

B. Classification of Time-Varying Flows

It follows from the discussion above that when using chromatography as a method of characterization, all time-varying substance flows can be divided into rapidly or slowly changing ones, depending on the time required for the chromatographic separation of components in the column. If the time resolution of the process exceeds the time required for the separation of flow components, then we call the flow a *slowly changing flow*, and the flow component concentration changes in time can be exactly

reconstructed using component peak areas. Otherwise, we call the time-varying flow a *rapidly changing flow.*

Flows can also be characterized on the basis of those of a high or low concentration of components, depending on the detection limit of the detector used by a chromatographer. If all of the time-varying flow components can easily be detected with a particular detector, we call the flow *a high concentration flow.* When the opposite is true, the flow is *a low concentration flow.*

Taking $\Delta t = 10$ s and recalling the requirements set on time resolution by a Gaussian peak, we can conclude that the reliable analysis of substance bands with a half-width narrower than tens of seconds is hardly possible. If the required detection limit is too low for a given detector and sample, the situation can be improved by concentrating the sample. This works well for stationary samples. When the sample composition changes rapidly, the concentration procedure is often not possible or too inconvenient — requiring very many concentrating units to be filled during a given analysis time — to follow this change.

Using a new, computerized approach to a chromatographic input, the time resolution and detection limits can be decreased significantly without a drastic redesign of the chromatographic equipment. Table 1 lists the input methods developed in computer chromatography together with the time-varying processes investigated so far. These methods are discussed in the following paragraphs.

C. The Time-Varying Flow as a Two-Dimensional Object of Analysis

Analysis of the time-varying flow results in a set of chromatograms. The intensity of peaks on different chromatograms expresses the concentration changes of different components involved in the process at particular moments of sampling. If the chromatograms are arranged according to the running time of the process, the response surface in space is obtained. It means that, depending on two independent variables (the chromatographic running time and process running time) the result can be considered two dimensional. Using the terminology of Kowalski et al.,[9] these instruments are called second-order instruments, and these generate second-order data. Such 2D data cannot be very easily analyzed manually, but computerization in the data handling of the re-

TABLE 1
The Sampling Methods Used in Multiinput Computer Chromatography and the Processes Investigated

Component concentration	Rate of component change	
	Slow	**Rapid**
	Equiinterval	**Stroboscopic**
High	2D chromatography Thermochromatography	Catalytic reactions Ignition of organic substances
	Random	**?**
Low	Evolution of polymer additives Monitoring of organic pollutants	No applications yet

sults opened new opportunities for 2D data representation using the algorithms developed in the graphics-*emphasis* areas of computer science. The surfaces can easily and conveniently be represented in a computer screen at different angles of view with hidden lines removed, and thus the whole process can be obtained by an analyst. This form of data representation is usually called a stack plot. Another important method of 2D data representation is a contour plot. Examples of the stack and contour plot are presented in Figure 2. Chromatographers working with diode array detectors in LC are familiar with this kind of data processing and representation. In this case, two independent variables are the chromatographic running time and th wavelength of the spectra.

Thus, treating the flow analysis as a two-dimensional task gives the advantage of a clear overview of the process. This approach may be elaborated even more by abstract or target factor analysis of the output data. This approach enables one to suppress data several times without sacrificing the information obtained in measurements, and find the number of overlying parameters that determine the process run. Some examples of the 2D flow data treatment are given in Section VI.

III. THE PRACTICAL IMPLEMENTATION OF MULTIINPUT COMPUTERIZED CHROMATOGRAPHY

A. Interfacing Computers to Chromatographic Experiment

To realize the goal of computerized experimentation, the computer must be able to control several functions of the chromatograph work and acquire data from detectors and sensors as an ordianry task of automation. Different ways of realizing a particular interface construction have no principal significance from the point of view of computerized experimentation — the desired aim can be achieved by different setups. For example, the development of a large number of suitable interfaces for the modern IBM PC family of computers has opened new ways of using them as flexible and powerful measurement and control devices;[10,11] even more they have become integral to chromatography.

Typical interface devices required in computerized multiinput chromatography are an analog-to-digital converter (ADC) for recording the detector signal and a digital-to-analog converter (DAC) to obtain control signals for relays.[12] The relays output current, which, in turn, controls the solenoid valves used for direct sampling of the probe, or the valves used for controlling the pressure on the mechanical valve actuators. In addition to solenoids, the other electrically driven output devices can be controlled. Thus, the interface port of computerized input chromatography is usually rather sophisticated.[13]

As a typical example of the interfaces used in computerized chromatography, the authors present the thermochromatographic equipment used presently in their laboratory (Figure 3). In this system, the pyrolysis reactor temperature is controlled by an IBM PC by reading the reactor temperature from a thermocouple, and the computer regulates the reactor temperature by applying current pulses to the heating elements. Sampling is done by the pneumatic switch controlled by the solenoid valve. The thermocouple signal and two detector signals are read by an ADC. The equipment is supposed to sample the gases evolved from the solid probe heated under the linearly programed temperature to the chromatograph and record the detector signal at fixed time moments. If the pneumatic switch has to be replaced by the mechanical valve, the interface chain will require a valve actuator as well.

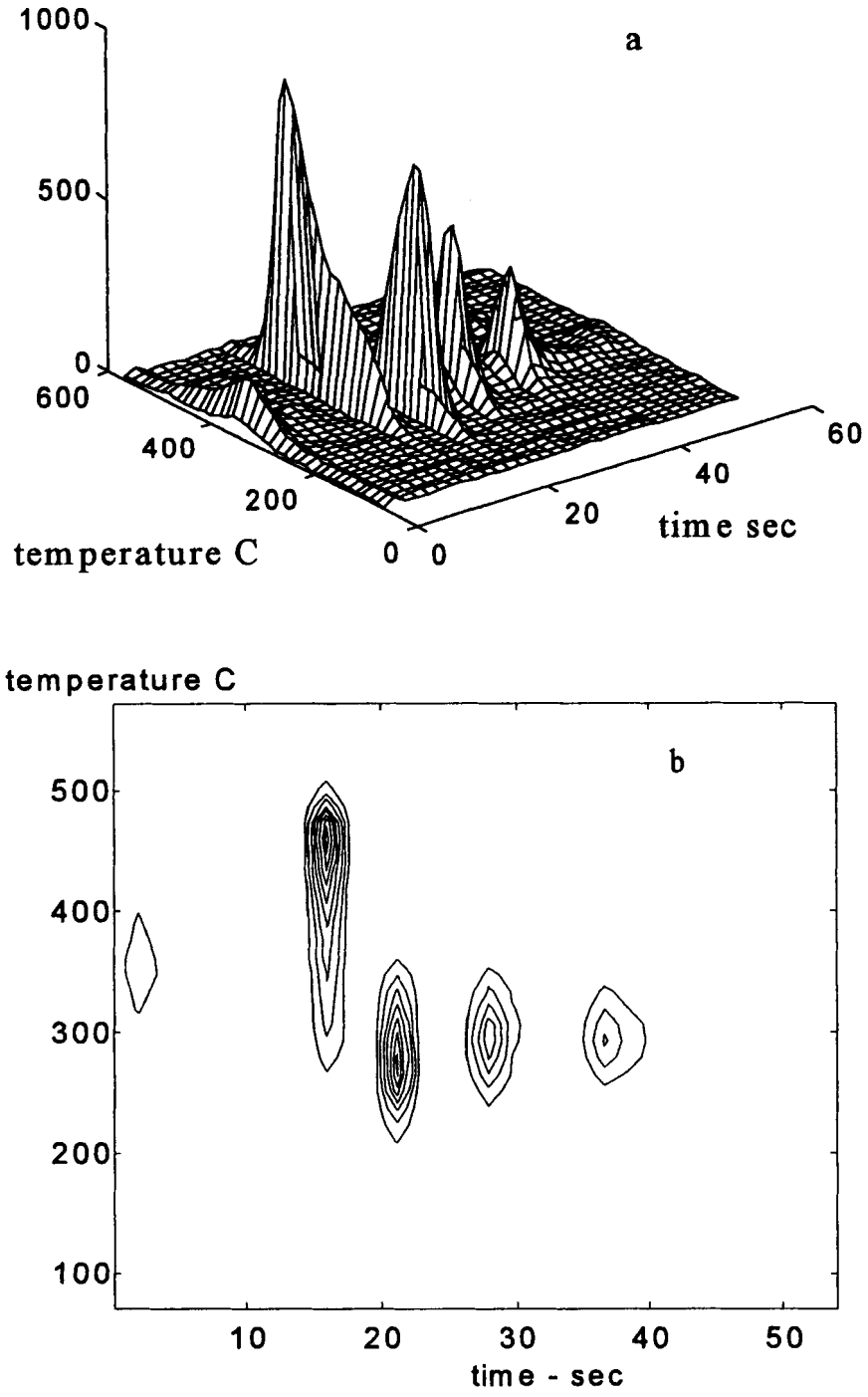


FIGURE 2. 2D representation of data from the chromatographic separation of headspace gases at certain temperature during heating of natural polymeric sample (70 to 500°C, with 15°C/min rate in inert atmosphere). (a) Stack plot called thermochromatogram; (b) Contour plot of same.

B. Input Devices

Electronic circuits for interfacing are usually commercially available. There are A/D converters available on the chromatographic equipment market capable of simultaneously recording signals from several detectors and with built-in event control

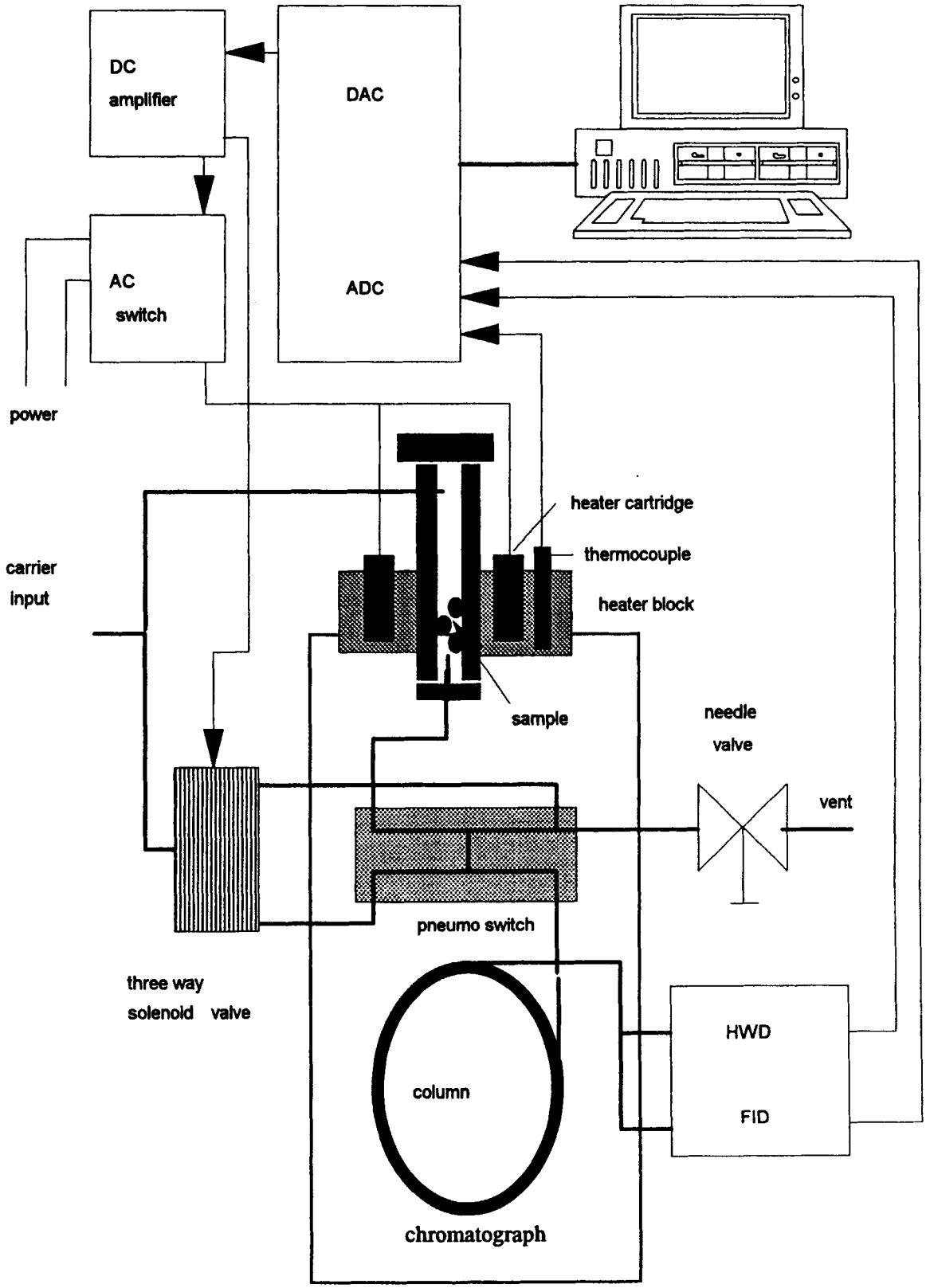


FIGURE 3. Schematic of thermochromatographic equipment as an example of computerized chromatography.

possibilities. This makes it easier to assemble the interface setup for single-input computerized chromatography. In contrast, the input devices used in multiinput chromatography are frequently unique, special-purpose devices. An important requirement is that the computer control of input devices must be easy. That is why a common syringe is not the best device to be used in computerized chromatography. Input devices must also guarantee required accuracy and precision. The latter requirements are even more severe in multiinput computerized chromatography than in common chromatography, as shown below.

Autosamplers have a complicated mechanical construction, with a syringe used as an injection instrument and a rigid working program with a long duty cycle not very suitable for computerized chromatography. If the syringe is fixed to the hand of a laboratory robot, a rather flexible input device is obtained.[14,15] The advantage of this system is that almost no reconstruction of the equipment available is needed to realize a large variety of computerized chromatographic experiments. Of course, the robotic cost becomes part of the cost of the analytical result and must be weighed as such.

Mechanical rotary valves have undergone a long period of development, and at the present time, several companies can provide valves able to withstand temperatures up to 300°C and pressures up to 50 MPa. They can also be applied to, for example, correlation chromatography, which requires thousands of injections to be performed during one experiment.[16] There is the "wear and tear" associated with any moving component device and the cost of maintenance. The more important consideration is that the switching time of a mechanical valve can become critically long in some applications.

Pneumatic switching valves are also popular in gas chromatography. There are two approaches. One uses the switching devices developed in fluid automatics or "fluidics".[17,18] The fluid switches are very rapid but consume much carrier gas. The second approach is based on the pressure-balancing principle proposed by Deans.[19] Several chromatographic equipment-making companies (e.g., SGE and Chrompack) produce pneumatic switches. The latter can also be locally made in a few hours following the original description.[20] Flow rates in pneumatic switches must be carefully balanced; otherwise, the switching will not take place. This is a real disadvantage of this input device. Any change in the flow rate in some part of the equipment (e.g., when replacing a column) requires a new measurement and setup of flow rates in the system. Another disadvantage of this input device is that the sample is diluted with carrier gas in the switch's flow channels. The magnitude of dilution is not known precisely. Extensive studies on the performance of this sampling device design have not yet been done, and no engineering-mathematical model exists. The switching time can be decreased to 50 ms, and this is a powerful advantage of the Deans-type switch.

Recently, J. B. Phillips proposed a new approach to the sampling in chromatography. Let us assume that the sample flow is directed through the column. If at a certain time moment a physical or chemical action is applied to the flow for a short period of time that removes a sample from the flow, the sample deficiency will undergo a common separation process and a vacancy chromatogram at the end of the detector will be obtained. The physical and chemical processes used so far for sample modulation are thermal desorptive,[21,22] spark,[23] electrochemical,[24,25] thermal-destructive hot wire,[26] optical,[27] and oxidative.[28] The idea of using modulation in analytical chromatography is very promising because there is a hope of constructing the input device with no moving mechanical parts. The performance of several modulators has been studied extensively by Engelsma.[29] Two drawbacks of

modulators are that they do not react immediately to the input excitation and there are memory effects. The action of modulation can be very compound specific and selective (sometimes this can also be considered an advantage). In modulators involving some chemistry, new compounds are generated that may lead to confusion in interpreting chromatograms. In general, the performance of chemical modulators requires further study before their extensive application can be recommended.

The characteristics of the input devices described in Section 3.B are summarized in Table 2.

C. Software

No universal, ready-made software is available for computerized chromatography; every investigator must write or compose his own using commercial tools. One possibility is using LabVIEW[30]from National Instruments. Some important conclusions about the programing and software based on the authors' experience are presented below.

The rates of detector signal digitization and input device switching in computerized chromatography are not very high, which means that not very sophisticated programing is needed to design these computerized systems. High-level programing languages can easily be used for this purpose, with common facilities for programing the interface (RS-232, IEEE488, or directly through the computer bus).

The software of multi-input computerized chromatography can be divided into three modules:

- The generator of a particular input sequence. Depending on application either (1) a pseudorandom sequence in correlation chromatography or (2) a periodic sampling with a moving shift in stroboscopic input. The simplest sequence form is (3) equiinterval
- Control of the working of the input device, the detector signal digitization process, and some other working parameters of the instrument
- Detector output data saving, processing, and presentation in a user-friendly form

All these modules have a different relationship to a particular application. First, to

TABLE 2
The Input Devices Used in Computerized Multiple Input Chromatography

Input device	Switching rate(s)	Reproducibility	Ref.
Robot (autosampler) with syringe	1.0	1.5	14, 15
Mechanical rotary valve	0.5	0.1	16
Deans-type pneumatic switch	0.05	0.1	19, 20
Fluid switch	0.01	0.1	17, 18
Modulators			
Oxidative	—	—	20
Thermal desorptive	0.04	—	21, 22
Thermal degradative	—	—	26
Hot wire	—	—	26
Spark	—	—	23
Electrochemical	—	—	24, 25

study the same process, different sequences may be used. This means that it is possible to choose the sequence needed and the right timing parameters, depending on the expected kinetic parameters of the process under study.

In the case of low-level signals, correlation chromatography (CC) with complicated sequences and transforms into chromatograms can be used. The random input sequence used for CC is generated by the computer. Two algorithms for determining the sampling moments are popular: random-number generators or shift registers. Shift registers are more popular. We found that the most economical way to obtain a chromatogram is to use the fast Hadamard transform, whose programing is even simpler than that of the Fourier transform.

If the same process is somehow slowed down or has an increased signal level, the equiinterval sequence can be successfully used. Sometimes it is important to integrate signal filtering and baseline drift removal.into this step. The fast Fourier transform is very effective for this purpose.The input device itself and its control are different in every chemical application. The development of drivers for input devices may require low-level programing, but not in every application, and we used to do this through high-level language facilities.

The third part of the software is the same for all applications with the same data-processing features, including digital filtering and smoothing of the detector signal.

For visualizing and plotting the data, three common displays are in use: (1) chromatograms, plotted in a sequence one after another, (2) the stack plot in which chromatograms are plotted one above the other and are shifted, and (3) a top view of chromatographic peaks showing a contour plot. The algorithms for constructing these plots can be found in many textbooks on computer graphics (simple algorithms are given in Reference 1). Statements about the build-up of the stack and contour plots are also included in contemporary data-processing packages.

After the data have been displayed, the next step is measuring the peak retention time, height, and area on chromatograms. Peak parameters can be determined either automatically by the predetermined parameters of peak integration or manually via the mouse. The result of this processing is a table of peak areas (or maximums) according to retention times and is related to the reaction time. This table can be stored in a separate file for further processing by a spreadsheet program. It will be convenient to complete the program by displaying the concentration curves as a function of reaction time. In our locally written software, the first two modules are connected into one experimental control program and the third is a separate data-handling and -presenting program. The last one is equipped with file transport capabilities. The flexibility of postexperiment data processing can be improved by adding options for chemometric calculations. For example, a good software package for a PC is MATLAB (The Math Works, South Natick, MA) with its toolboxes enabling one to build up interactive, display-oriented, fast-processing procedures.

The sophisticated interfaces are equipped with software packages for quite complicated experimental control and for the ADC of several measuring channels. The only part of the software for which a commercial program is not available is a generator of an input sequence, which must be written by the user.

IV. RANDOM INPUT AND CORRELATION CHROMATOGRAPHY

In the analysis of time-varying flows, random input is an alternative to the common regular input chromatography. According to Table 1, this input technique proved to be suitable for slowly varying low-level concentration flows.

In contrast to common chromatography where a sample is introduced as a single, narrow concentration impulse to the top of the column, in multiple input chromatography it is introduced semicontinuously or as a sequence of impulses during the whole course of chromatographic analysis of the varying flow. Possible sampling strategies include sampling at randomly chosen times and sampling according to specially chosen pseudorandom binary sequences (PRBS) at regular intervals that are comparable to the detector signal digitization interval. By this method, a continuous sample flow is modulated by a predetermined function. The modulation is performed by one of the automatic sampling devices described in paragraph 3. The detector signal is recorded and transformed by the computer to a common form of the chromatogram, which has, however, a lower level of noise than the corresponding chromatogram obtained by a single injection of sample under equivalent conditions. For stationary signals, these sampling schemes lead to CC (also referred to as "multiplex" by some authors), a method for decreasing detection limits and improving signal-to-noise ratios. CC indeed belongs to the family of 'multiplex methods'. In the latter case, instead of a direct measurement of the required results, their linear combination is obtained. Recent trends in the field of multiplex measurements have been summarized in Reference 31. The best example of the multiplex technique is NMR-FFT.[32] FT-infrared spectroscopy is also routinely used.[33] The multiplex methods based on the Hadamard transform are known in infrared[34] and Raman[35] spectroscopy.

A. Correlation Chromatography

Several research groups are actively working in this field, and the number of publications on CC has reached 100. The results of CC studies until the mid-1980s have been summarized in Reference 1. Since then, many interesting results have been obtained in the theory and application of CC, which should at least partly dispel a suspicion about the uselessness of CC in general.[36–38] Also, recent technological developments in sampling device design should facilitate introducing CC into everyday practice. A specific example of noise reduction in capillary zone electrophoresis is presented in Figure 4. Details of sampling device are presented in Reference 39.

1. Decorrelation of the Detector Signal

As explained above, a CC sample is introduced as a sequence of impulses or continuously by modulating the sample stream with a proper function. Theory says that the best function for modulation purposes is a random one. The detector response y(t) can be expressed in general as the following convolution integral

$$y(t) = \int_{-\infty}^{\infty} x(t-\tau)h(\tau)d\tau \qquad (1)$$

where h(t) is a chromatogram and x(t) is an input signal. When the chromatogram is recorded by computer, the detector signal is digitized at intervals Δt, which means that instead of a continuous function y(t), the detector signal vector is measured. It can be proven that the convolution integral in Equation 1 transforms to the following matrix equation[1]

$$\begin{bmatrix} y_i \\ y_{i+1} \\ \vdots \\ y_{i+n-1} \end{bmatrix} = \begin{bmatrix} x_1 & x_{n-1} & \cdots & x_2 \\ x_2 & x_1 & \cdots & x_3 \\ \vdots & \vdots & \cdots & \vdots \\ x_n & x_{n-1} & \cdots & x_1 \end{bmatrix} \begin{bmatrix} h_1 \\ h_2 \\ \vdots \\ h_n \end{bmatrix} \qquad (2)$$

or

$$y = Xh$$

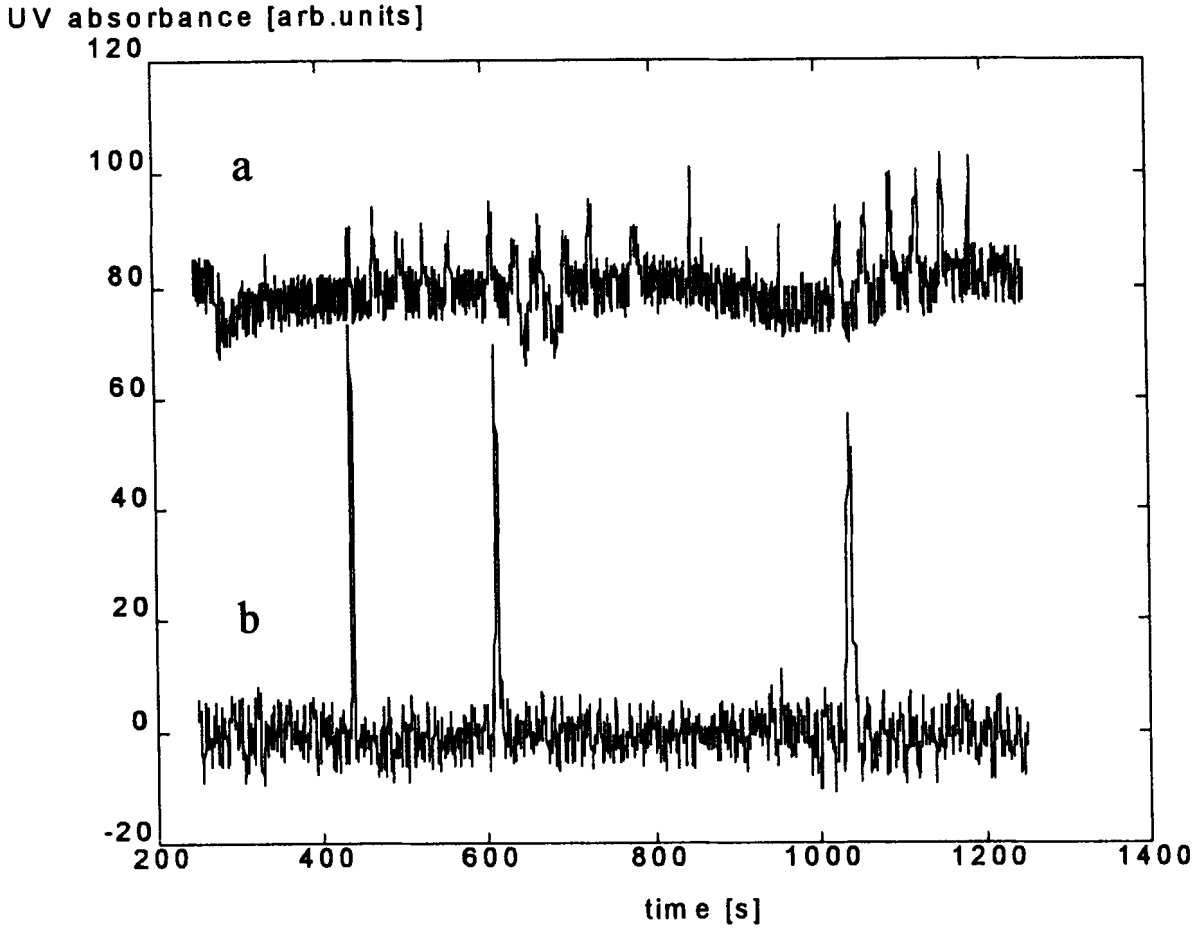


FIGURE 4. Example of noise reduction in CZE of mixture of phenoles at a concentration of 5×10^{-7} *M* by multiple random input. UV detector λ = 212 nm; 50-μm I. P. Polymicro capillary column, voltage 18 kV. (a) Detector output of five single injections (electrostatic injection, 3 s) sequentially; (b) correlogram of same sample from input by PRBS of n = 511 elements. (provided by A. Ebber.

where $h = \{h_1, h_2, \ldots, h_n\}$ is the chromatogram vector and $\{y_{i+n}, y_{i+1+n}, \ldots, y_{i+2n-1}\}$ is the detector signal vector window with length n and starting from point i > n. X is a circulant matrix whose first row is a vector of digitized input functions and the following rows are obtained by cyclic permutation of the first row. In this paper, we consider that first-row elements of the X matrix are derived using the feedback shift register[1] and that the elements of this row have values of 1 or 0. This sequence is commonly referred as PRBS. The number of elements in PRBS is equal to n = 2P-1, where p- is an integer. A typical seven-element ($7 = 2^3 - 1$) PRBS is, for example, 1,1,1,0,1,0,0.

In practical measurements, the input function x(t) controls the sampling as follows: an injection is made into the column at every time moment i during the time interval Δt when the corresponding PRBS element is equal to 1. The sampling device is switched off when the corresponding element is equal to 0. Thus, the input function in this case has two levels, 1 and 0, and changes between those two level occurs pseudorandomly after every interval Δt. The chromatogram h can be found easily by inverting the X ma-

trix in Equation 2 because it allows a fast Hadamard-type transform for the inverse computation. h is also called a correlogram.

An input sequence with elements {1,–1} can also be considered. Experimentally, it can be realized in a two-column, two-detector system. The 1 means injection into the first column and –1 means injection into the other column. The noise reduction properties for a {1,–1}sequence are better than the {1,0} sequence by a factor of two. However, no experimental reports of using {1,–1} sequences have been published.

In the second approach, the sample introduction moments are randomly distributed over the measurement period.[40] The input vector can be considered a "purely" random sequence of "ones" and "zeros". "Purely" is in quotation marks because, in fact, the sequence is generated according to the strict rules of generating random numbers in the computer. The ratio of "ones" to "zeros" in the input sequence determines the injection probability. It is well known that a circulant matrix can be inverted by the Fourier transformation.[41] Then it follows from Equation 1. that in this case

$$h = IFT\ (FT(y)/FT(x)) \qquad (3)$$

where FT and IFT denote the Fourier and inverse Fourier transformations. This approach is conceptually simple and straightforward. The serious drawback of the pure-random input approach is that, being very conventional, it does not provide much material for building up any substantial theory that should predict and explain several phenomena appearing in CC.

PRBS has several interesting properties that make its application to CC extremely useful. First, the influence of several forms of input sequence imperfections on the final result can be predicted. Second, the computation of the chromatogram via the fast Hadamard transform (FHT) becomes possible,[1] which is about 30 times faster than using the FFT.[42]

2. Correlation Noise and Ghost Peaks

When the input concentration signal is not stationary, the deconvolution procedure (decorrelation) is still possible, but output is an averaged chromatogram over the input sequence length. The variations of the sample are transformed to the chromatogram baseline in the form of a signal disturbance known as *correlation noise*. The standard deviation of the correlation noise is proportional to the input concentration variation rate.

A standard deviation of the correlation noise, $s_{\Delta h}$, can be calculated for the Gaussian peak[1]

$$s_{\Delta h} = 2\sqrt{\frac{\sqrt{\pi}\sigma}{n\Delta t}}s_e \qquad (4)$$

where σ is the peak standard deviation and s_e is the standard deviation of the injected sample amount (in peak area units).

When this rate is large enough (input signal variances over 30% of the mean value), the chromatogram is lost in the correlation noise. Direct decorrelation algorithms have been developed that have produced correlation noise-free chromatograms for the case in which all the sample components change linearly.[43] Algorithms also exist for the exponential input function case. However, decorrelation algorithms have not yet been extended to the general case.

Ghost peaks that sometimes appear on correlograms are one of the most intriguing phenomena in CC. Being interesting from a theoretical point of view, their appearance is a remarkable obstacle to the application of CC because they can be confused with real peaks (i.e., the peaks for which the corresponding substance exists in the sample). It is generally recognized that the main reason

for the appearance of ghost peaks is the imperfectness of the input system. In general, random deviations from the average injected amounts result in a random baseline distribution of the correlogram, not ghost peaks. If the input system has a systematic deviation from the ideal, the ghost peaks appear, and their intensities and positions can be associated with a particular form of the systematic input system error.

A mathematical explanation for the origin of ghost peaks is rather complicated, requiring knowledge of algebra and coding theory. This has been done in References 40 and 44]. The main ideas of ghost peak prediction, however, can easily be explained. First, the position of ghost peaks can be predicted only for the PRBS input. Using a "pure" random input with an imperfect input system is probably affected only by the correlation noise. If the input is given in the form of a PRBS, the deterministic deviation from the required input pattern usually can be decomposed as a linear combination of several PRBSs that are shifted relative to the main sequence. Decorrelation of the detector signal evidently calculates a "correlogram" for each member of this decomposition. One example of appearing ghost peaks are presented in Figure 5. Experimental details of this correlation ion chromatography are given in Reference 45.

Although the best way to eliminate ghost peaks is by designing perfect sampling devices, there are also methodological possibilities for removing ghost peaks. One effective way to remove the influence of ghost peaks is to perform each measurement twice: first with a normal PRBS and then by inverting bits in the PRBS (i.e., introducing the sample when PRBS is equal to "zero"). Different types of ghost peaks cancel when correlograms of both experiments are subtracted or added.[44]

The properties of the input systems used in CC have been studied extensively by Mars,[41] Engelsma,[23,46] and Phillips et al.[16–19] When the ghost peaks appear on the correlograms, their positions agree with good precision with the theoretical predictions derived from the properties of the input devices. Thus, there is a good understanding of the ghost peak generation mechanism in CC theory. In addition, CC can be a good method for testing the sampling devices in chromatography because of its sensitivity to input system imperfection. An important point is that systematic errors can be discovered in sampling devices that are usually not recognized when using common standard deviation tests with sampling at regular intervals by detecting ghost peaks on correlograms. Understanding the origin of the correlation noise and ghost peaks is a good introduction for time-varying flow analysis by multiple input chromatography.

B. Monitoring of Time-Varying Flows by CC

The application of CC to nonstationary flow studies is based on a certain controversy. CC theory says that the input to the chromatograph must be stationary; otherwise, correlation noise will appear. On the other hand, the sample is introduced to the column after intervals that are far shorter than those determined by the time of separation of sample components in the column. Hence, the detector output somehow reflects changes in the composition of the sample. In general, the correlation procedure acts as an averaging boxcar filter with a length of n points with respect to the time-varying process. All nonstationarities are transformed into the correlation noise. When the change is insignificant, the case is represented in Table 1 as slowly varying low-concentration flows. Nonstationarities are then considered a disturbance that can be neglected in the first approximation.

Analysis of the correlation noise form can tell us much about the function accord-

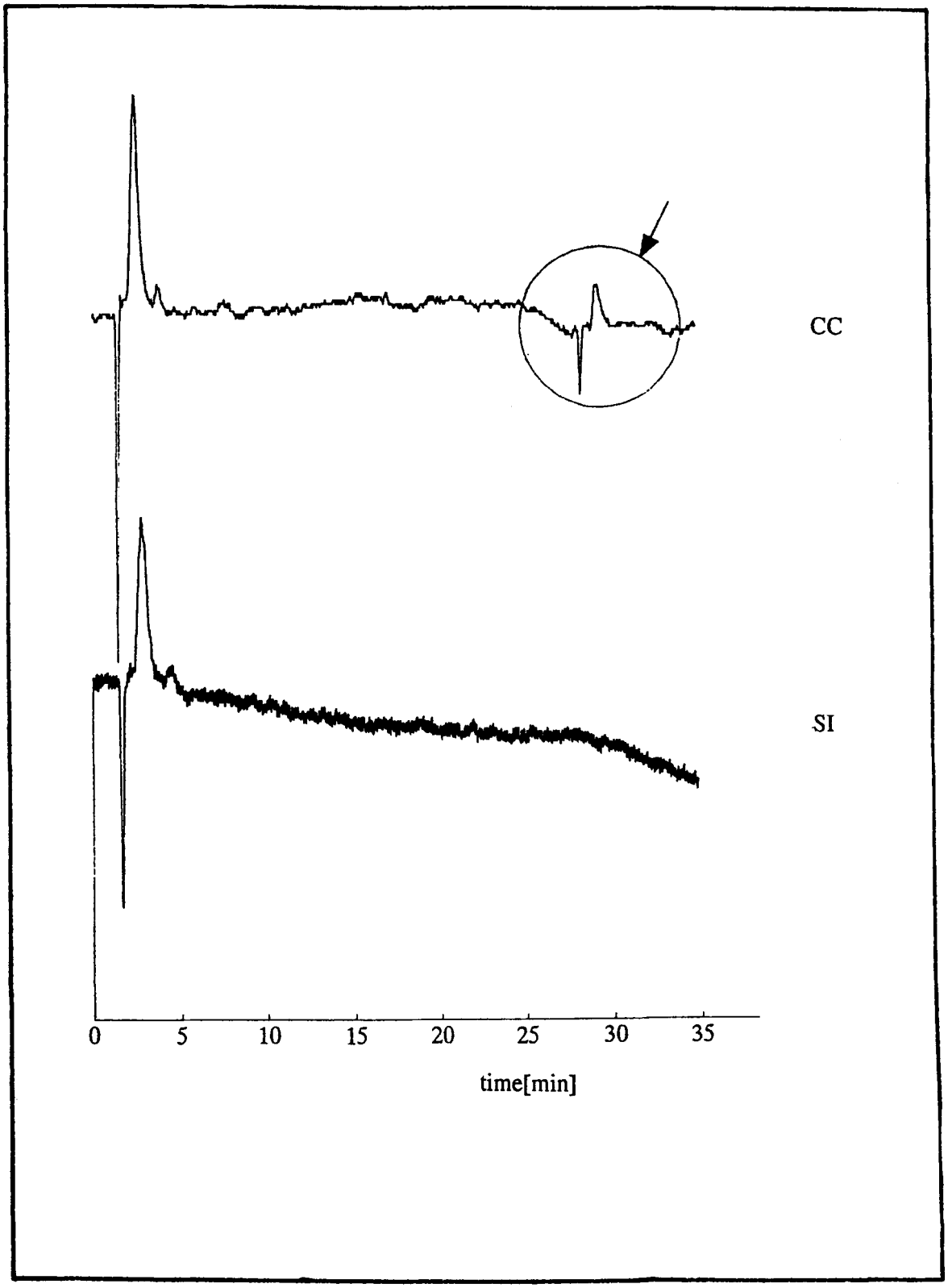


FIGURE 5. Ghost peaks (in circle) in real correlation ion-chromatography experiment (UV detection λ = 260 nm) resulting from imperfection of input system. Correlogram (CC with PRBS n = 511) is compared with single injection (SI) of same sample.

ing to which the time-varying process is changing, but, so far, this field has received little attention. Only Frazer and Burke [47] have made some correlations between the correlation noise and linearly varying input CC. Their idea was to use the calibration technique to compare the recorded correlogram with a set of correlograms with the known time-varying input. Their approach was completely empirical.

Recently, Louwerse proved theoretically that by using pseudorandom input, it is possible to monitor twice as fast as can be done by single injection chromatography.[48] This result was later confirmed by Smit and Kaljurand[49] by computer simulations. A twofold increase is not a terribly remarkable result, and at this point one can ask whether the measurement of time-varying substance flows by CC makes any sense at all. Instead of trying to decorrelate the detector output signal, one can attempt to solve the problem by calculating the possible theoretical detector output and fitting it to the experimental output by a linear least squares procedure (as proposed in Reference 48.. Preknowledge about column properties, input sequences, and samples are taken into account as much as possible. We demonstrate that if the peak shape function is known beforehand and there is preknowledge about the number of sample components then the functions describing the changing of sample components concentrations in time (concentration functions) can be reconstructed for many practical situations. We also show that for PRBS, the necessary preknowledge can be extracted directly from the detector signal for classes of functions that exhibit the shape of a peak.

1. Nonstationary Input Concentrations.

If the sample concentration varies in time, the X matrix cannot be considered as having elements with only two possible values (1 and 0) anymore. Considering a sample in which all components change with the same function and assuming that l PRBS inputs with a period of n elements are performed, the input sequence will now be

$$\underbrace{\{x_1(1),\ldots,x_n(n)}_{\text{1th PRBS}},\underbrace{x_1(n+1),\ldots,x_n(2n)}_{\text{2nd PRBS}},\ldots,$$

$$\underbrace{x_1((l-1)n+1),\ldots,x_n(ln),}_{\text{lth PRBS}}$$

Indices in the brackets denote the running time of the measurement. It can be shown[50] that the detector output can be presented as

$$\begin{bmatrix} y_i \\ y_{i+1} \\ \vdots \\ y_{i+n-1} \end{bmatrix} = \begin{bmatrix} \tilde{x}_1(i) & \tilde{x}_{n-1}(i-1) & \cdots & \tilde{x}_2(i-n+1) \\ \tilde{x}_2(i+1) & \tilde{x}_1(i) & \cdots & \tilde{x}_3(i-n+2) \\ \vdots & \vdots & \cdots & \vdots \\ \tilde{x}_n(i+n-1) & \tilde{x}_{n-1}(i+n-2) & \cdots & \tilde{x}_1(i) \end{bmatrix} \begin{bmatrix} h_1 \\ h_2 \\ \vdots \\ h_n \end{bmatrix} \quad (5)$$

or $y(i) = \tilde{X}h$. Assuming that the sample change is a smooth function, the $\tilde{X}$ matrix elements can be expanded into a Taylor series up to the second-order term as follows

$$\tilde{X}_k(i \pm m) = X_k\left(a_i \pm b_i m + c_i m^2\right)$$

where k = 1,..., n–1; m = 1,..., n – 1. By substituting these values into Equation 5, it also can be expanded into series

$$y(i) = a_i Xh + b_i Bh + c_i Ch$$

where matrixes B and C have a simple structure

$$B = \begin{bmatrix} 0X_1 & -1X_n & \cdots & -(n-1)X_2 \\ 1X_2 & 0X_2 & \cdots & -(n-2)X_3 \\ \vdots & \vdots & \cdots & \vdots \\ (n-1)X_n & (n-2)X_{n-1} & \cdots & 0X_1 \end{bmatrix}$$

$$C = \begin{bmatrix} 0X_1 & 1X_n & 4X_{n-1} & \cdots & (n-1)^2X_2 \\ 1X_2 & 0X_1 & 1X_n & \cdots & (n-2)^2X_3 \\ 4X_3 & 1X_2 & 0X_1 & \cdots & (n-3)^2X_4 \\ \vdots & \vdots & \vdots & \cdots & \vdots \\ (n-1)^2X_n & (n-2)^2X_{n-1} & (n-3)^2X_{n-2} & \cdots & 0X_1 \end{bmatrix}$$

Matrices B and C can be expanded further as follows

$$\begin{aligned} B &= NX - XN \\ C &= N(NX - XN) - (NX - XN)N \end{aligned} \tag{6}$$

where N is a diagonal matrix

$$N = \begin{bmatrix} 0 & 0 & \cdots & 0 \\ 0 & 1 & 0 & 0 \\ \vdots & \vdots & \cdots & \vdots \\ 0 & 0 & \cdots & n-1 \end{bmatrix}$$

We obtain the following expression for the detector output

$$\begin{aligned} y(i) = {} & a_i Xh + b_i(NX - XN)h \\ & + c_i\left[N(NX - XN) - (NX - XN)N\right]h \end{aligned} \tag{7}$$

These expressions can all be easily proven by direct matrix multiplication.

Assuming that the chromatogram shape h is known beforehand (in many cases, this is not a very restrictive assumption), the products Xh, (NX – XN)h and [N(NX – XN) – (NX – XN)N]h can be calculated easily by several FHTs of h, Nh, and NNh. Factorization of matrices B and C (Equation 6) is useful because it enables one to further speed up calculations and save computer memory because the matrix multiplication on the order of n^2 operations can be replaced with vector operations (such as FHT and multiplication with diagonal matrices) on the order of n operations. Determination of the concentration function of the sample is now reduced to determining the coefficients, a_i, b_i, and c_i of the Taylor expansion in Equation 7, which is an [n × 3] set of linear equations that can be solved by standard methods. For this purpose, Equation 7 can be written as

$$y(i) = Zq(i) \tag{8}$$

where Z is an [n × 3] matrix with three columns Xh, (NX – XN)h and (N(NX – XN) – (XN–NX)N)h; and q(i) = $[a_i,b_i,c_i]$. The standard least squares solution for q(i) is $q(i) = (Z^TZ)^{-1}Z^Ty(i)$. The generalization for many concentration functions is straightforward. Assuming that the chromatogram can be divided into k groups of peaks with peak shape functions $h^{(1)},h^{(2)},\ldots,h^{(k)}$, Equation 8 can be written as follows

$$y(i) = \begin{bmatrix} Z^{(1)} & Z^{(2)} & \cdots & Z^{(n)} \end{bmatrix} \begin{bmatrix} q^{(1)} \\ q^{(2)} \\ \vdots \\ q^{(k)} \end{bmatrix} \tag{9}$$

Thus, by solving Equation 9, the concentration function values for $i\Delta t$ can be found. By scanning the i value from n to (l – 1)*n and solving Equation 9 for each i value, the concentration curves for all the components can be found with time resolution Δt.

A typical case with simulated data is presented in Figure 6, where the total chromatogram is presented as a three-peak chromatogram together with peak shape functions, concentration curves, and the detector output signal for 511 points. As one can see in the figure, the total chromatogram is almost correlation-noise free, thus providing a good opportunity to obtain preinformation about peak number and positions. This was the case in most of the simulations. The reconstructed concentration curve width appears to be so small that only two to three concentration values could be measured by single injections. Figure 7 shows that the reconstruction from the PRBS input is still noisy but closely follows the given time function on average. (Single-injection reconstruction does not demonstrate noise because it its just an interpolation by exact formulas.) Such features as the position of the maximum, width, and amplitude of the concentration curve were easily estimated from the PRBS input reconstruction. The origin of the noise was probably due to the fact that the approximation of the Gaussian with the square polynomial was not exact. Single-injection interpolation reconstruction worked well for the slowly changing concentration functions (wide peaks), as it was supposed to, but failed to reconstruct the positions and amplitudes of the narrower peaks (rapidly changing concentration functions). An astonishing example is peak "a" in Figure 7. For wide peaks, the advantage of the PRBS input was not very evident.

2. Finding Peak Shape Functions from Total Chromatogram

As already pointed out above, sample composition is usually known beforehand in many monitoring applications and therefore the peak shapes $h^{(1)},h^{(2)},\ldots,h^{(k)}$ can be measured before the experiment. Even when this is not the case, the number of compounds can still be estimated when the concentration functions are "peak like", that is, when the sample concentration is negligible before a certain time, then goes through a maximum and decreases again to a very low value. Estimation of the chromatogram by direct decorrelation of the detector output signal is difficult because of the nonstationary input-generated correlation noise, as pointed out earlier. However, if l PRBS inputs are performed, it is possible to divide the detector output to l parts of length n points and compute the "total" detector output by summing the corresponding detector signal windows obtained. If we consider only one component, Equation 7 develops into the linear members

$$y_{tot} = \sum_k y(kn) = \left(\sum_k a_{kn}\right) Xh + \left(\sum_k b_{kn}\right)(NX - XN)h \qquad (10)$$

For the peak-like functions, all a_{kn} values are positive when the b_{kn} values have as many positive values (ascending part of the curve) as negative values (descending part of the curve) of the same magnitude. Thus, the second term in Equation 10 cancels out. This term is responsible for the correlation noise, as we can easily see if we decorrelate y_{tot} by multiplying it by X^{-1}. Thus, the total decorrelated chromatogram has reduced correlation noise value and can be used to estimate the number of components in the sample and their corresponding peak shapes.

3. Expanding Detector Output To a Matrix

The form of Equation 2 suggests another possibility for the treatment of time-varying

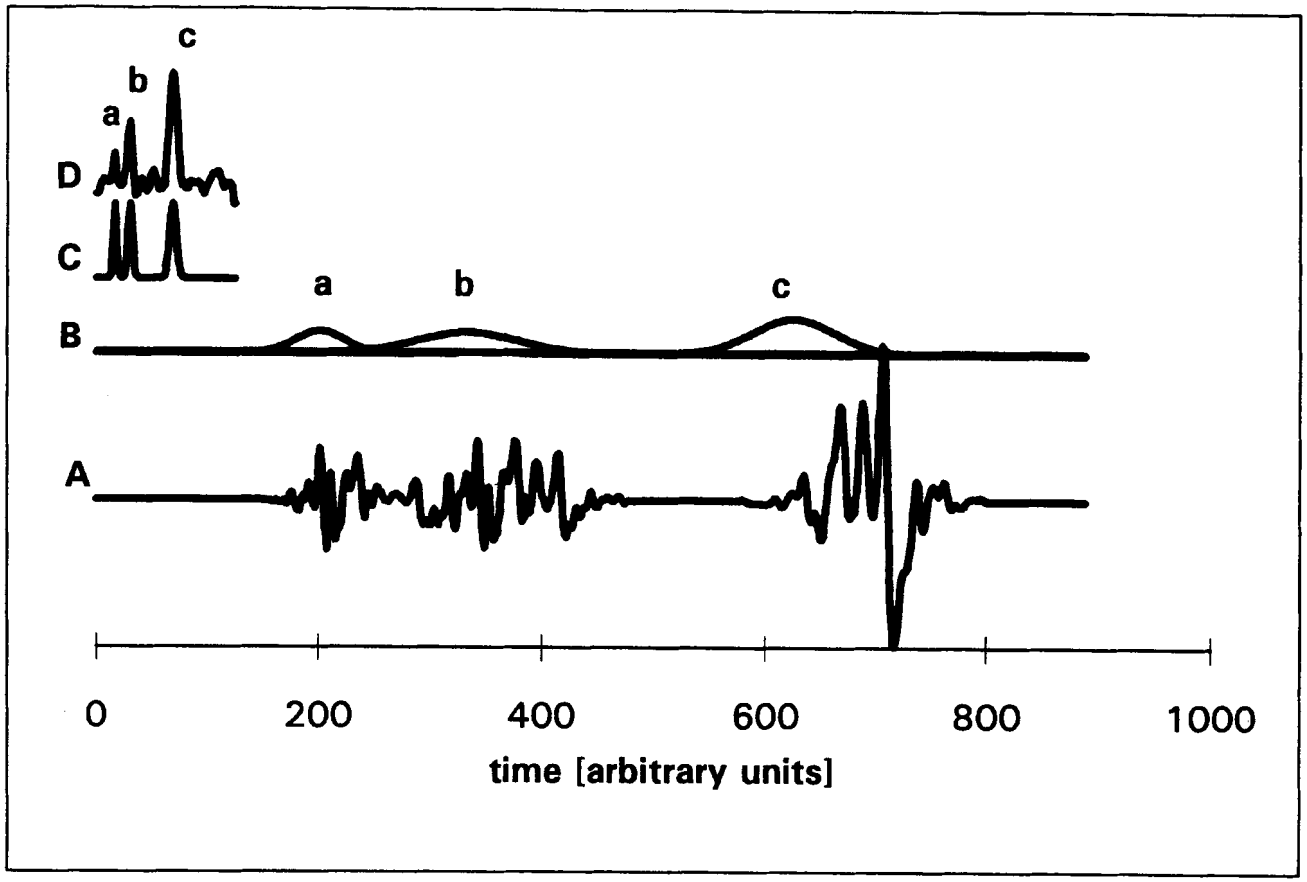


FIGURE 6. Estimation of total chromatogram and peak shape functions. Input PRBS, with 511 {1,–1} elements. A, detector output; B, given concentration curves; C, peak shape functions; D, total chromatogram.

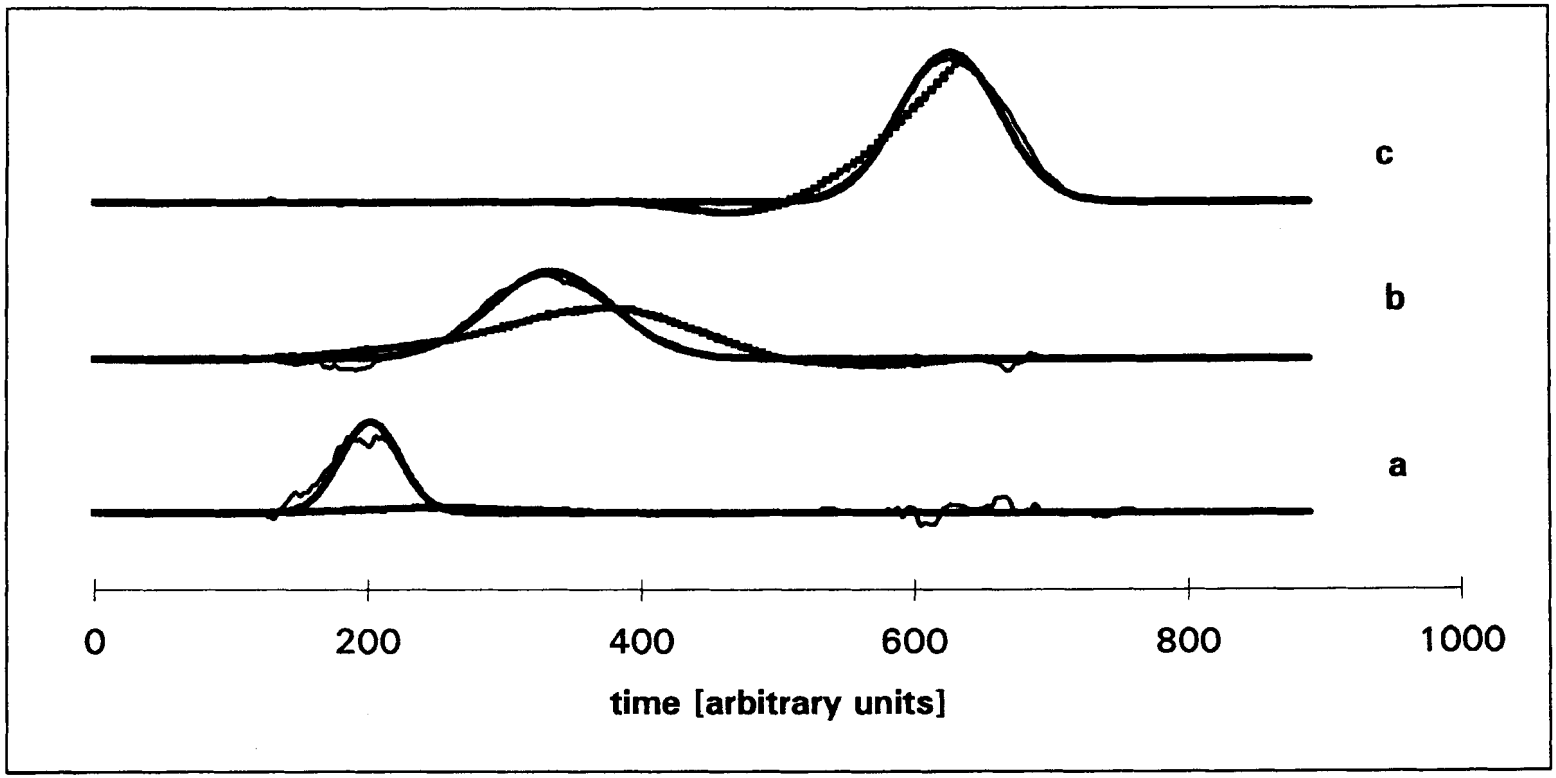


FIGURE 7. Reconstruction of concentration curves using expansion of detector output to Taylor series. (a–c) Concentration curves corresponding to peaks in Figure 6. Thick solid line, ideal concentration curves; thin "noisy" line, PRBS input reconstruction; thin "smooth" line, interpolation from single injections.

concentrations in multiinput chromatography. This method was proposed by Hoffmann[51] as a solution for the general problem of identifying time-varying processes, and its applicability for reducing correlation noise in CC was studied by Kaljurand et al.[50] Because a full description of the algorithm is available,[50] it is only briefly outlined here. In the previously described algorithm, the chromatogram shape was assumed to be constant and the input matrix was developed into a series. In this case, the input sequence is considered to be constant and the chromatogram is considered as a matrix with elements $H_j(i)$, where j is a chromatogram run-time index and i is a process run-time index. Let us consider a matrix of order of $[n \times n(l-1)-1]$ where the elements of the vector y are located at the main diagonals separated by n elements. It can be demonstrated that

$$\begin{bmatrix} y_1 & * & \cdots & * & y_{n+1} & * & \cdots & \cdots & \cdots & * & y_{ln+1} & 0 & \cdots & 0 \\ 0 & y_2 & * & \cdots & * & y_{n+2} & * & \cdots & \cdots & \cdots & * & y_{ln+2} & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ 0 & \cdots & y_{n-1} & * & \cdots & * & y_{2n-1} & * & \cdots & \cdots & \cdots & * & * & y_{ln+n-1} \\ 0 & \cdots & 0 & y_n & * & \cdots & * & y_{2n} & * & \cdots & \cdots & \cdots & * & 0 \end{bmatrix}$$

$$X \begin{bmatrix} H_1(1) & H_1(2) & H_1(3) & \cdots & H_1(n) & H_1(n+1) & \cdots & H_1(ln) & 0 \\ 0 & H_2(1) & H_2(2) & \cdots & H_2(n-1) & H_2(n) & \cdots & H_2(ln-1) & H_2(ln) \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ 0 & \cdots & 0 & 0 & H_n(1) & H_n(2) & & H_n(ln-n+1) & H_n(ln-n+2) \end{bmatrix}$$

or

$$Y = XH \qquad (11)$$

This equation could provide a fine solution for finding the exact solution form of a 2-D chromatogram by multiple input chromatography if the nondiagonal elements (marked with asterisks) are known. Those elements are not measured, however, and must somehow be estimated from the main diagonal elements. A straightforward way is to interpolate nondiagonal elements in a particular row using the main diagonal elements of the same row. If the concentration of all the peaks changes linearly or exponentially, it is easy to demonstrate that, by using the definition of matrix multiplication, the solution is exact. For other cases, it gives some approximation.

C. Practical Results of Using CC in Analysis of Time-Varying Flows.

The setup of thermochromatographic equipment,[52] schematic of which is presented in paragraph 3, is a very good source of time-varying gas flows. Flow of volatiles evolving during the heating of organic or inorganic samples in different temperature regions and rate changes in time qualitatively and quantitatively. The solid sample is located in a flow-through thermal degradation reactor, and the gases are swept, by helium, from the reactor to the vent through the Deans-type pneumatic sampling valve. The valve samples the evolved products into a gas chromatographic column. The instrument is capable of performing either the regular injection sequence or the pseudorandom injection sequence needed in CC.

For real data, the peak-shape functions were not available beforehand and were estimated from the total chromatogram. Total

chromatogram and peak-shape data are presented in Figure 8 for the 1- to 2-mg pieces of polypropylene (PP) sample with a molecular weight of 300,000 located in a flow-through thermal degradation reactor and heated from 100 to 550°C at a 5°C/min rate. The total chromatogram indicates the presence of five peaks. Under such thermal conditions, PP evolves a mixture of hydrocarbons. The hydrocarbons were analyzed by a 15 m × 0.5 mm OV101 metal capillary column (Perkin-Elmer). Peak locations were used as initial data to fit the total chromatogram with a model chromatogram of four Gaussian peaks to obtain peak-shape functions. The peak-area functions were then calculated. Because the concentration variations were estimated to be relatively slow, we limited our calculations to the linear term in Equation 7. The results are presented in Figure 9. Because the real concentration curve is not known, these results were compared with the results obtained from the use of the algorithm that converts the detector output vector to a matrix (Equation 11). As can be seen in Figure 9, the time functions obtained by both methods are close enough to conclude that both algorithms give approximately the same result. The polynomial approximation is much noisier than the detector matrix algorithm, in which the concentration curves are flatter. This is because the algorithm that transforms the detector output to polynomials benefits from the preknowedge of the individual peak shapes, whereas the algorithm that transforms the detector output to a matrix assumes that the concentration curves are smooth functions.

Figure 10 shows the ThGC results from samples of Hawaiian volcano rock (HVR) soils that were heated from 100 to 550°C at a rate of 10°C/min. HVR evolves a mixture of H_2O and CO_2. Water and carbon dioxide were analyzed by a 10 m × 0.5 mm Poraplot column (Orion Ltd., Finland). Again, the reconstruction by Equation 11 is much smoother than the Taylor series expansion reconstruction but tends to have increased width.

The reconstruction of an extremely narrow gas evolution curve is given in Figure 11. The sample was calcium oxalate, a common thermal analysis standard, which releases water and carbon oxide between 100 and 500°C. As can be seen in Figure 11, the experimental peak shapes (or total chromatogram) are very noisy, which is a direct result of the extreme narrowness of the evolved gas peaks. The reconstruction by both methods is not very good, and "leakage" of the intensity from one peak to another can be observed because water evolution is not present at high temperatures or any carbon oxide evolution in the low-temperature region. Comparison with the detector signal reveals that the Taylor series expansion method gives a better reconstruction of the evolved gas curves in general despite the noise. Due to the water peak retention time, the single injections in this case could have been performed at a time interval of 2 min, which means that about two or three points could have been measured on evolved compound concentration curves.

Here, two calculation methods are proposed. Expansion of a detector signal to a matrix method (Equation 11) appears to give much smoother results than the expansion of input matrix to a Taylor series (Equation 7). However, this drawback is balanced by the fact that, in general, reconstruction by the Taylor series follows the real curve more precisely, as can be seen in the calcium oxalate example in Figure 11. The origin of the noise can be attributed to errors in determination of the experimental peak shapes, which in turn might have been amplified by computational instabilities. Further studies are needed to establish whether more exact measurements of peak shapes or more elaborate computational approaches to the problem will reduce the noise on peak-area curves.

The multiplex advantage inherent to the PRBS input is still present when the time-

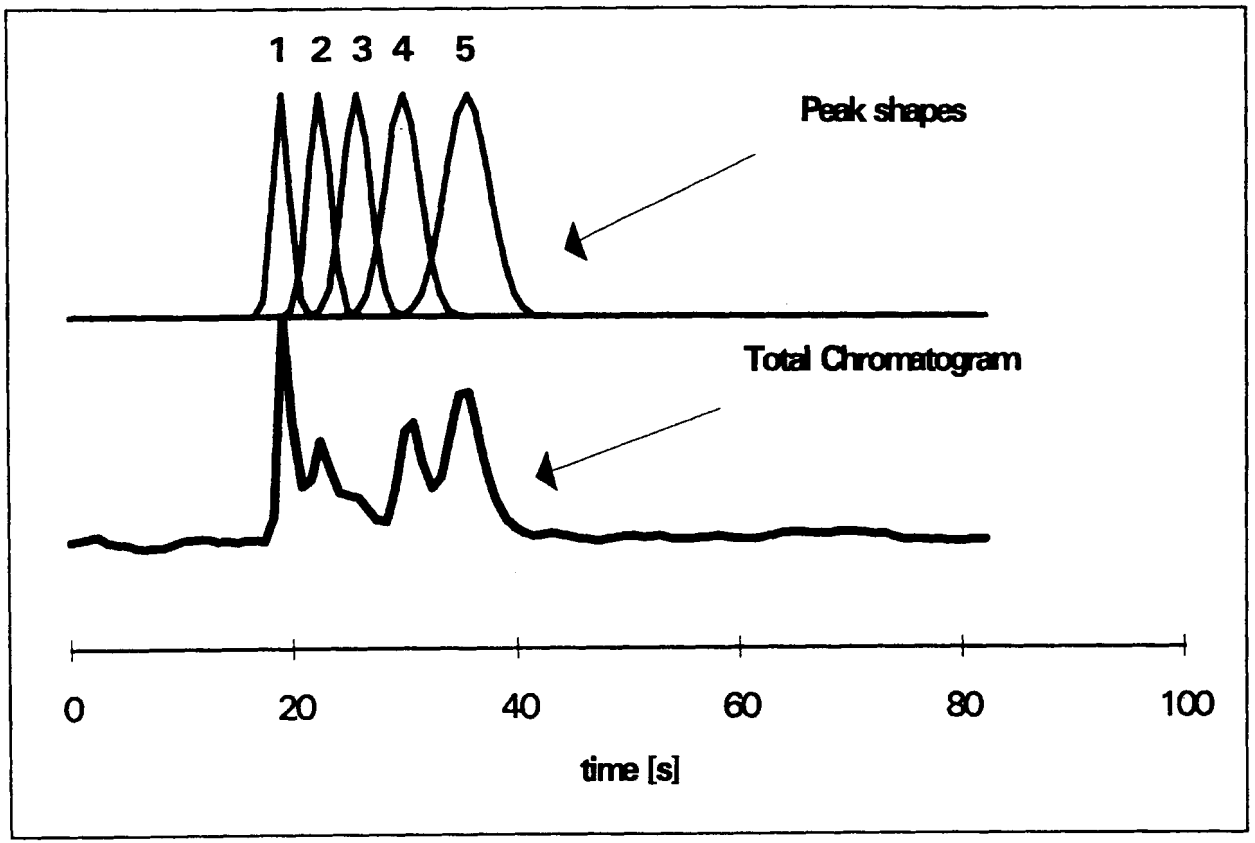


FIGURE 8. Total chromatogram and peak shape functions of thermal degradation products of polypropylene. Heating rate, 5°C/min; digitization interval, 0.5 s.

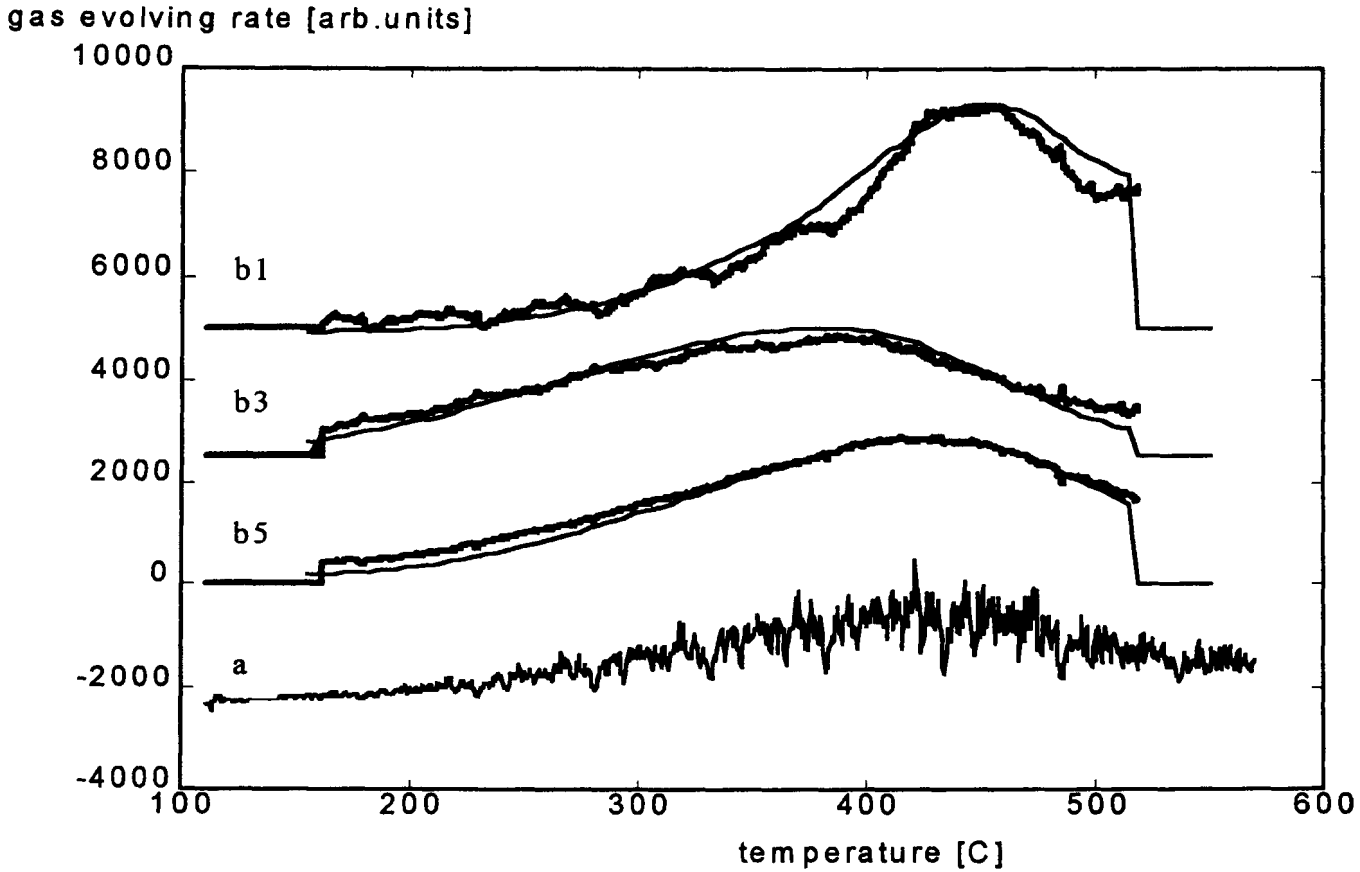


FIGURE 9. Reconstruction of evolving curves of polypropylene sample. Comparison of two reconstruction techniques: "smooth" line, expansion detector output to matrix; "noisy" line, expansion detector output to linear function. a, detector output; b1, b3, and b5, concentration curves corresponding to peaks in Figure 8.

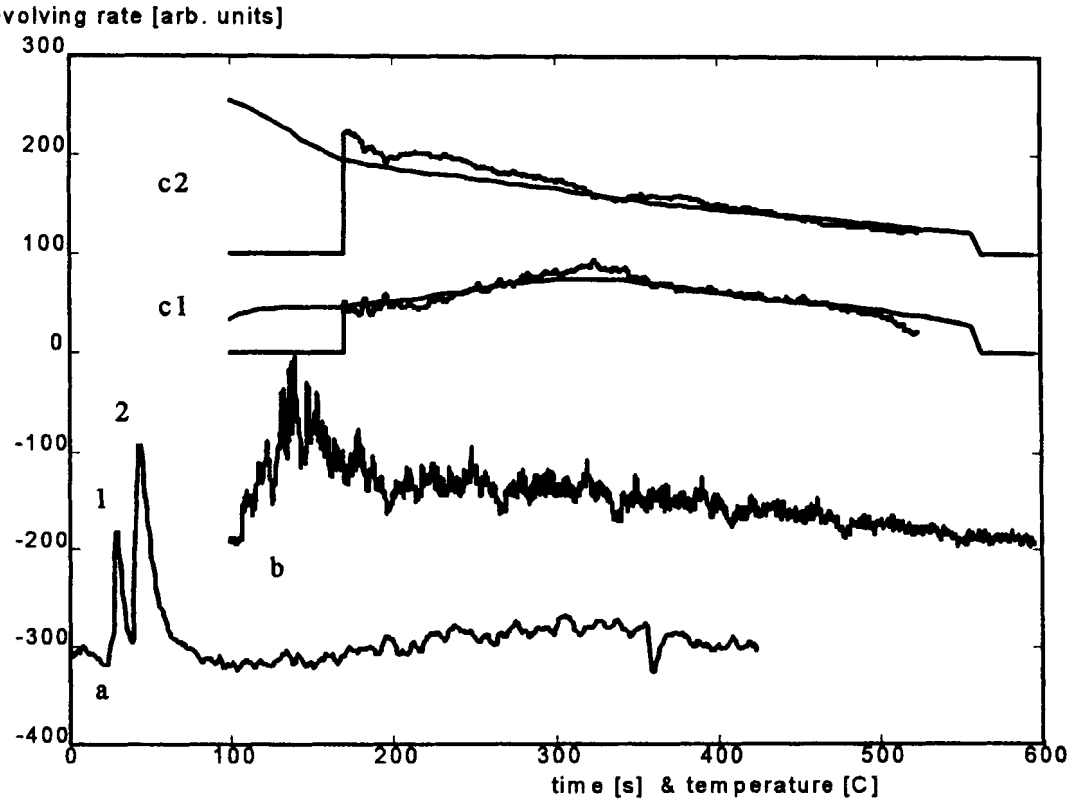


FIGURE 10. Reconstruction of evolving curves of heating of Hawaian volcano rock sample. Heating rate, 10°C/min; digitization interval, 0.859 s. a, total chromatogram; 1, CO_2 peak (retention time, 35 s); 2, water peak (retention time, 55 s); b, detector output; c1, CO_2 peak area function; c2, water peak area function. Comparison of two reconstruction techniques: "smooth" line, expansion detector output to matrix; "noisy" line, expansion detector output to linear function.

varying concentrations are analyzed. An experimental proof is also given in Figure 12, where the 2-D thermochromatograms of an HVS sample are presented. Here, single-injection reconstruction is very noisy and CO_2 evolution cannot be detected at all. The termochromatogram, which is reconstructed from the detector signal of PRBS input (Figure 10a) using Equation 11, has a reduced noise level factor of about 10, which is in agreement with theoretical predictions for 511-element PRBS input sequences.

Although in the case of monitoring using multiple input chromatography the main task is focused on the time characteristics and not the multiplex advantage, the results can be successfully used for the analysis of low-level gas flows in which the concentration varies slowly. Figure 12 shows that correlation noise can be significantly reduced without a significant effect on the monitoring properties of multiple input chromatography.

Another application of CC to the time-varying problem was in studies of several samples of PP headspace composition dependence on sample temperature.[53] This case is very important in the study of polymer behavior when working at elevated temperatures and in characterizing the lifetime of material. The existing alternative method, the evolved gas concentration on sorbent and subsequent release, was found to be rather unreliable because of irreversible adsorption and possible operator errors. Figure 13 demonstrates that the sample concentra-

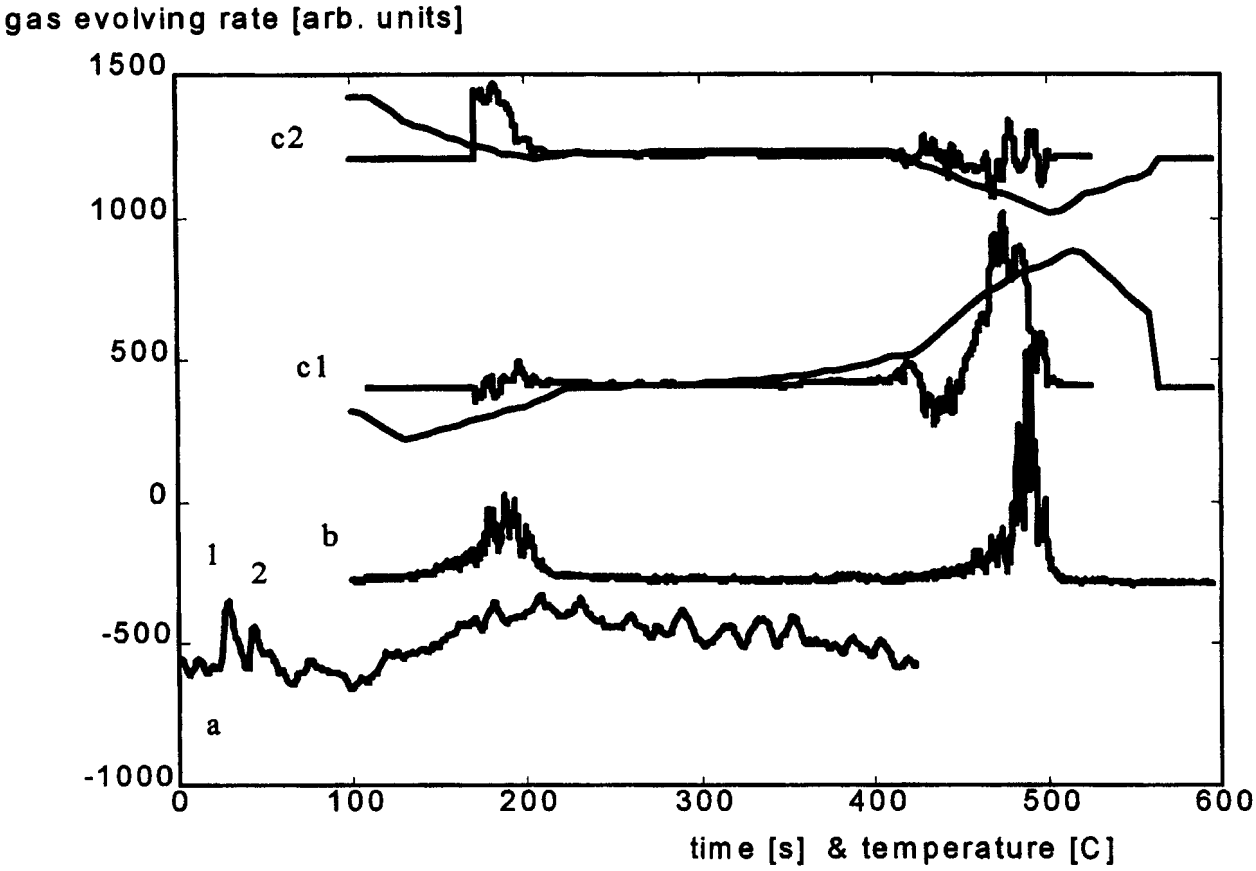


FIGURE 11. Reconstruction of evolving curves of heating of calcium oxalate sample. Heating rate, 10°C/min; digitization interval, 0.859 s. a, total chromatogram; 1, CO peak (retention time, 31 s); 2, water peak (retention time, 55 s); b, detector output; c1, CO peak area function; c2, water peak area function. Comparison of two reconstruction techniques: "smooth" line, expansion detector output to matrix; "noisy" line, expansion detector output to linear function.

tion on the trap fails to represent correctly the qualitative and quantitative composition of a headspace sample when compared with CC if single-injection chromatography is considered a "true" reference sample. In this work, the good interlaboratory reproducibility of the CC method was demonstrated.

Implementation of CC seems to require substantial effort, and because of that, the general chromatographic community has not been very enthusiastic about introducing correlation chromatographs in its labs, preferring either sorbents for low-concentration samples or equiinterval sampling for monitoring. The situation might change when measurements have to be performed in extreme conditions. A good example is space studies. The instruments used for this purpose must fulfill the following general requirements: low weight, low electric power consumption, small sizes, and high mechanical strength and reliability (to withstand vibrations and shocks). Because of these constraints, there has always been a gap between the technology for chemical analysis used on the ground and that used in space. However, space applications often induce new developments. Gas chromatography is a powerful technique for *in situ* analysis of planetary atmospheres, but the main constraint is the time limitation for analysis. The total time available for GC analysis during the

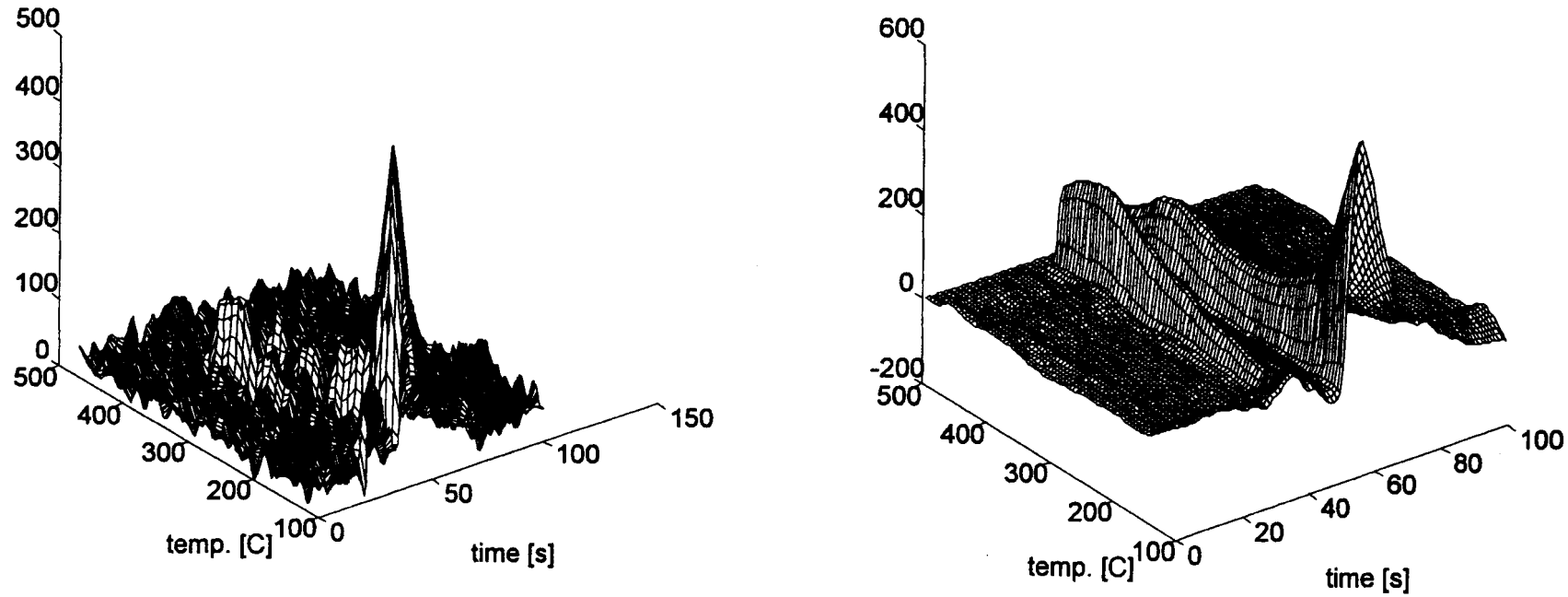


FIGURE 12. Comparison of reconstruction of 2D thermochromatogram of Hawaiian volcano rock sample by (A) single injection chromatography and (B) multiple injection chromatography.

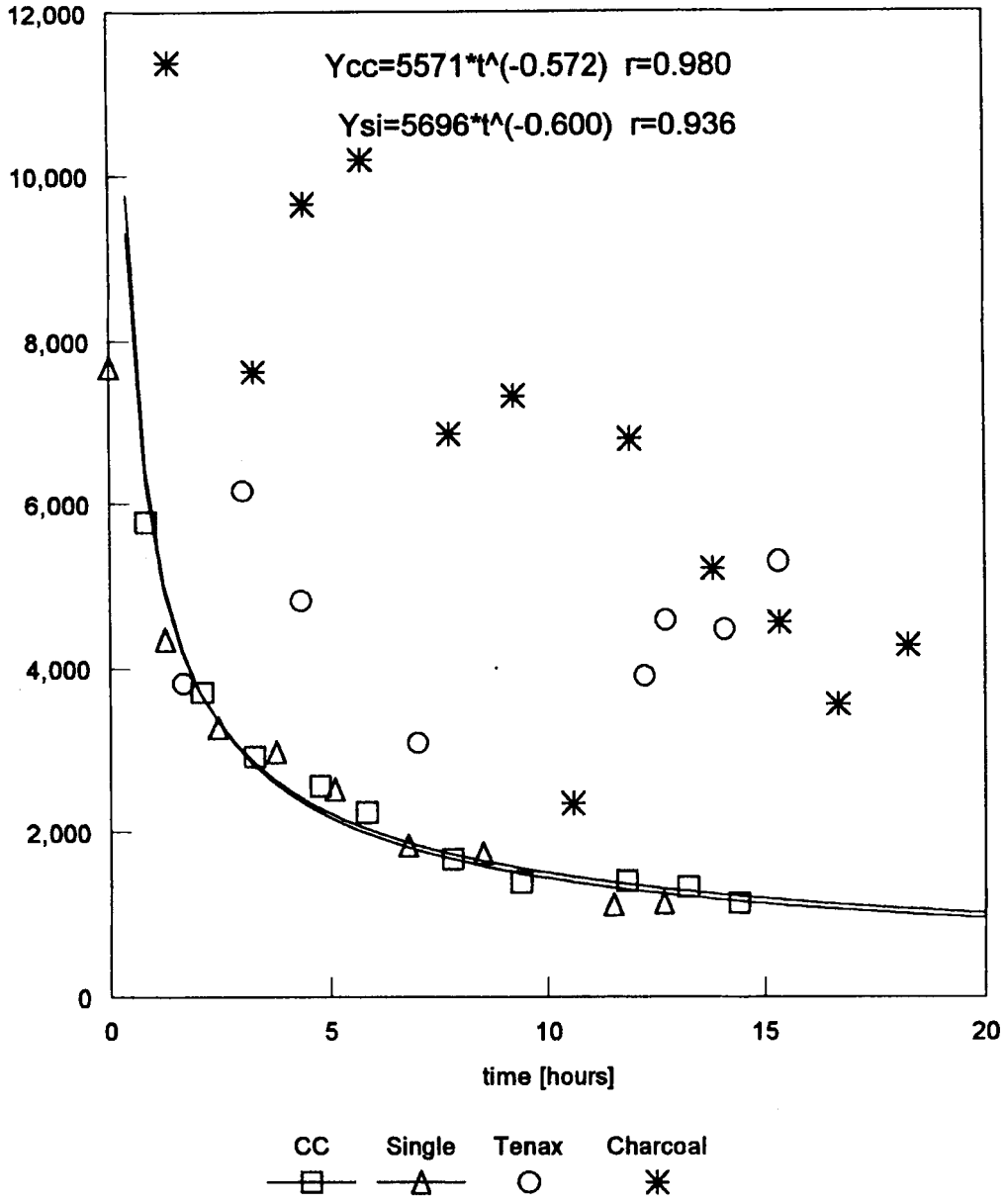


FIGURE 13. Comparison of concentration on traps with correlation chromatography in polymer (polypropylene) headspace kinetics studies. Results are compared with single injection chromatography.

landing of the probe unit is about 30 min.[54] It is clear that some interesting clouds may be easily passed without any response.

The possibilities of CC for space studies have been extensively studied by Valentin at the NASA Ames Research Center.[55–58] He has demonstrated the feasibility of using a mechanical valve as an input device for CC at a subatmospheric pressure of 3 torr. Also, he simulated the planetary atmosphere environments by an exponential dilution of light hydrocarbon samples and used them as an input to CC. In this work, the correlation noise form will not be analyzed, but the dependence of the standard deviation of the correlation noise on the sample dilution rate.

The results demonstrate good agreement with the theoretical prediction (see, e.g., Equation 2 in Reference 1) that the standard deviation of the correlation noise increases linearly with increasing sample concentration. The studies were performed in a "slow-rate and low-concentration" region (according to Table 1).

CC has its challenge here: automatic sampling for the probe unit chromatograph can easily be made pseudorandom, and then a sophisticated detector response can be analyzed on the powerful computer later. Thus, the problem that otherwise may involve a remarkable sophistication of instrumentation on board a spacecraft can be solved just by reorganizing the measurement procedure. However, before the application of CC to space studies can be recommended, the main theoretical problem must be solved: how to interpret the correlation noise pattern. As follows from the theoretical part, making several not very restrictive assumptions, the theoretical two-fold monitoring speed advantage over common chromatography can be easily overcome at the expense of more elaborate computational procedures. Despite the not very restrictive limitations of the approach presented here, it is clear that reconstruction of concentration curves of varying sample components is possible.

When atmospheric gases are the analytes of interest, the possible number of candidate compounds rarely exceeds five to six, thus fitting the compound range of the simulations used in this work (Figures 6 and 7). Contemporary columns and analysis conditions developed by using sophisticated experiment-planning methods predict a typical separation time of about 10 min.[59] If the digitization interval is about 1 s, which is satisfactory for such a chromatogram, the number of points on the digitized chromatogram is about 600, which again is within the size of the chromatograms used in simulations presented in Figures 6 and 7. These considerations are given to show that these simulation results indicate what can be expected in real applications of the time-varying concentration restoration methods discussed in this paper. The separation time of 10 min (proposed for the separation of Titan atmosphere gases for instance) would enable about three samples by single injections to be recorded during probe descent. In addition, the equipment on board might break between the two consecutive analyses. This possibility cannot be completely ignored if one considers past experiences in space exploration studies. Because the multiple input chromatography output is semicontinuous, the results are available right up to the unfortunate event, which is not the case with single-injection chromatography.

V. REGULAR INPUT CHROMATOGRAPHY

The more common chromatographic experiment with input sequences where single injections are separated at regular (equal or linearly increasing) time intervals has been reorganized according to the possibilities of computer control and with a view to applying chemometric methods to data processing.

A. Stroboscopic Input

As already demonstrated in Section I, the time of component separation in the column sets the limits to the time resolution of processes that can be studied by chromatography. We can roughly consider the lower limit for the separation time to be about 10 s. On this basis, the processes of interest are divided into rapid or slow ones. For rapid processes, chromatography cannot be considered a proper method of analysis. However, for reproducible processes, the limitation imposed by the separation time can be easily overcome by implementing an old idea of visualization of rapid periodic movements using a stroboscope.

1. Principle of Stroboscopic Input

In chromatography, the idea of *stroboscopic sampling* is as follows: the process under study (e.g., a reaction) is initiated many times in the same way, and at a certain time interval after each initiation, the sample is removed from the reaction vessel or the flow and introduced into the column. By scanning the interval between the initiation and sampling moments from zero to the end of the reaction, it is possible to record concentration curves for reagents and products with adequate time resolution. The major requirement is process reproducibility. For most chemical reactions, the latter can be realized at the expense of the more or less sophisticated experimental setup. The idea of stroboscopic sampling is illustrated in Figure 14.

Measurement of the kinetics of a chemical reaction with stroboscopic sampling is a typical computer-based experiment. This can also be done manually, but human capabilities of generating precise time intervals and complicated sequences of injections are rather limited. Automatic devices can be built, but they lack the required flexibility in generating time sequences provided by computerized experimental control. The term "boxcar" in chromatography was proposed by Snyder et al.[60] for the technique that can be considered as a first, fully-automated realization of stroboscopic sampling, but the interval between initiation and sampling could not be scanned and remained fixed during the whole course of the measurement. Like in CC, for stroboscopic sampling method one meets again the problem of generating a special form of the input sequences. Using a computer, it is very easy to produce the particular sequence with proper timing.

It is evident that the time resolution in stroboscopic sampling is determined mainly by the experimental possibilities of interrupting the process. In turn, this is frequently determined by how fast one can remove the aliquot of the sample from the reaction vessel (i.e., the time resolution is determined by the functioning speed of the sampling devices). (Other process termination possibilities can also be considered, such as stopping the thermochemical reaction by switching off the heating radiation.) Table 2 shows the functioning times of the commonly used automatic sampling devices. It follows that the time resolution in stroboscopic sampling can be decreased to tens of fractions of seconds, thus enabling a study of most macrochemical reactions (i.e. including transport and diffusion phenomena).

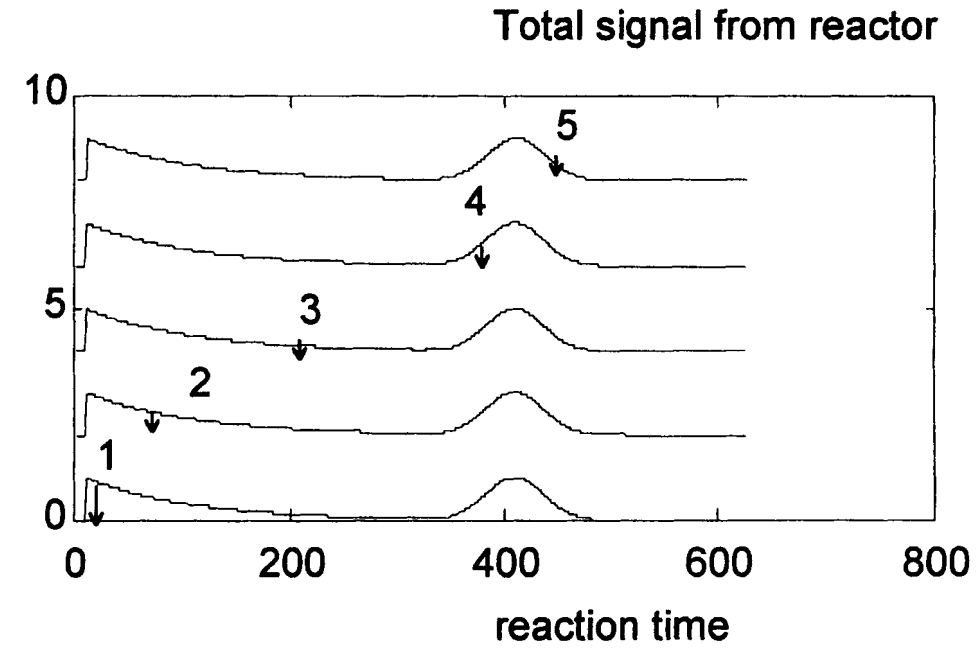
A typical experimental setup in chromatography for heterogeneous catalysis studies with stroboscopic sampling is simple, and is explained later. A process is initiated and sampled by computer, which also records the detector signal. The data are generally presented as a 2D response surface with axes of reaction running time and chromatographic retention time.

2. Examples of Stroboscopic Sampling in Kinetic Studies

As an example of several possibilities and limitations of the stroboscopic sampling technique, a study of hydrocracking of hydrocarbons to methane as a final product is presented. For example, for the Ni-SiO_2-catalyzed alkanes, the following reaction mechanism is valid:

$$n\text{-}C_n + H_2 \rightarrow n\text{-}C_{n-1} + CH_4 \quad (12)$$

Recently, this reaction was found to be suitable for designing a novel type of total organic carbon (TOC) analyzer, an alternative to the existing TOC analyzers.[61] The latter instruments convert organic matter to CO_2. Measuring the methane amount would enable the use of flame ionization detectors instead of the thermal conductivity ones required for CO_2 detection to detect lower quantities of TOC. The optimal temperature of the reaction is important to achieve a complete conversion of all reactants to methane. Other-



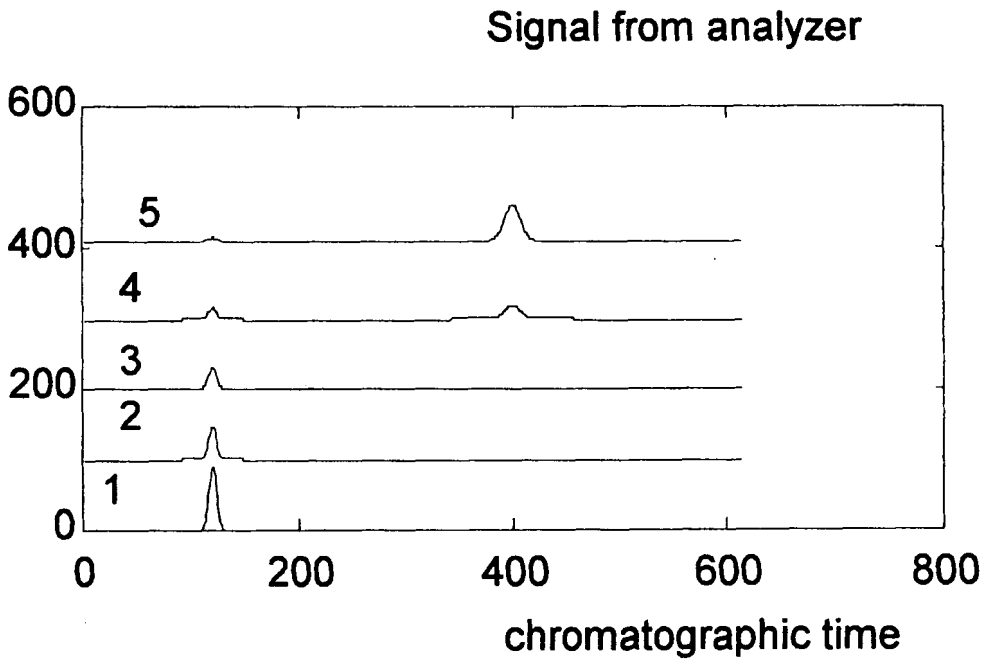


FIGURE 14. Explanation of idea of stroboscopic sampling in case of hypothetical A → B type reaction occurring in chromatographic column. (A) Total concentration function of exponentially decreasing product B and spreading reactant A; (B) related chromatograms taken at determined time moments.

wise, according to the reaction sequence predicted by Equation 12, the whole set of hydrocarbons will be detected, and because of different response factors, retention times in the reactor column, and several other parameters, the result will be inaccurate. Thus,

the composition and form of the narrow substance impulse passing the catalytic column at different temperatures are important. Stroboscopic sampling would provide a convenient method for such studies.

The experimental setup for hydrocracking reaction studies is based on two 6-port mechanical Valco valves (Figure 15). The reactant vapor flow was generated in a thermostated vessel and periodically sampled from the flow with a loop and carried by the H_2 flow to the reactor column (10 × 3 mm stainless steel, filled with Ni-SiO_2 catalyst). The impulse of the reactant and the products that passed the reactor were carried through the second valve and sampled to the analytical column. The reaction initiation and products sampling moments were generated by computer following the idea of stroboscopic sampling. Details of the experiment procedure, setup, and catalyst preparation can be found in References 62 to 64.

A set of chromatograms appearing in heptane hydrocracking is given in Figure 16, which corresponds to the schematics presented in Figure 14. Increasing the temperature favors a more complete conversion of the reactant to methane when the impulse passes through the catalyst column, and at a certain temperature all peaks, except for CH_4, disappear from the chromatogram. Thus, the stroboscopic sampling method provides the possibility of finding the optimal reactor temperature when other working parameters of the reactor have been fixed (hydrogen flow rate, dimensions, catalyst nature, and state). The other parameters mentioned above can also be optimized using stroboscopic sampling as easily as the temperature for the methane peak area/other peak area ratio maximization.

Besides solving practical problems, a study of the hydrocracking reaction is of academic interest as well. The concentration curves for different products can be given in the first approximation as follows

$$C(t) = C_0(e^{-kt} - e^{-at})$$

where C(t) is the product concentration in the end of the reaction column at time moment t and k and a are chemical reaction and transport rate constants. From the Arrhenius plot of log(k) vs. the reciprocal temperature 1/T, the activation energy of reaction can be easily found by the standard methods used in kinetics. For the dehydration reaction on Porasil catalyst coated with phosphoric acid[63] the activation energies appear to be as follows: *n*-butanol, 123 ± 10 kJ/mol, *n*-heptane, 30 ± 8 kJ/mol; acetone, 92 ± 25 kJ/mol, and benzene, 94 ± 16 kJ/mol.

Another application of stroboscopic sampling is a study of the thermooxidative degradation of alcohols.[65-67] The experimental setup is similar to that of Figure 15, where the only difference is that the catalytic reactor is replaced by the flow-through reactor. The reactant vapors flow through the reactor. Computer controlled thermal pulses, by passing current through the platinum wire rounded over the reactor, with a duration 1 ÷ 20 s and a maximum temperature of 900°C, are delivered to the sample. The sampling valve for the reaction products to the column is a pneumatic switch. A typical result is shown in Figure 17.

Other applications of stroboscopic sampling for various reaction studies are listed in Table 3.

So far, the application of stroboscopic sampling to thermooxidative processes has demonstrated its capabilities more than have fundamental investigations. The first results are promising, determining important points in the temperature scale where the reaction course changes significantly. On the other hand, kinetics studies of the dehydration reaction by stroboscopic sampling enabled us to find the activation energy of reaction, compare different mathematical expressions for concentration curves with respect to the best fit, and speculate about reaction mechanisms. Thus, strobochromatography provides a good opportunity for a quick screening of the properties of different catalysts.

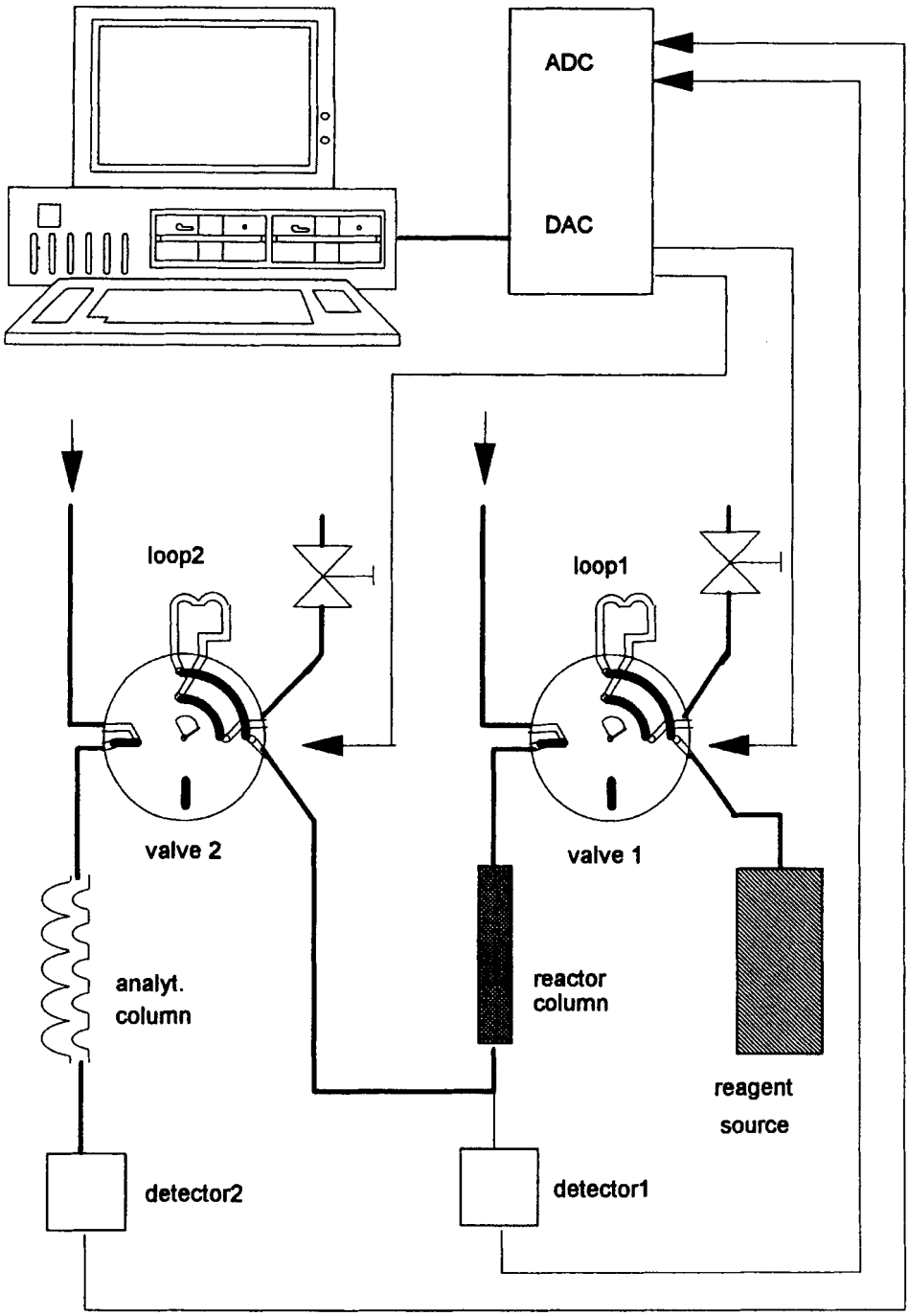


FIGURE 15. Schematic of chromatographic equipment with stroboscopic sampling.

B. Equiinterval Sampling

The simplest multiinput chromatographic approach is equiinterval sampling. According to Table 1, equiinterval sampling can be applied to slow processes where high concentrations of components are involved. This is the most common application of chromatography to reaction studies. In computerized applications of this kind of study, the

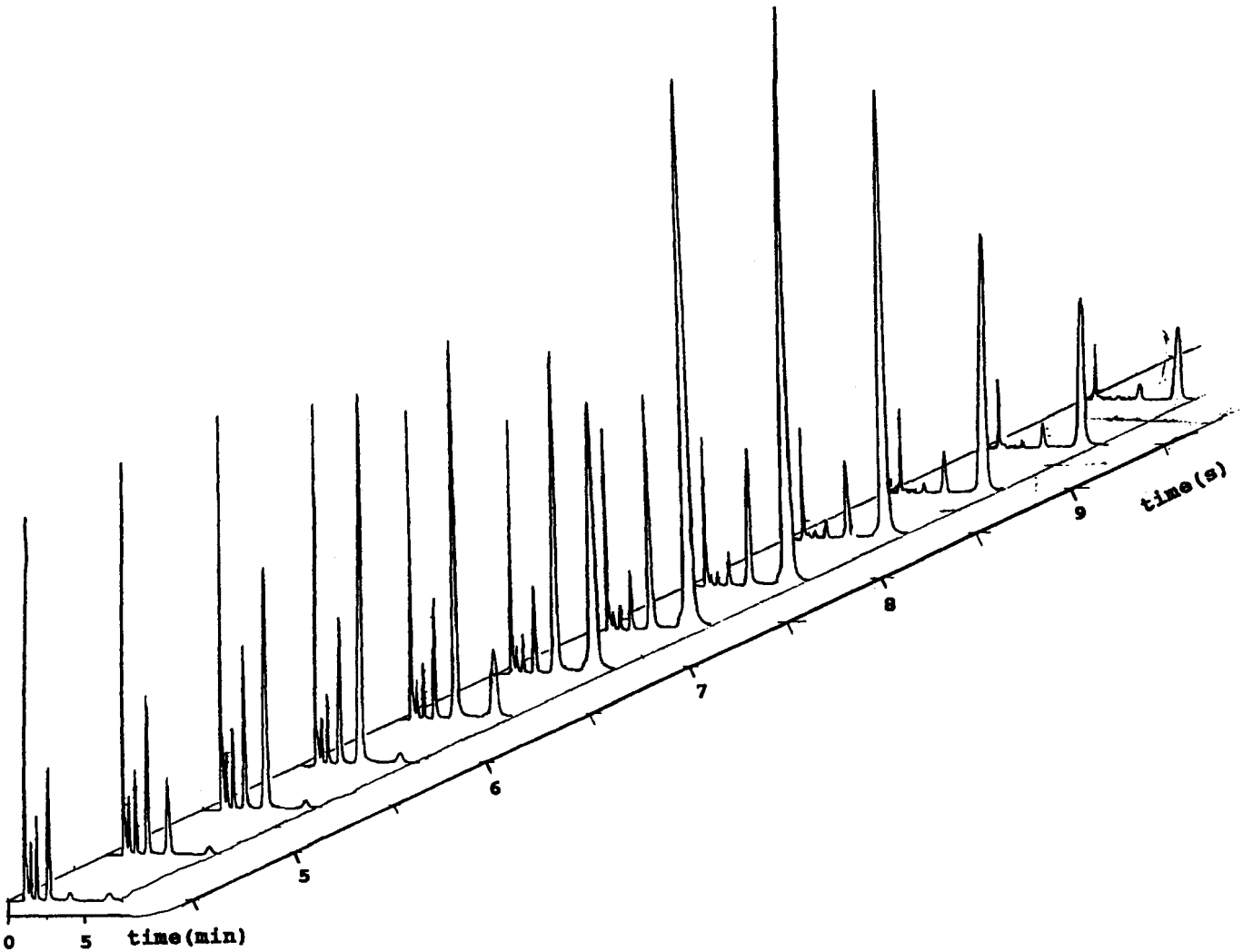


FIGURE 16. Set of chromatograms from heptane hydrocracking experiment. Peaks are n-hydrocarbons from C_1 to C_7.

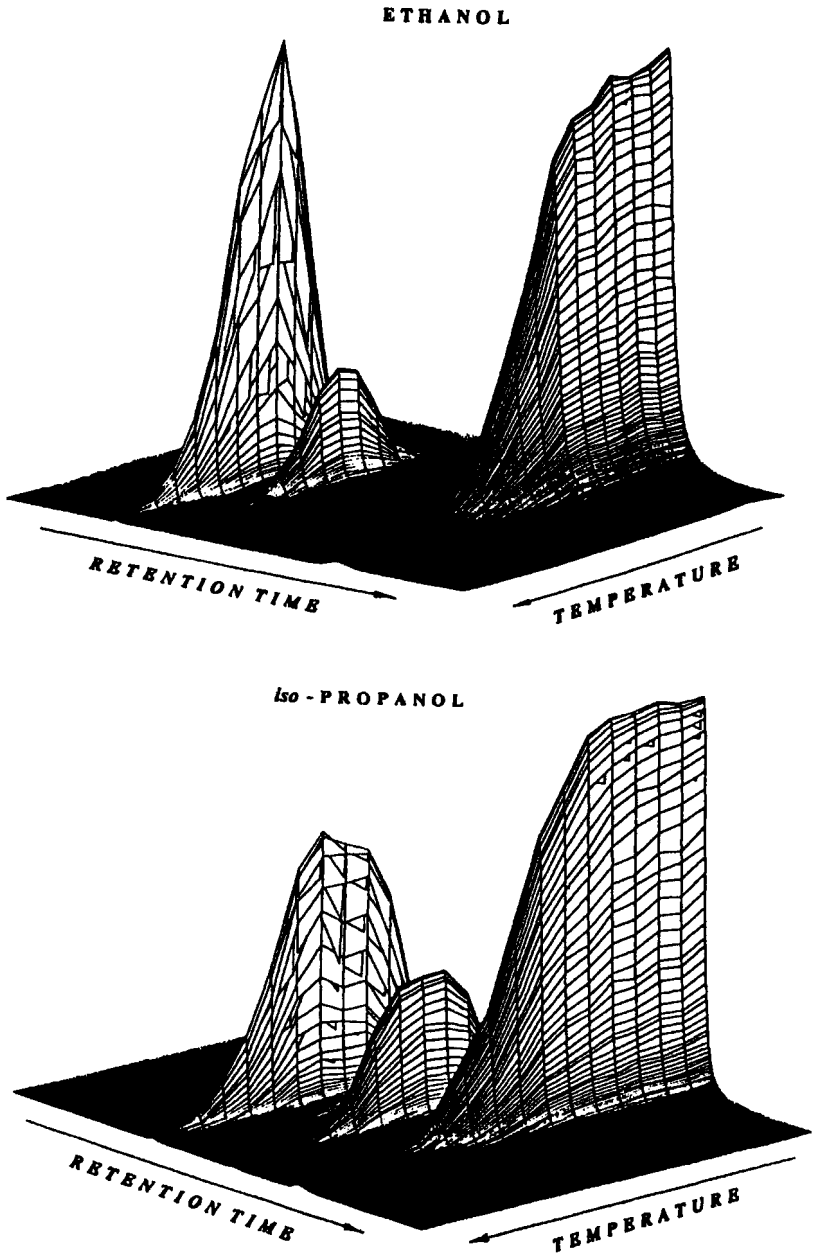


FIGURE 17. 2D output of thermooxidative degradation of alcohols using stroboscopic sampling. Retention time runs from 0 to 60 s; temperature runs from 100 to 800°C, with 25°C.

selection of possible input systems is wider, so that almost all common computer-driven equipment and autosamplers may be used. In the simplest form, it means withdrawing a sample from the reaction vessel with a syringe and injecting it into the chromatograph by the laboratory robot hand. More elaborate techniques such as stop flow or reversed flow[68] can be included in this classification. As this technique is rather straight-

TABLE 3
Application of Stroboscopic Sampling to Reaction Kinetic Studies

Reaction	Reactor	Initiation method	Sampling device	Time resolution(s)	Ref.
Dehydration of *n*-butanol	20 × 3 mm stainless steel tube with H_3PO_4 on Teflon	Injection of reagent pulse into reactor	Mechanical 6-port valve	1.0	64
Thermo-oxidative degradation of ethanol	50 × 2 mm quartz tube	Microwave heating of Cr/Ni wire	Deans-type pneumatic valve	0.15	64
Catalytic conversion of alcohol to olefins	70 × 3 mm glass tube with H3PO4 on Porasil	Injection of reagent pulse into reactor	Mechanical 6-port valve	1.0	62–64
Ignition of poly-propylene	30 × 3 mm quartz tube	Ignition of pyrolysis products with thermal pulse	Deans-type pneumatic valve	0.05	62, 66
Ignition of ethanol	30 × 3 mm quartz tube	Ignition of ethanol vapor with thermal pulses	Two mechanical 6-port valves	0.1	67

forward, the role of the computer is in improving the precision of the sampling interval, the probe quantity sampled (compared with human effort), and automation of analysis. Also, the computer has an important role in the handling and final presentation of the results. Table 4 shows the variety of processes that can be studied by equiinterval sampling.

Table 4 deserves comment. The thermochromatography of polymers is a rather old characterization of the light gases evolved from polymers during heating. The technique consists of sampling evolved gases from the reactor to the GC column during the programed heating of a sample. The long separation time of products in packed columns used in the earlier variants of thermochromatography[77] was a disadvantage of the technique. It led to coarse temperature resolution. Researchers attempted to overcome this disadvantage by trapping the evolved components[78] or by using the pulse heating of a sample ("stepwise" pyrolysis).[79,80]

Short capillary columns enabled separation of the evolved products in 1 to 2 min[52,69,70] when using a 1 to 5°C/min heating of polymer samples. This gives about a 1 to 10°C temperature resolution, which is enough for practical purposes because the evolved gas peaks in the temperature scale are hardly narrower than, say, 10°C. Thus, combining the thermal method with chromatographic methods of separation and identification of volatile products should give a thermal analysis method that in principle combines the advantages of the two systems as well as pyrolysis as thermogravimetry. Some drawbacks of this method are that (1) very polar and high boiling compounds are not detected due to trapping in the chromatographic column and (2) there is no temperature programing of the chromatographic column, and therefore most chromatographic peaks overlap, and (3) the interpretation can be based on groups of evolving products. Neverthe less, the technique should compete well with common thermogravimetry because the price of the system (GC, interfaces, reactor, valve, and computer) is far lower than that of a contemporary thermogravimeter, and the thermogravimetric curve can be easily synthesized from the evolution curves (assuming that a proper detector sensitivity to all compounds is used). Moreover, presenting the result of ThGC as a 2D surface gives at a glance a visualization of the complexity of a degradation reaction.

Recently, an interesting application of equiinterval sampling GC to supercritical

TABLE 4
Some Applications of Equiinterval Sampling

Problem	Sampling device	Ref.
Transesterification of alchohols	Laboratory robot	15
Thermochromatography of polymers	Deans-type pneumatic valve	52, 69, 70
2D HPLC & CZE	Mechanical valve with internal loop	71, 72
2D gas chromatography	Thermal modulator	73, 74
Continuous monitoring at trace levels	Sequential valve-microtrap with thermal desorbtion	75
Monitoring of hydrodesulfurization kinetics	Thermal modulator	76
Monitoring of supercritical fluid extraction kinetics	Thermal modulator	22

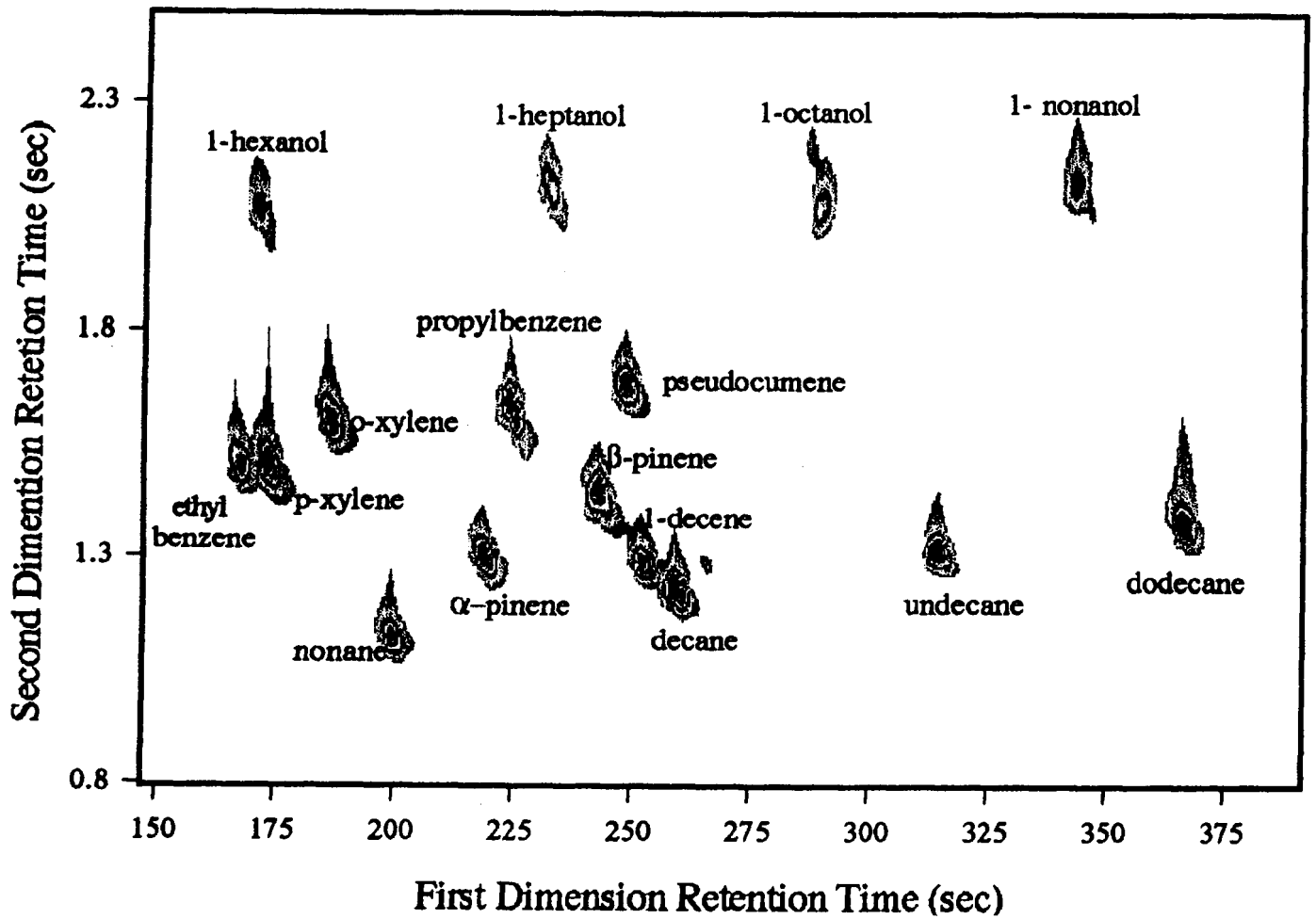


FIGURE 18. Complete 2D chromatography of mixture containing 14 components. For experimental conditions, see Reference 73. (From Liu, Z.; Phillips, J. B. *J. Chromatogr. Sci.* **1991**, *29*, 227. With permission.)

fluid extraction (SFE) kinetics was published.[22] SFE emerged recently as an alternative method of preparing samples before analysis. This extraction method is more efficient than the commonly used Soxhlet extraction, but little is known about the extraction process itself. The system proposed by Liu provides semicontinuous monitoring of the SFE process by using a thermal modulator for sample introduction into a short capillary column.

Other equiinterval sampling applications deal with comprehensive 2D gas chromatography (HRGC)[73] and capillary zone electrophoresis (CZE).[71,72] These systems are similar to the 2D, two-column chromatographic system. If the separation in the second medium is carried out fast enough (a few seconds), it becomes possible to sample and separate the overlapping bands emerging from the first column very rapidly. Thus, comprehensive 2D chromatography becomes possible[74] as shown in the form of contour plot in Figure 18. Identified in the literature as conventional 2D gas chromatography, the commonly known method of heart cutting also couples serially two different chromatographic columns. Heart cutting, however, is not a real 2D method because the secondary instrument (column) cannot be applied to the entire primary process (chromatogram eluting from the inlet GC).

The time interval between sampling time resolutions in all applications listed in Table 3. has always been chosen intuitively, demonstrating an analyst's ignorance of the possibilities of estimating and optimizing the time resolution using the results of the Nyquist sampling theorem.

VI. ANALYSIS OF 2D CHROMATOGRAMS

As pointed out earlier, the result in all applications is the raw data array that can be reshaped into a matrix. This gives better visibility and the possibility of using chemometric methods for data analysis to full advantage. The output for display also is reconstructed from the sequence of chromatograms of the second column as a 2D contour or stack plot.

Estimation of component number and the functions of component variations in time are usually the aim of data processing. 2D processing is trivial when the primary process separation in time and the secondary chromatographic separation is complete, but this happens very rarely, and the rerË®t is a 2D surface with overlapping peaks. In this case, chemometric methods such as factor analysis could be of help. The same approach used for GC-MS, FT-IR, and LC-diode matrix detector signals can be used to process the 2D data obtained by random or regular sampling.

As an example of 2D data obtained by regular sampling consider the case of ThGC, where the "component" in the primary dimension means a set of chemical compounds released in the same (thermal) process (see Figure 2). This means, for example, that every process can be described by a single chromatogram when all peak intensities are changing by the same law (called a "thermogram" in the case of thermal processes). To be more specific, let us assume that a sample degrades in two steps, and during the first step a set of compounds is released. This set has a chromatogram $h_1(t)$, where t is time. The second step releases a set of compounds that has a chromatogram $h_2(t)$. Let us assume that the first set of compounds evolves by the function $c_1(T)$, where T is the temperature, and the second set by the function $c_2(T)$. Digitizing the data for computer treatment, the functions $h_1(t)$ and $h_2(t)$ are measured with the sampling interval Δt. This results in vectors

$$h_1' = \{h_{11}, h_{12}, \ldots, h_{1n}\} \quad \text{and} \quad h_2' = \{h_{21}, h_{22}, \ldots, h_{2n}\}$$

where $'$ means the transpose.

The functions $c_1(T)$ and $c_2(T)$ are recorded with the temperature resolution ΔT. The resulting vectors are

$$c_1' = \{c_{11}, c_{12}, \ldots, c_{1m}\}$$

and

$$c_2' = \{c_{21}, c_{22}, \cdots, c_{2m}\}$$

Taking, for example, n = 4 and m = 3, the following equation for the data matrix D is obtained:

$$D = \begin{bmatrix} h_{11}h_{21} & \\ h_{12}h_{22} & c_{11}c_{12}c_{13} \\ [h_{13}h_{23}] & [c_{21}c_{22}c_{23}] = h * c' \\ h_{14}h_{24} & \end{bmatrix}$$

$$= \begin{bmatrix} h_{11}c_{11} + h_{21}c_{21}h_{11}c_{12} + h_{21}c_{22}h_{11}c_{13} + h_{21}c_{23} \\ h_{12}c_{11} + h_{22}c_{21}h_{12}c_{12} + h_{22}c_{22}h_{12}c_{13} + h_{22}c_{23} \\ h_{13}c_{11} + h_{23}c_{21}h_{13}c_{12} + h_{23}c_{22}h_{13}c_{13} + h_{23}c_{23} \\ h_{14}c_{11} + h_{24}c_{21}h_{14}c_{12} + h_{24}c_{22}h_{14}c_{13} + h_{24}c_{23} \end{bmatrix} \quad (13)$$

Equation 13 reflects the hypothesis that a thermochromatogram consists of bilinear datum that occurs when the detector responds in a linear fashion to each detectable species and the signals of these species are linearly additive.

Generally, neither the number of components nor the functions $h_i(t)$ and $c_i(t)$ are known beforehand, and they are to be found by analyzing the data matrix D. The discovery of independent but overlapping evolution processes demands that one operate on raw data in order to deconvolute the contribution of each process to the observed response surface.

The first attempt to factor analyze ThGC patterns as a set of chromatograms based on their different origin in temperature in the matrix presentation was made by Elomaa.[81] He demonstrated that, like the diode array detection in HPLC, the number of components in thermochromatography can be found by abstract factor analysis (AFA). He also demonstrated that the original ThGC data can frequently be represented by two principal components only, and the first eigenvector for polymers was comparable to the differential TG curve of the material. However, in general, the results of AFA are principal components of the ThGC pattern that have no chemical meaning (e.g., the "chromatograms" and "thermograms" obtained by AFA contain peaks with negative intensities). AFA "absorbs" only the ThGC pattern variability into a few eigenvectors (and its designees), protecting at the same time the information that the pattern may contain. The variability due to noise is transformed to the rest of the eigenvectors. Thus, AFA is extremely useful for data compression.

More information about gas evolution processes in a sample may be obtained from 2D data by the target transformation of abstract factors[82] (see Chapter 6 in Reference 82 and related literature). This procedure, however, depends on the investigator's intuition and experience. The aim of target transformation is to transform abstract "chromatograms" and "evolution curves" to real ones (i.e., to find the h and c matrices in Equation 13). Obviously, this solution may not be unique — chemometricians talk about rotational ambiguities of bilinear data, and several constraints should be applied. One such constraint is, of course, the fact that, as in the case of spectrometry/chromatography, chromatograms, spectra, and evolution curves cannot contain regions with negative intensities.

The second approach is to use the intrinsic order of the data matrix. Evolving factor analysis (EFA)[83] is a general method for the analysis of these kinds of data. In ThGC, the output data have these kinds of intrinsic order, and EFA was used to resolve overlapping chromatograms based on their different origin in pyrolysis temperature.[84] The result was a set of temperature profiles and their corresponding chromatograms of evolving headspace gases, as presented in Figure 19.

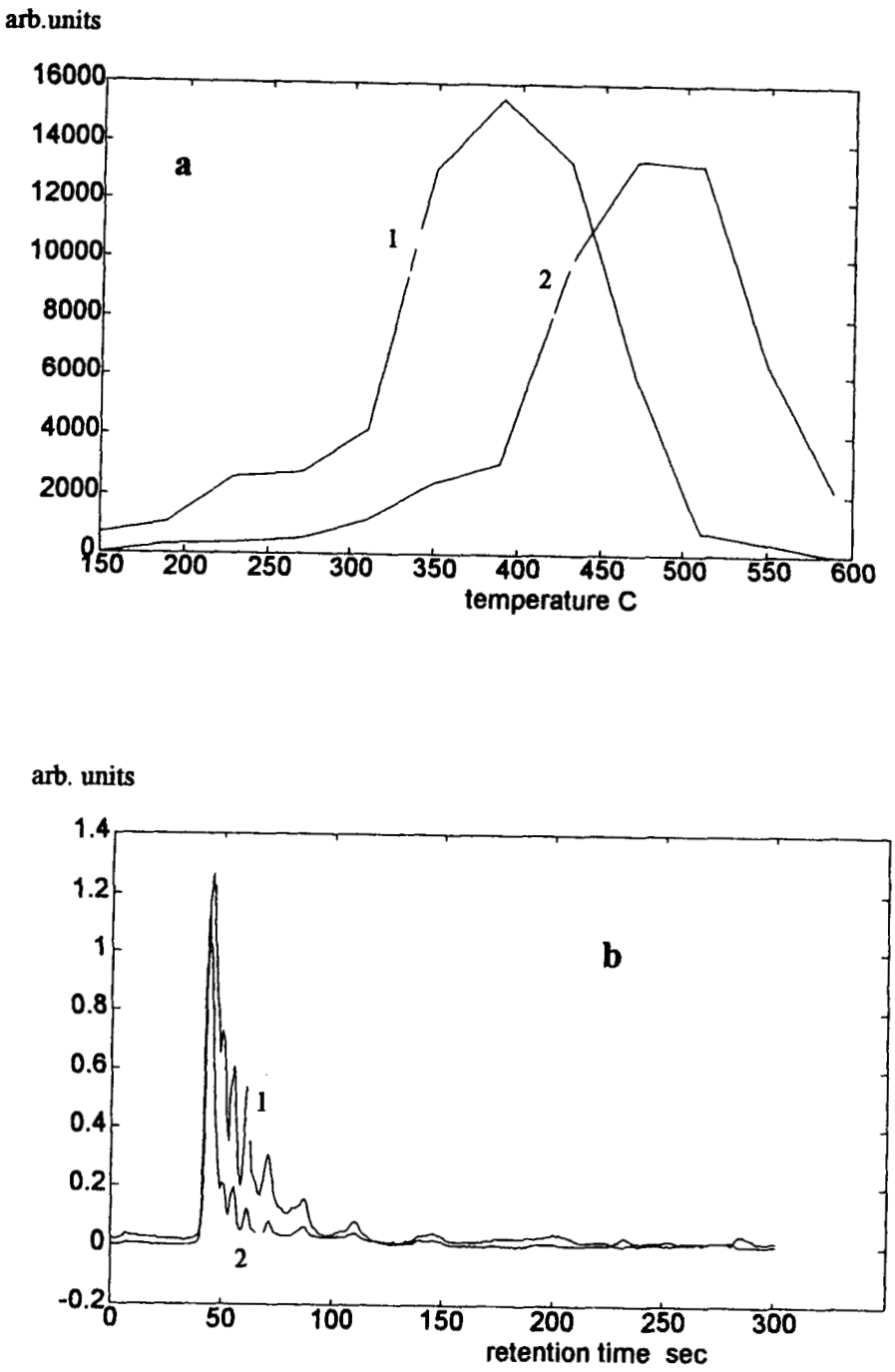


FIGURE 19. Results of evolved factor analysis of thermochromatograms of Kukersite (Estonia) oil shale sample. (A) Temperature profiles; (B) calculated chromatograms of evolving volatiles related to thermal profiles.

If there are some areas where only one component on the 2D surface can be considered to dominate the region of possible solutions can be narrowed even more. The heuristic evolving latent projections (HELP)[85] add some useful insights toward resolving multidimensional data into pure components. From the whole data matrix, the latent variables are found by principal component decomposition. Selective regions are visually detected through inspection of bivariate score and loading plots.

The selective regions appear as straight lines through the origin in all combinations of principal components. In the case of ThGC[86] these graphs did not show enough temperature resolution to obtain details in the temperature region where most of the degradation was taking place (450 to 550°C).

Frequently, the 2D surfaces obtained are a unique "fingerprint" of the sample. In this case, the conditions for chromatographic and thermal resolution are not so rigid. The stability of the chromatographic conditions as well as the temperature at which sampling is done are more important. The samples are differentiated by the use of pattern recognition algorithms,[87] where a parameter is calculated to evaluate the similarity (distance) of two thermochromatograms. Two examples of such different parameters are (1) the Euclidean distance, d, between two 2D chromatograms

$$d_{xy} = \left[\Sigma_{ij}\left(X_{i,j} - Y_{i,j}\right)^2\right]^{1/2}$$

where d_{xy} is changing between the limits $0 < d < \infty$ (when $d = 0$, then the chromatograms are identical) and (2) the Pearson product-moment correlation coefficient (r) of two 2D chromatograms[88]

$$r_{xy} = \frac{\Sigma_{ij}X_{i,j}Y_{i,j} - 1/N\left(\Sigma_{ij}X_{i,j}\right)\left(\Sigma_{ij}Y_{i,j}\right)}{\left\{\left[\Sigma_{ij}X_{i,j}^2 - 1/N\left(\Sigma_{ij}X_{i,j}\right)^2\right]\left[\Sigma_{ij}Y_{i,j}^2 - 1/N\left(\Sigma_{ij}Y_{i,j}\right)^2\right]\right\}^{1/2}}$$

In this case, r_{xy} varies within $-1 < r < 1$, and when $r = 0$, the chromatograms are not correlated.

In both cases, $X_{i,j}$ and $Y_{i,j}$ denote the intensity of the chromatograms at the corresponding temperature and retention times, t_i and $_j$, respectively. The parameter N is the number of data pairs. The values of d and r provide quantitative measurements of differences and similarities of thermochromatograms, respectively, which are useful for comparison.

Using other chemometrical tools, one can correlate the 2D patterns obtained for the set of objects with some physical or chemical parameters of interest for the same samples (e.g., multivariate calibration techniques[89]). When using this approach, it is not necessary to have a good chromatographic resolution (the dream of every chromatographer), and interesting properties can be derived from low-resolution chromatograms.

Decreasing the resolution in the case of ThGC provides an opportunity to decrease the GC analysis time and more chromatograms can be obtained during heating, which means better resolution in the temperature scale. This approach outlines the new possible trend in evolved gas analysis where the entire evolution pattern is related to the property of the sample by chemometrical means. Much remains to be done.

VII. CONCLUSIONS

Multiinput chromatography can be considered the next step in the automation of chromatographic analysis. This article attempted to provide some direction to this step by discussing the instrumental and computational aspects of the topic. The method does not require extensive hardware modification of the chromatographic apparatus but is computationally intensive. Many results of contemporary chemometrics have been found to be applicable to computerized chromatography, and this was the main reason for considering computerized multiinput chromatography as part of chemometrics. Being in its infancy, the chemometric analy-

sis of 2D data raises more problems than it solves.

The practical applications presented in this paper demonstrate that computerized chromatography is not just redoing old things by using computers instead of a ruler and calculator, it really opens new possibilities for the application of chromatography.

For the analysis of rapid time-varying processes two main approaches are possible:

1. In the case of reproducible processes, stroboscopic sampling with time resolutions starting from 50 ms and longer, with no limitations on process composition or functional form imposed
2. For unique processes (such as passing the probe with GC through the planet atmosphere), random sampling with sophisticated data processing (changes in concentrations must be smooth or the sample compositions should be known beforehand)

One interesting question is how much can one sacrifice to instrument performance and data production quality. It is evident that increasing the measurement precision to a new qualitative level requires much investment in equipment design. Computerized experiments and data processing give an opportunity to raise measurement quality without redesigning or rebuilding the experimental setup. The best example of this is the use of correlation procedures to improve the performance of modestly sensitive chromatographic detectors.

REFERENCES

1. Kaljurand, M.; Küllik, E. *Computerized Multiple Input Chromatography;* Ellis Horwood: Chichester, 1989.
2. Kelly, P. C.; Horlick, G. *Anal. Chem.* **1973,** *45,* 518.
3. Gaspar, G. *J. Chromatogr.* **1991,** *556,* 331.
4. Schutjes, C.; Vermeer, E.; Cramers, C. *J. Chromatogr.* **1983,** *253,* 49.
5. van Es, A.; Janssen, J.; Cramers, C.; Rijks, J.; *J. High Resolut.Chromatogr.* **1988,** *11,* 852.
6. Annino, R. *J. High Resolut.Chromatogr.* **1996,** *19(5),* 285.
7. Chen, D. J.; Swerdlow, H. P.; Harke, H. R.; Zhang, J. Z.; Dovichi, N. J. *J. Chromatogr.* **1991,** *559,* 237.
8. Haab, B. B.; Mathies, R. A. *Anal. Chem.* **1995,** *67(18),* 3253.
9. Booksh, K. S.; Kowalski, B. R. *Anal. Chem.* **1994,** *66(15),* 782A.
10. Data Acquisition Control System S, Series 500, Hardware, Software and Accessories, Catalog and Reference Guide 1993-1994, Keithley Instruments ThC.
11. Instrupedia 96, Interactive Encyclopedia for Instrumentation, National Instruments Corporation, 1996.
12. Dessy, R. *Anal. Chem.* **1986,** *58(7),* 793A.
13. Noble, D. *Anal. Chem.* **1995,** *65(17),* 617A.
14. Lochmüller, C. H.; Lung, K. R.; Lloyd, T. L.; Kaljurand, M. *Zymark Lab. Autom. Newsl.* **1985,** *2,* 6.
15. Lochmüller, C. H.; Lloyd, T. L.; Lung, K. R.; Kaljurand, M. R. *Anal. Lett.* **1987,** *20,* 1237.
16. Mars, C.; Smit, H. C. *Anal. Chem. Acta.* **1990,** *228,* 193.
17. Gaspar, G.; Arpino, P.; Guiochon, G. *J. Chromatogr. Sci.* **1977,** *15,* 256.
18. Gaspar, G.; Olivo, J. P.; Guiochon, G. *Chromatographia.* **1978,** *11,* 321.
19. Deans, D. R. *Chromatographia.* **1968,** *1,* 18.
20. Deans, D. R. *J. Chromatogr.* **1984,** *43,* 189.
21. Phillips, J. B.; Luu, D.; Pawliszyn, J.; Carle, G. C. *Anal. Chem.* **1985,** *57,* 2779.
22. Liu, Z.; Farnsworth, P. B.; Lee, M. L.; *J. Microcol. Sep.* **1992,** *4,* 199.
23. Engelsma, M.; Smit, H. C. *Chromatographia.* **1991,** *31,* 393.
24. Engelsma, M.; Louwerse, D. J.; Boelens, H. F. M.; Kok, W. Th.; Smit, H. C. *Anal. Chem. Acta.* **1990,** *228,* 209.

25. Carney, D. P.; Phillips, J. B. **1986,** *58,* 1251.

26. Valentin, J. R.; Garle, G. C.; Phillips, J. B. *HRC CC.* **1983,** *6,* 621.

27. Laster, W. G.; Pauliszyn, J. B.; Phillips, J. B. *J. Chromatogr. Sci.* **1982,** *20,* 278.

28. Valentin, J. R.; Garle, G. C.; Phillips, J. B. *Anal. Chem.* **1985,** *57,* 1035.

29. Engelsma, M. Chemical Concentration Modulation in Correlation Chromatography; Ph.D. dissertation, University of Amsterdam, 1991.

30. Data Acquisition and Analysis, National Instruments Catalog, 1995.

31. Treado, P. J.; Morris, M. D. *Anal. Chem.* **1989,** *61,* 723 A.

32. Farrar, T.; Becker, E. D. *Pulse and Fourier Transform NMR;* Academic Press, 1971.

33. R.J. Bell, *Introduction to Fourier Transform Spectroscopy;* Academic Press: New York, 1972.

34. Harwit, M.; Sloane, N. J. A. *Hadamard Transform Optics;* Academic Press: New York, 1979.

35. Tilotta, I. C.; Hammaker, R. M.; Fatley, W. G. *Appl. Spectrosc.* **1987,** *41,* 727.

36. Smit, H. C.; Kaljurand, M. *J. Chromatogr.* **1993,** *642,* 53.

37. Kaljurand, M.; Urbas, E.; Haldna, Ü. *J. Chromatogr.* **1994,** *660,* 147.

38. Yang, M. Y.; Pawliszyn, J. *Anal. Chem.* **1993,** *65,* 1758.

39. Kaljurand, M.; Ebber, A.; Sõmer, T. *J. High Resolut. Chromatogr.*, **1995,** *18(4),* 263.

40. Phillips, J. B. *Anal.Chem.* **1980,** *52,* 468.

41. Mars, C.; Smit, H. C. *Anal. Chem. Acta.* **1990,** *228,* 193.

42. Kunt, M. *IEEE Trans. Comput.* **1975,** *C-24,* 1120.

43. Kaljurand, M. *J. Chromatogr.* **1979,** *186,* 145.

44. Mulder, R.; Hoogerbrugge, P.; Smit, H. C. *Chem. Int. Lab. Syst.* **1987,** *1,* 243.

45. Kaljurand, M.; Urbas, E.; Haldna, U. *Chromatographia.* **1992,** *34,* 417.

46. Engelsma, M.; Louwerse, D. J.; Boelens, H. F. M.; Kok, W. Th.; Smit, H. C. *Anal. Chem. Acta.* **1990,** *228,* 209.

47. Frazer, S.; Burke, M. *Anal. Chem. Acta.* **1985,** *177,* 15.

48. Louwerse, D. J. Correlation Techniques in Chromatography: Extending the Scope and Limits of Measurement, Ph.D. dissertation, University of Amsterdam, 1995.

49. Smit, H.; Kaljurand, M. *Chromatographia.* In press.

50. Koel, M.; Kaljurand, M.; Küllik, E. *Proc. Est. Acad. Sci. Chem.* **1983,** *32,* 125.

51. Hoffmann, R.; Gupta, M. M.; Nikiforuk, P. N. *Proc. IEE,* **1972,** *19,* 237.

52. Küllik, E.; Kaljurand, M.; Lamberg, M. *Int. Lab.* **1987,** *17(1),* 72.

53. Kaljurand, M.; Takjas, V. *Proc. Est. Acad. Sci. Chem.* **1992,** *41,* 73.

54. Raulin, F.; Vanssay, G.; Do, L.; Paillous, P. *LC GC.* **1992,** *5,* 22.

55. Valentin, J. R. *LC GC.* **1989,** *2,* 32.

56. Valentin, J. R. *Multiplex Gas Chromatography for Use in Space Craft;* NASA Tech. Memo 8 6668, January 1985.

57. Valentin, J. R.; Hall, K. W. *HRC-CC.* **1989,** *12,* 53.

58. Valentin, J. R.; Hall, K. W.; Becker, J. F. *J. Chromatogr.* **1990,** *518,* 199.

59. de Vanssay, E.; Zubrzycki, S.; Sternberg, R.; Raulin, F.; Sergent, M.; Phan-Tan-Luu, R. *J. Chromatogr. A.*, **1994,** *688,* 161.

60. Snyder, L. R.; Dolan, J. H.; Van der Wal, Sj. *J. Chromatogr.* **1981,** *203,* 3.

61. Stolyarov, B. V.; Galev, E. E. *Russ. Water Chem. Technol.* **1988,** *10,* 34.

62. Küllik, E.; Kaljurand, M. J. *Chromatography.* **1990,** *517,* 175.

63. Bureiko, A.; Kaljurand, M.; Marinichev A.; Stolyarov, B. *Proc. Est. Acad. Sci. Chem.* **1991,** *40,* 157.

64. Kaljurand, M.; Lamberg, M.; Küllik, E. *Proc. Est. Acad. Sci. Chem.* **1988,** *37,* 78.

65. Kaljurand, M.; Ebber, A. *Proc. Est. Acad. Sci. Chem.* **1996,** *45,* in press.

66. Kaljurand, M. *Proc. Est. Acad. Sci. Chem.* **1990,** *39,* 218.

67. Kaljurand, M. *Russ. J. Phys. Chem.* **1990,** *64,* 187.

68. Stolyarov B.; Kartsova, A. L. *Russ. Adv. Chem.* **1987,** *56,* 1024.

69. Küllik, E.; Kaljurand, M.; Lamberg, M. *Russ. J. Anal. Chem.* **1986,** *41,* 1425.

70. Elomaa, M.; Salo, S.; Linberg, J.; Pilt, L.; Kaljurand, M.; Küllik, E. *Kemia Kemi.* **1988,** *15,* 875 (in Finnish).

71. Bushey, M. M.; Jorgenson, J. W. *Anal. Chem.* **1990,** *62,* 978.

72. Hogan, B. L.; Lunte, S. M.; Stobaugh, J. F.; Lunte, C. E. *Anal.Chem.* **1994,** *66(5),* 596.

73. Liu, Z.; Phillips, J. B. *J. Chromatogr. Sci.* **1991,** *29,* 227.

74. Phillips, J. B.; Xu, J. *J.Chromatogr. A.* **1995,** *703,* 327.

75. Mitra, S.; Lai, A. *J. Chromatogr. Sci.* **1995,** 33(6), 285.

76. Mitra, S.; Phillips, J. B. *Anal. Instr.* **1989,** *18,* 127.

77. Liebman, S. A.; Bestrom, D. H.; Foltz, C. R. *J. Polym. Sci.* **1978,** *16,* 3139.

78. Yamada, K.; Oura, S.; Hasuki, T. *Proc. 4th Int. Conf. Thermal Analysis*, Budapest, 1974, 1029.

79. Ericsson, J. *J. Chromatogr. Sci.* **1978,** *16,* 340.

80. van Lieshout, M.; Janssen, H-G.; Cramers, C. A.; Hetem, M.; Schalk, H. *J. High Resolut. Chromatogr.* **1996,** *19(4),* 193.

81. Elomaa, M. *Pyrolysis and Combustion Studies of Polymers by Gas Chromatography and Thermal Analysis, Comment;* Physico-Mathematical et Chemico-Medical No. 128, Finnish Society of Sciences and Letters: Helsinki, 1991.

82. Malinowski, E. R.; Horwey, D. O. *Factor Analysis in Chemistry;* John Wiley & Sons: New York, 1980.

83. Maeder, M.; Zuberbühler, A. D. *Anal. Chim. Acta.* **1986,** *181,* 287.

84. Elomaa, M.; Lochmüller, C. H.; Kaljurand, M.; Koel, M. *Chem. Int. Lab. Syst.* **1995,** *30(1),* 129.

85. Kvalheim, O. M.; Liang, Y.-Z. *Anal. Chem.* **1992,** *64(8),* 936.

86. Koel, M.; Kaljurand, M.; Lochmüller, C. H.; Moebus, M. *Chem. Int. Lab. Syst.* **1995,** *30(1),* 173.

87. Jurs, P. C.; Isenhour, T. L. *Chemical Applications of Pattern Recognition;* John Wiley & Sons: New York, 1975.

88. Tanabe, K.; Saeki, S. *Anal. Chem.* **1975,** *47(1),* 118.

89. Sharaf, M. A.; Illman, D. L.; Kowalski, B. R. *Chemometrics;* John Wiley & Sons: New York, 1986.