Continuous bed technology is a promising area which allows convective flow in wider channels and at the same time smaller diffusion limitations in microspheres since they have a very small diameter.


Further Reading


Correlation Chromatography

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Principles

Correlation chromatography (CC, multiplex chromatography, multiple input chromatography) belongs to the family of multiplex methods and is essentially statistical in nature. It is a typical example of an integrated product of chemometric principles and an analytical technique. A schematic set-up of a CC system is shown in Figure 1.

In conventional chromatography the sample is injected over a short time, and the response of the chromatographic system – the chromatogram – can be considered as an impulse response. In CC the input

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In conventional chromatography the sample is injected over a short time, and the response of the chromatographic system – the chromatogram – can be considered as an impulse response. In CC the input
flow of the column is rapidly switched between the sample and the eluent, according to a (pseudo) random pattern. A cross-correlation function (correlogram) of the random input signal and the resulting very complex detector signal is also identical to the impulse response (chromatogram) of the chromatographic system, if the input satisfies certain conditions. In other words, the correlogram is identical to a conventional chromatogram obtained by a pulse-shaped injection.

In CC the sample is usually injected according to a pseudo random binary sequence (PRBS) pattern \( p(i) \), where \( i \) is the discrete time. A PRBS is a binary noise with a specific length \( M \), the sequence length, of \( 2^n - 1 \) periods (\( n \) is a positive integer) controlled by a clock; the only levels are \( +1 \) and \( -1 \), or \( 1 \) and \( 0 \). The \( M \) clock periods correspond to \( I = 2^n - 1 \) injections. The periodic nature of the PRBS input signals yields low estimate variance of the estimation of statistical quantities such as correlation functions if taken over an integer number of sequences.

The signal power of a PRBS, determining the final intensity of the detector response, is much higher than that of an impulse-like injection function with similar amplitude, and it is equally spread over the frequency range of the chromatographic system. This ‘white noise’ property is essential for the application of CC. In addition the levels can be used to control simple on/off valves, corresponding to injection of sample or mobile phase.

The detector signal \( y_i \) is built up of noise-free chromatograms \( b(j) \) shifted in time, according to the PRBS pattern, plus detector noise \( n(i) \):

\[
y(i) = \sum_{j=0}^{M-1} [b(j), p(i-j)] + n(i) \quad [1]
\]

The PRBS length is chosen to be equal to or longer than the time duration of the comparable chromatogram obtained from a single injection. The detector signal becomes circular after one PRBS sequence, the so-called presquence. The calculation of a correlogram comparable with a similar chromatogram requires the inverse of the PRBS, defined as the function \( p^{-1}(i) \), producing a Kronecker delta function \( \Delta(i) \) after circularly cross-correlating with \( p(i) \):

\[
R_{p^{-1}} (i) = \frac{1}{M} p^{-1}(j + i) \cdot p(j) = \Delta(i)
\]

\[
\Delta(i) = \begin{cases} 1 & \text{for } i = 0 \\ 0 & \text{for } i \neq 0. \end{cases} \quad [2]
\]

Owing to the special properties of a PRBS, the inverse calculated from a PRBS with one point per period and levels \( 1 \) and \( 0 \) gives the same PRBS, but with levels \( +M/I \) and \( -M/I \) instead of \( +1 \) and \( 0 \). Cross-correlating the detector signal \( y(i) \) with the inverse \( p^{-1}(i) \) results in a correlogram with a reduced noise level. In the calculations, non-correlated (white) noise is assumed:

\[
R_{p^{-1}, y}(k) = \frac{1}{M} \sum_{i=0}^{M-1} [p^{-1}(i+k) \cdot y(i)]
\]

\[
= \frac{1}{M} \sum_{i=0}^{M-1} \left\{ \sum_{j=0}^{M-1} [b(j) \cdot p(i-j)] + n(i) \right\}
\]

\[
= \frac{1}{M} \sum_{j=0}^{M-1} \left\{ b(j) \cdot \frac{1}{M} \sum_{i=0}^{M-1} [p^{-1}(i+k) \cdot p(i-j)] + n(i) \right\}
\]

\[
+ \frac{1}{M} \sum_{i=0}^{M-1} p^{-1}(i+k) \cdot n(i) \quad [3]
\]

Considering the levels \( +M/I \) and \( -M/I \) for \( p^{-1}(i+k) \), \( p^{-1}(i+k) \cdot n(i) \) can be replaced by...
The correlation procedure can be continued for an arbitrary integer number of sequences. Theoretically, noise not correlated with the input pattern can be reduced to any desired level – but at the cost of time – assuming that the chromatographic system is stationary and that enough sample is available. The noise reduction in only one sequence is about a factor of 10 to 20.

Correlation techniques can be applied in different column separation methods, applications in gas chromatography (GC), liquid chromatography (LC) and capillary zone electrophoresis (CZE) are known. Particularly in LC and CZE, the detection limit can be a problem and correlation techniques in principle offer possibilities to increase the signal-to-noise ratio considerably without preconcentration of the sample. Another feature is the possibility of using CC for continuous monitoring; CC allows a fast and almost continuous updating of the value of the varying concentrations to be monitored. However, the moving average effect, typical for the correlation procedure, limits the highest frequency that can be monitored. CC permits to monitor a frequency about a factor of 2 higher than conventional chromatography. A considerable improvement is possible, if the multiple injection (PRBS) input is maintained, but the correlation procedure is replaced by non-linear fitting. The time-varying concentrations are described as functions of the time and a number of parameters are optimized in the fitting procedure. A detector signal is calculated using the parameters, the known PRBS and known peak shapes; the squared differences with the real detector signal are minimized. The maximum frequency is not determined by the chromatogram length but by the peak width, orders of magnitude better.

**Instrumental Requirements**

The separation system, column, detector and separation conditions are similar in CC and in conventional chromatography. Nonlinearity of both the column and the detector, and poor stability, i.e. changing chromatographic conditions during the procedure, influence the correlation procedure, resulting in a disturbed baseline. Modern chromatographic systems fulfill the stringent demands of CC in this respect. An important modification is the special injection device required. Such a device has to meet several demanding requirements such as high reproducibility, absence of memory effects, high switching speeds, rugged design (no wear and tear problems) and controllability by a computer. Incorrect injection will cause disturbances (ghost peaks) are specific relative positions on the time axis, and in general cause so-called ‘correlation noise’ proportional to the amplitude of the real peaks. This therefore limits the determination of traces in the presence of the main components. Injection of sample for one clock period of the PRBS ideally results in an amount of sample transported to the column that is solely dependent on the value of the PRBS in the clock period concerned; 1 represents injection of sample and 0 represents no injection of sample. However, in general the injection is not ideal. After each 0 to 1 transition less than 100% of the ideal amount of sample is injected; and after each 1 to 0 transition a percentage of the sample is still fed into the column. This phenomenon gives rise to PRBSs shifted in time, resulting in the ghost peaks mentioned. Disturbances due to nonsymmetric and reproducible nonideal injection can be corrected. A reliable, accurate and simple injection system, meeting all demands of correlation LC, is obtainable. It is based on a common 8-port or 10-port LC valve, two equal sample loops and a valve actuator.

**Computer Requirements**

The appropriate hardware and software for injection control and data processing are essential in CC. Off-line data processing is possible, but a flexible and user-friendly program running on a microcomputer is to be preferred. The computer requirements
are modest: a standard PC with 640-kbyte memory is sufficient. A hardware card for controlling the valves, and an A/D converter (12 bits, minimum sampling frequency 10 Hz), including an anti-aliasing filter to prevent back-folding of high-frequency noise, are necessary. The software includes the generation of an arbitrary number of PRBSs, with selectable duration of the clock period and $2^n - 1$ clock periods in a sequence; $n$ is integer number mostly between 5 and 12. The standard deviation of the smallest (first) peak and the desired resolution determine the clock period.

The data processing is relatively simple. Straightforward cross-correlation may be replaced by the application of an off-line Hadamard transform procedure, speeding up the calculation. Display of the selected parameters (clock period, sequence length, number of sequences), the time varying detector signal, the on-line calculated correlogram, and possibly the injection PRBS are indispensable for application in practice.

Advantages and Disadvantages Compared with Conventional Chromatography.

The main advantage of CC is the improvement of the signal-to-noise ratio without preconcentration in a relatively short time. The resulting lower detection limit makes the technique very suitable for trace analysis. Another important advantage is that the compounds to be analysed remain on the average in their original chemical environment. This property of CC is particularly useful if compounds present in the sample degrade easily once isolated from their matrix. Another feature is the possibility of continuous monitoring of the varying concentrations encountered in process analysis. An interesting possibility is the strong reduction of effects due to nonlinear behaviour of the chromatographic system. When using CC, non-linear affected separations may improve drastically, although correlation noise may arise. Chromatographers need no special knowledge or skill to use CC in daily practice.

Possible disadvantages are the high demands on the chromatographic system, particularly the injection (reproducibility, wear and tear); the possible correlation noise, mainly due to injection errors; the extra sample required; and the extra time. In addition, the separation conditions are not exactly the same as in conventional chromatography, because of the continuous presence of sample throughout the column. The improvement of the signal-to-noise ratio is less than when the analytical signal itself is increased by a comparable preconcentration of the sample. However, preconcentration is often cumbersome or even undesirable, for example because of poor reproducibility.

The principles of CC are based on the assumption of stability of the system. Therefore, the application of gradient elution or programmed temperature techniques is out of the question in normal CC.

Modifications

Differential CC

The distortions of the correlogram caused by imperfect injection and nonlinearities are proportional to the concentration differences between the compounds in the sample and the eluent. CC can be used in a differential mode. The differences mentioned can be made much smaller by making the eluent almost equivalent to the sample by adding the (known) main components, present in the sample, to the eluent. Also, another sample – possibly modified for optimum separation conditions – can be used as eluent; only differences between the samples are measured, resulting in positive and negative peaks in the correlogram. This can be very useful in environmental analysis and trace analysis of samples with a relatively complex matrix.

Simultaneous CC (SCC)

In principle, it is possible to determine simultaneously $n$ different samples on the same column, each injected according to its own mutual uncorrelated random pattern, using multiplex techniques. Completely uncorrelated (pseudo) random patterns in one sequence are impossible. However, the use of only one long PRBS for each sample, with a time shift equal to an integral number of chromatogram duration different for each sample, allows simultaneous CC. The correlation time is equal to $n + 1$ chromatogram lengths. This technique can be used for high precision chromatography. Unknown sample and calibration standards can be processed under exactly the same conditions, resulting in a very accurate calibration. The determination of pure compounds simultaneously with a complex sample gives the exact place and peak shape of these compounds in the correlogram. This can be used for optimization purposes, for optimum intensity estimation, and for resolving strongly overlapped peaks.

Single-Sequence CC (SSCC)

Single-sequence correlation chromatography is an intermediate between single-injection chromatography and CC. The injection volume is significantly enlarged in comparison to single-injection chromatog-
raphy. However, the rectangular input function is modulated with a fine structure; one sequence of a PRBS. A deconvolution procedure allows the preservation of good peak resolution for fast-elution narrow peaks, while the other more broadened peaks are processed in a conventional way. In both cases the signal-to-noise ratio is improved. Gradient elution liquid chromatography (or programmed temperature GC) is still applicable, in contrast to conventional correlation and multiplex techniques.

Special attention has to be given to deconvolution errors due to the transient nature of the signals. Some noise frequencies may be amplified enormously if the number of points used for deconvolution is not optimally chosen.

**Chemical Concentration Modulation CC**

Chemical concentration modulators can be used in CC instead of mechanical injection valves. They can be considered as a chemical switch positioned at the head of the column. The eluent, which is continuously pumped through the system, contains the sample. In ‘off’ position of the switch nothing happens and a constant sample concentration is introduced into the column. In the ‘on’ position a chemical reaction takes place in the cell, causing certain components to react, yielding reaction products with different chemical structures and different retention and detection characteristics. The chemical reaction may add selectivity to the method. Nonreacting components will not influence the correlogram, because of the different properties of CC. **Figure 2** shows a set-up of a chemical modulation CC system.

Several modulators in gas CC, both destructive and nondestructive, are known. Examples, particularly suited for trace analysis in air, are the hot-wire modulator, the spark modulator, and the thermal desorption modulator. In correlation LC, electrochemical concentration modulation has been tried successfully.

**Illustrative Examples**

The performance of correlation LC, compared with conventional LC, can be illustrated with a calibration graph as shown in **Figure 3(A)**. Phenol was measured...
at a five concentrations: 0.01 to 100 µg L\(^{-1}\). The three higher concentrations (1–100 µg L\(^{-1}\)) were determined by conventional reversed-phase chromatography, the three lower concentrations (0.01–1 µg L\(^{-1}\)) by CLC. Measurements at the 1 µg L\(^{-1}\) level were done by both techniques. The bars indicate the peak area ± 3σ\(_I\) (arbitrary units), where σ\(_I\) is the standard deviation of the integrated noise. The correlation time is 1, 3 and 16 sequences or chromatogram lengths, corresponding to 5, 15 and 80 minutes, respectively.

Figure 3(B) shows the correlogram of a very low concentration of phenol for 80 minutes of correlation. The detection limit in this case is approximately 0.003 µg L\(^{-1}\). For a comparison with conventional LC it must be noted that the injection volume injected in one clock period of the PRBS is 48 µL, a factor of 2.4 more than the 20 µL single injection.

Another example of the possibilities of CLC is shown in Figure 4. Here a diluted rather complex standard material was analysed, containing a number of compounds at different known concentrations. The separation is not optimal, but the conditions are effective for examining the behaviour of CC in the case of more complicated mixtures.

Table 1 gives the composition of the samples, consisting of a mixture of polynuclear aromatic hydrocarbons (PAHs), prepared from standard reference material SRM 1647 (National Bureau of Standards). The improvement by application of CC, particularly in case of the diluted sample, is considerable.

A typical application of simultaneous correlation chromatography (SCC) is accurate calibration in LC, as is shown in the following experiment. Three different samples, each composed of naphthalene, anthracene and 1,2-benzanthracene, were prepared (Table 2). The concentration of anthracene was kept constant. Anthracene was used as an internal standard to correct for variations in the injected volumes of the different samples. The samples were injected according to a PRBS of 127 clock periods; the starting points of the injection patterns of the different samples were shifted over one-third of the sequence length. The clock period was divided into three subperiods, one for the injection of each sample.

Figure 5 shows the simultaneous correlogram. The peak areas were determined and corrected for systematic errors with the internal standard anthracene. The calibration graph is shown in Figure 6; the calculated correlation coefficient of the linear fit was 0.999 97 – a very good fit.

In principle SCC allows excellent quantification. Deterministic disturbances and drift influence

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### Table 1 Composition of the PAH sample

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (µg L(^{-1}))</th>
<th>Compound</th>
<th>Concentration (µg L(^{-1}))</th>
<th>Compound</th>
<th>Concentration (µg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>18.0</td>
<td>Fluoranthene</td>
<td>8.08</td>
<td>Benzo[k]fluoranthene</td>
<td>4.02</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>15.3</td>
<td>Pyrene</td>
<td>7.87</td>
<td>Benzo[a]pyrene</td>
<td>4.24</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>16.8</td>
<td>Benz[a]anthracene</td>
<td>4.02</td>
<td>Benzo[ghi]perylene</td>
<td>3.21</td>
</tr>
<tr>
<td>Fluorene</td>
<td>3.94</td>
<td>Chrysene</td>
<td>3.74</td>
<td>Dibenzo[a,h]anthracene</td>
<td>2.94</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>4.05</td>
<td>Benzo[db]fluoranthene</td>
<td>4.09</td>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>3.25</td>
</tr>
<tr>
<td>Anthracene</td>
<td>2.63</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
measurement and calibration samples in exactly the same way. Also, the noise-reducing property of CC is maintained. A comparison with sequential calibration can be made by successively performing independent experiments. Each calibration experiment yields an almost perfectly fitting linear calibration plot, but the points for the same concentration, measured successively, are distributed with rather large standard deviations. The bars in Figure 6 indicate the standard deviations of the measurements.

An illustrative example of the application of chemical concentration modulation correlation chromatography is the selective determination of traces of phenol. An electrochemical modulation cell (EMC) and a fluorescence detector are used. This combination, together with a suitable column, is both selective and sensitive to phenol.

Figure 7 shows log-log calibration graphs for conventional loop injection and EMC-CC, respectively. The signal-to-noise enhancement of EMC-CC is a factor of 11 higher at the most, equal to the theoretically predicted value.

Correlation Capillary Zone Electrophoresis (CCZE) Micromachinery

CZE is known for its high detection limits. Correlation techniques, as used in chromatography, can be applied in CZE as well. The goal and basic principles are the same. The main problem is the high demand on the injection system, just as in CC. However, in CCZE the injection system can relatively easy be modified, because CZE is electrically – rather than pressure – driven.

Microchip technology is very well suited for application in CZE systems and particularly in correlation CZE. A high quality injection device on a microchip, connected to a fused silica capillary and particularly usable for correlation CZE, is reported. The speed of separation in a microchip CZE system can be increased due to higher accessible field strengths. Detection is the major problem, because of the smaller channel dimension. The application of
correlation techniques drastically reduces the high
detection limit in a modest time.

See also: I/Chromatography. II/Chromatography:
Liquid: Theory of Liquid Chromatography. Electrophor-
esis: Capillary Electrophoresis.

Further Reading
Engelsma M, Kok WT and Smit HC (1990) Selective deter-
mination of trace levels of phenol in river water using
electrochemical concentration modulation. Journal of
cross correlation electrophoresis on microdevices. Ana-
lytical Chemistry 71: 4460–4464.
Input Chromatography. Chichester: Ellis Horwood.
cross-correlation high performance liquidchromatogra-
phy, a method of establishing small concentration dif-
fences in samples of similar origin. Analytica Chimica
Louwerse DJ, Boelens HFM and Smit HC (1992) Single-
sequence correlation chromatography. A novel tech-
nique to decrease detection limits. Analytica Chimica
Louwerse DJ, Smit HC and Kaljurand M (1997) Monitor-
ing time-varying concentrations in sample streams by
multiple input chromatography. Analytica Chimica
Mars C and Smit HC (1990) Sample introduction in cor-
relation chromatography. Application, properties and
working conditions for a novel injection system. Ana-
Smit HC, Mars C and Kraak JC (1986) Simultaneous cor-
relation chromatography, a new technique applied to
calibration in high performance liquid chromatography.
Analytica Chimica Acta 181: 37–49.
van der Moolen JN, Louwerse DJ, Poppe H and Smit HC
(1995). Correlation capillary zone electrophoresis,
a novel technique to decrease detection limits. Chromatographia, 40: 368.
van der Moolen JN, Poppe H and Smit HC (1997)
A micro machined injection device for CZE, Applica-
tion to Correlation CZE. Analytical Chemistry 69:
4220–4225.

Countercurrent Chromatography and High Speed
Countercurrent Chromatography: Instrumentation

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Introduction

This article presents a brief overview of the most
significant aspects of the history, apparatus, theory
and practice of countercurrent chromatography
(CCC). CCC is primarily a preparative technique for
the isolation and purification of chemicals on a milli-
gram to multigram scale. It has been broadly applied
to natural products, pharmaceuticals and other syn-
thetic organic and inorganic chemicals.

What is Countercurrent
Chromatography?

Countercurrent chromatography can be broadly
characterized as a form of liquid–liquid chromato-
graphy (LLC) in which two mutually saturated immis-
cible liquids are employed. One phase is retained
in the chromatograph as a long continuous or
segmented stationary bed without the use of an
absorptive matrix; the second phase passes through
the stationary bed and is efficiently equilibrated
with it by means of either hydrodynamic or turbulent
mixing.

In earlier forms of liquid–liquid partition
chromatography introduced by Martin and Syng, where one phase is retained in a porous matrix such
as diatomaceous earth or cellulose, significant peak
tailing is often seen and some analytes are lost by
irreversible adsorption on the supporting matrix. In
CCC, the stationary phase is retained by grav-
tational, inertial or capillary forces and adsorption is
precluded by construction of the apparatus from
polytetrafluoroethylene (PTFE) or other inert, usually
polymeric, material. Thus analyte migration in CCC
is determined only by its partition coefficient in the
two-phase system and peaks are typically quite
symmetrical.

CCC differs from countercurrent distribution
(CCD) of the type introduced by Craig in the 1940s in
that CCD is a discontinuous process based on attain-
ment of partition equilibrium prior to phase transfer,
whereas CCC is a continuous dynamic or steady-state
process which characterizes all forms of chromato-
graphy. Both CCC and CCD may employ the same
solvent systems and both achieve separations based
on the partition coefficient, but the apparatus