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Theoretical description of a new analytical technique: Comprehensive online multidimensional fast Fourier transform separations

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ABSTRACT

Comprehensive multidimensional separations are today dominated by systems that are fundamentally limited to highly asymmetrical online separations sacrificing separation space, or to lengthy, time consuming offline separations. With the exception of pulse-modulated methods, separations have thus been limited to two dimensions. It is proposed that some of the limitations and shortcomings of these methods may be ameliorated or overcome by employing multi-dimensional detection whereby each analyte is effectively labelled in the frequency domain by a series of pulsed-injections, and a symmetrical, comprehensive online analysis performed with the resulting signal processed by sequential Fourier analysis. A semi-empirical computer model of this system was developed and its feasibility positively demonstrated in simulations of high-efficiency separations in two dimensions. Separations of higher dimensionality were shown to be possible but involved signal-processing challenges beyond the present work. By eliminating wrap-around effects and enabling the separation of physically unseparated peaks, the technique facilitates significant improvements in peak capacity per unit of analysis time as well as greatly improved signal to noise ratios. Because these comprehensive online multidimensional Fourier transform separations depend heavily upon the practical lifetime of imposed injection pulses, it is envisaged that this method will leverage emerging high-efficiency micro- and nanoscale separations technologies.

1. Introduction

Online comprehensive separations have been demonstrated in multi-dimensional gas chromatography via the use of inter-dimensional pulse modulation [1,2] and statistical analyses of the resulting signal. Other mathematical methods have also been applied to chromatography and analytical signal processing for many years, and of the transform methods, the Fourier transform is the most important and finds considerable application [3], albeit in various guises and differing purposes. The advantages of pulse-modulation and frequency domain analysis to achieve comprehensive online multidimensional separations have not hitherto been combined.

Allegri et al. [4] used Fourier analysis as a means of deconvolution to resolve partially overlapped peaks in time-dependant (detector) signals. Fourier transforms have also found use in improvements to signal-to-noise ratios, peak resolution and the speed of in-process chromatography by either analysing the results of multiple overlapped sample injections (correlation chromatography [5,6]) or measuring analyte column migration speeds by multiple-point detection such as in Shah Convolution Fourier Transform (SCOFT) detection [7] and combinations of such methods [8]. Over time, these methods have been refined to include the application to correlation chromatography of Hadamard transforms (a discrete Fourier transform in two variables) by employing complicated sample-injection protocols of pseudo-random binary sequences [9,10]. Further enhancements include the use of multiple parallel sample injection as in Fourier Transform Capillary Electrophoresis (FTCE) [11] to achieve the same result as that generated by SCOFT, but without the need for multiple-point detection: translation of the migration speed of an analyte into a frequency domain signal. Each of these applications has achieved notable improvements in signal to noise ratios and, in some cases, improved chromatographic resolution [11,12]. Each one replaces time-domain detection with frequency-domain detection. Most of these methods are used in process control analyses, in industrial environments and are unknown by most laboratory chromatographers.

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The present work suggests a further enhancement to the repertoire of frequency domain methods by simultaneous application of both time- and frequency-domain detection to pulse-modulated separations in multiple dimensions, using only one single-point detector.

1.1. Purpose of the proposed method

Multidimensional chromatography has arisen as an increasingly important technique in fields requiring the analysis of highly complex mixtures, usually of natural origin. The fields of proteomics, metabolomics, and natural drug discovery, for example, deal with samples containing hundreds or even thousands of compounds that cannot be adequately or practically separated in one dimension (D_1). In such cases, further separation may be achieved by serial application of separations in further dimensions ($D_2 \cdots D_n$), which may be carried out in either time (S_t) or space (S_s) or combinations of both (such as $S_t \times S_s$).

1.1.1. Current methods of implementation of multidimensional chromatography

In practice, it has been found that multiple time-domain dimensions ($S_t \times S_t$) provide the simplest and most efficacious separations [13]. The analytical requirements, constraints and limitations placed (by choice or necessity) on multidimensional chromatography may be summarised as follows:

1) The separation must be "comprehensive" in that all components pass through all dimensions.
2) No separation achieved in the series of previous dimensions ($D_1 \cdots D_n$) may be lost in any of the subsequent separation dimensions ($D_2 \cdots D_{n+1}$)
3) In the most common case of multiple time-dimension separations ($S_t \times S_t$), practical application may be achieved in a variety of modes:

a. Online:
N-fractions per peak in D_n are collected, transferred to the next separation level, and directly subjected to analysis in D_{n+1} in real-time. In this case, the analysis time (T_{n+1}) in D_{n+1}, must be less than the time between subsequent fractions transferred to the second dimension, ideally less than one standard deviation of the peak distribution eluting from the first dimension [14]. Otherwise chaotic band displacement may result. In HPLC, this may require D_{n+1} run times of only a few percent of the D_n run time.
b. Offline:
N-fractions in D_n are stored temporarily until subjected to analysis in D_{n+1} such that the run rime of D_{n+1} is no longer subject to the same constraint as in (a), but analyses are time consuming.
c. Stop–Start:
After an eluate plug has been delivered directly to D_{n+1} from D_n, the flow in D_n is stopped and the separation is then run in D_{n+1}. Upon completion of the D_{n+1} separation, the flow is resumed in D_n to deliver another eluate plug, and so on. The need to collect, store and manage large numbers of fractions, free of contamination, is thereby obviated. There is, however, no reduction in the overall analysis time compared with (b).

In offline analysis, relaxation of the constraint on D_n run times comes at the cost of a very large increase of the total analysis time, which is given by the series:

$$T_A = T_1 + N_1 T_2 + N_2 T_3 + \ldots \quad (1)$$

where T_A is the total analysis time, T_1, T_2, …, T_n represent the analysis time in each respective dimension and N is the number of fractions transferred to each dimension. This drawback has been addressed by subjecting only specific fractions of interest from D_n to analysis in D_{n+1}: 'Heart-cutting' [15] – but this method can no longer be termed a 'comprehensive' separation. For comprehensive separations, the online method 3a is preferred due to its simplicity and speed of analysis. Unfortunately, the constraint placed on the analysis time in the second dimension is severe, and necessitates highly optimised, asymmetrical analyses that sacrifice much of the separation space that could otherwise be available in a multidimensional system [13]. Parallelisation of online second-dimensions has been employed as a means of achieving rapid symmetrical comprehensive separations [16], but remains limited in its D_1 retention time resolution and is a very expensive approach that necessitates significant optimisations.

Now, the crux of these difficulties – and the reason for the development of the above techniques – lies in the problem of being able to identify in D_n, the results of separations that have taken place in $D_{<n}$, such that requirement (2) may be met. In time-domain separations (such as in liquid chromatography and electrophoresis), components are identified by their retention time. If there were another way to persistently 'label' the components previously separated and thus to identify and maintain the achievements of the $D_{<n}$ time-domain separations, the analysis-time constraint for online D_{n+1} could be removed and there would be no need for time-consuming offline separations.

1.1.2. Introduction of the frequency domain in multidimensional chromatography

To date, the frequency domain has been applied in chromatography essentially as a simple translation of retention time to migration speed through one dimension, providing improvements in signal to noise ratios. In multidimensional separations it would be possible for two components that would otherwise be separated in the time domain, to present the same frequency at the detector. Similarly, components migrating at different speeds may elute at the same time in later dimensions. Rather than using the frequency domain or time domain alone, if we were to separate components in both the time and frequency domains, the separation of coincident components in subsequent dimensions becomes a problem of the separation of the frequencies that are coincident in time. This may be treated as a purely mathematical manipulation of the resultant signal by Fourier analysis (Eq. (2))[17].

$$G(f) = \sum_{k=1}^{N} g(t) \cdot \cos\left(\frac{2\pi k}{N}\right) - j \cdot \sum_{k=1}^{N} g(t) \cdot \sin\left(\frac{2\pi k}{N}\right) \quad (2)$$

where $G(f)$ is the discrete Fourier transform of a real, time dependant function ($g(t)$): N is the number of data points and $k = 1, 2, \ldots, N$ [17]. Solution of the discrete Fourier transform is highly computationally intensive and, in practice, is achieved by using Fast Fourier Transform (FFT) algorithms that exploit Euler's theorem [18] (Eq. (3)) and the transform's symmetries. These are known as radix-2 algorithms and the Cooley–Tukey FFT algorithm (Eq. (4)) is probably the best known and most widely used example [19,20].

$$e^{i\theta} = \cos\,\theta + i \sin\theta \quad (3)$$

$$X_k = \sum_{m=0}^{N/2-1} x_{2m} e^{-(2\pi i/(N/2))mk} + e^{-(2\pi i/N)k} \sum_{m=0}^{N/2-1} x_{2m+1} e^{-(2\pi i/(N/2))mk} \quad (4)$$

where N is the number of data points and must be an integer power of 2, i.e.: $N = 2^x$ where x is a positive integer. The Fourier transform is symmetrical about the Nyquist limit, N_f (Eq. (5)):

$$N_f = \frac{N}{2} + 1 \quad (5)$$

where N_f is the number of frequencies that may be represented by a Fourier transform using a given number ($N = 2^x$) of data points and the '1' represents 0 Hz [17]. The FFT (Eq. (4)) thus calculates only the first half of the transform. Furthermore, Euler's relationship