Increasing Sample Throughput of NanoLC-MS through Hadamard Multiplexing

S. L. Staats, A. J. Fogiel, Jr., D. Cripps and A. Suna, Phoenix S&T, Inc. Chester, PA, USA

Overview

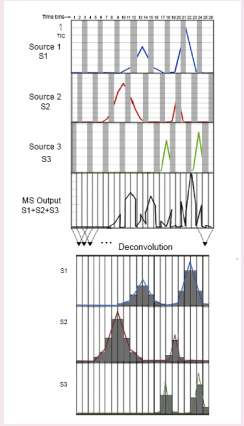
The growing use of LC-MS in drug discovery, biomarkers detection, and proteomics research has made increasing sample throughput without compromising sensitivity extremely desirable for efficiency and cost considerations. Waters' MUX™ platform, which sequentially samples 4 or 8 LC inputs for a single mass spectrometer, is the state-of-the-art commercially available technology. Using similar sequential spray techniques, the eluates from two different columns have been sprayed into a mass spectrometer for detection by means of a robotic arm that positions the spray tips alternately in front of the mass spectrometer.[1] Hadamard Transform HT uses the cross correlation of pseudorandom sequences to encode samples and the subsequent deconvolution of the data to improve the sensitivity of the acquired data. This method has been shown to be effective in improving the sensitivity in TOF-MS[2] and in increasing sample throughput in GC[3] and CE.[4] However, the HT approach typically requires the number of sample injections, and thus the number of pseudorandom sequences, to be large (in the 1000's) in order to be effective because of noise reduction considerations. Moreover, because of the sequential injections of the Hadamard-sequence encoded samples into a single flight-tube, capillary, or column, this approach will not work for gradient LC-MS, the most widespread and powerful of the hyphenated mass spectrometry techniques. This report shows a novel application of Hadamard sequences and their subsequent deconvolution that can be applied to as few as three LC sample inputs to significantly improve sample throughput in NanoLC-MS with gradient elution.

The Hadamard Multiplexing Approach

The multiplexing scheme we propose is based on the mathematics of Hadamard matrices˙ and uses cyclic N by N Hadamard simplex matrices (consisting of 1's and 0's instead of the 1's and -1's in the regular Hadamard matrices) to define the on-off patterns that are sequentially and regularly applied to the nanospray devices or tips. These 0's and 1's represent the "off" or "on" states of the spraying of the spray tips: each column of the matrix gives the on-off patterns of the N spray tips at any given time interval or "time bin." Cyclic simplex matrices exist only for certain N (see Ref. 5). The lowest values are N = 3, 7, 11, 15, 19, 23, 31, 35 ... . Each column has exactly (N+1)/2 ones, so the duty cycle for each source is about 50%.

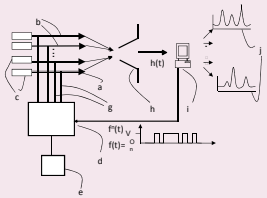
The differences between our proposed approach and the conventional Hadamard Transform (HT) technique are summarized in the following table. We use N to denote the dimensions of the simplex matrices used and n for the total number of time bins.

Property	Hadamard Multiplexing (this work)	Conventional Hadamard Transform
Dimension of simplexes	N << n, N=3, 7, 11, 15, 19, 23, 31, 35...	N , N=2^m-1, m=1, 2, 3,... is typically >1000
Number of distinct samples	N	1
Number of data points	n	n
Transforms used to solve	n – N + 1 ~ n	1
Calculated points	N × (n – N + 1) ~ N × n	n
Accuracy of calc. points	approximate	exact



The proposed Hadamard Multiplexing with Multiple LC Sources: For the case of N=3, the shaded portions of the top graphs indicate intervals during which the spray of the nanospray source is turned off. Reading vertically for time bin=1, the Hadamard sequence representing the on/off pattern of the nanospray sources is 110. For time bin=2, the Hadamard sequence is 101, etc. The data represented by the MS output are the sum of the three sources, only two of which are on for a given time bin. Data points are generated by averaging in each time bin. A transform of dimension N is then carried out for each data point, utilizing N neighboring data points of which that data point is the central one. The result is approximate and relies on the time variation of the neighboring points being small. We can correct exactly for errors caused by non-vanishing slopes and curvatures. Numerical simulations have shown that the deconvolution works well if the sharpest Gaussian peaks of the sources are at least N bins wide. In this diagram, the deconvoluted signal is represented by the shaded rectangular bars at each time bin. The signal from each source spraying separately and continuously is superimposed onto the deconvoluted signal to show the degree of approximation. It is clear from this diagram that the higher the number of time bins that fit into a peak, the better the approximation. The maximum number of columns or sprayers supported by this method is limited by the peak width, the scan speed of the mass spectrometer, the switching time of the high voltage for inducing the spray, and also the physical space available for placing the spray devices at the MS inlet.

Experimental



A schematic drawing of the new Hadamard Multiplexing technique that enables improved peak resolution for even a small number of separation columns and can be used for columns running different methods including gradient elution. a: spray devices; b: separation columns (or separation lanes of a multidimensional devices); c: LC pump devices; d: high speed high voltage switch box for supplying the spray voltages in the form of a Hadamard sequence; e: high voltage power supply; f: the Hadamard sequence applied to the spray devices; h: the mass spectrometer inlet; i: the computer control for generating the Hadamard sequence and the deconvolution; j: the deconvoluted mass chromatograms of the individual separations. This technique is patent-pending.

The spray devices used were polypropylene nozzles chosen because of their robust spray property. They were arranged in a circle as shown so that the spray from each device was equivalent in position and orthogonal to the MS (LCQ Advantage) inlet. An electronic circuit and the accompanying software that switched the high voltage (HV) required for nanospray (2.4 to 4 KV) on the ms time scale were constructed to apply the sequences to the three nozzles.

Method

The time bin sequences were applied as follows:

$T_n \longrightarrow$
N_1:01101101101101101011
N_2:11011011011011011010
N_3:10110110110110110101

The N=3 cyclic Hadamard S matrix:

$$\begin{pmatrix} 0 & 1 & 1 \\ 1 & 1 & 0 \\ 1 & 0 & 1 \end{pmatrix}$$

This series of on/off sequences was repeated for each time interval T_n until the end of the experiment. The time bin duration was 2 s in Experiment I below and 1 s in Experiment II. To obtain the deconvoluted results from experimental data, the MS data file from the data system was converted into a text file, and the repeated transforms were performed with Mathematica in order to obtain the chromatogram for each sample.

I. Hadamard Multiplexing Approach Validation

This experiment used the differences in the total ion current (TIC) of water vs. methanol to probe the validity of our proposed Hadamard multiplexing fundamentals. The different concentrations of H_2O in the samples were sufficient to generate TIC signals that were distinguishable from nozzle to nozzle.

Nozzle (time bin=2 S)	Sample	Flow rate
N1	50%H_2O/50%MeOH	~500 nL/min (split from 100 µL/min) Waters 510 pump
N2	50% H_2O/50% MeOH	~500 nL/min (split from 100 µL/min) Waters 510 pump
N3	90% H_2O/10% MeOH	500 nL/min (splitless pump) MicroTech XtremeSimple

II. Hadamard multiplexing for nanoLC-MS

For the nanoLC–MS experiment using Hadamard multiplexing, the following four peptides were selected for gradient and isocratic chromatography:

The peptide standards

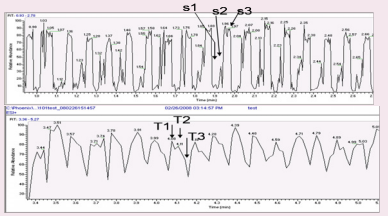
Name	Mass	[MH+2]2+
α-Endorphin	1744.82	872.4
γ-Endorphin	1857.90	930.0
Bradykinin1-9	1059.55	530.8
Bradykinin2-9	903.44	452.7

The endorphin peptides were obtained from American Peptide and the bradykinin peptides from Sigma. The peptides were dissolved in 100 mM ammonium bicarbonate and diluted with 0.1 % formic acid to ~0.1 pmol/µL. The run buffers were A: H_2O + 0.1% formic acid, B: Methanol + 0.1% formic acid. The methods and samples for the three sprayers are shown below:

N1	N2	N3
150µm i.d, 5 µm C18 capillary column	75 µm i.d., 5 µm C18 capillary column	A fritted capillary with ~1mm of 5 µm, C18 particles
4-peptide standard sample	4-peptide sample	40%A/60% B buffer, no sample
30 minutes of equilibration at 10% B Gradient Program: 0-10 minutes (load step) 10% B 10-15 minutes 10-40% B 15-35 minutes 40-70% B 35-40 minutes 70-90% B 40-50 minutes 90% B	Isocratic elution, 40%A/60%B	Direct infusion
500 nL/min splitless pump	~500 nL/min split from 100 µL/min	~500 nL/min split from 100 µL/min

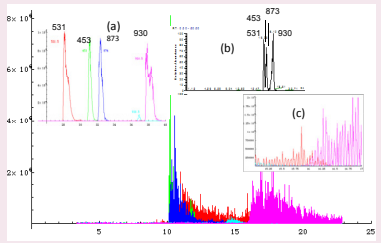
Results

I. Hadamard Multiplexing Validation



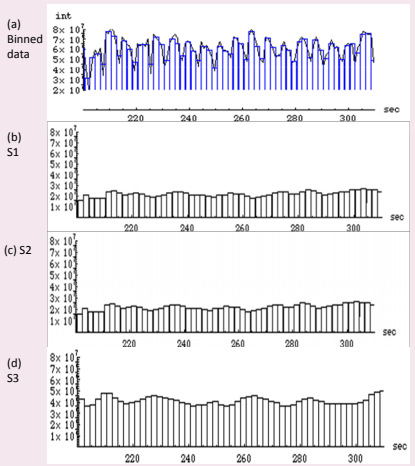
Above are preliminary results from the Hadamard multiplexed LC-MS apparatus. The top panel is the mass chromatogram of three inlets from sprayer N1 (~500nL/min 50/50 water/methanol), sprayer N2 (~500 nL/min 50/50 water/methanol), and sprayer N3 (500 nL/min 90/10 water/methanol). The spray order was sequential, i.e., 1, 2, 3, 1, 2, 3...every 2 s. The bottom panel is the Hadamard multiplexing result. T1 was the time bin containing the signal from the sequence 110. T2 was from the sequence 101, and T3 was the signal from 011. Each time bin was 2 s long. The simplicity of the design of the experiment allowed assignment of the spray sources by visual inspection.

II. Hadamard Multiplexing for Peptides Separations



Above is the mass chromatogram of the sum of the three multiplexed inputs of the peptides separations. The masses were color coded, indicating that separation of the peptides did occur. The gradient and isocratic separations appeared to have retention times that overlapped one another. The color coding to the mass peaks is shown in inset (a), which was the chromatogram taken with a single column and nozzle spraying continuously. The peptide concentration was also 10x higher than the multiplexed experiment. Inset (b) is the chromatogram of an isocratic separation of the peptides using the 150 µm i.d 5 µm C18 column pumped by the splitless pump. Two of the peptides co-eluted, but the whole group of 4 peptides eluted earlier than the gradient separation in the single nozzle experiments. The coincidence of the two samples in the mass chromatograms in the Hadamard experiment may have been due to the changes in the isocratic method. Inset (c) shows a magnified portion of the mass chromatogram with the characteristic discontinuities or "gaps" in the mass traces due to the turning off of the nozzles in each time bin according to the Hadamard sequence. Deconvolution of these data is underway.

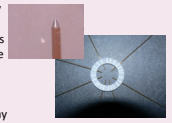
Deconvolution Results



(a) to (d) are the deconvoluted results of the Hadamard multiplexed sprays experiment. (a) shows the binning of the MS results superimposed on the raw data. (b) to (d) are the deconvoluted results assigned to the corresponding sources. When compared to the relative intensities of the TIC of sequential spray data, it is clear that the deconvoluted multiplexed data match the sources correctly.

Summary and Discussion

A Hadamard-based multiplexing scheme is presented and demonstrated to be feasible for increasing nanoLC sample throughput, even for gradient elusion. In the case of the 3 LC inputs shown in this report, the Hadamard duty cycle was 66% versus the sequential (as in MUX™) method's 33%. As N increases, the HT duty cycle will remain around 50%, while the sequential method will have a duty cycle too diminished for use.



The figure shown here is that of the 7 spray tips arranged in a circular pattern. Each spray tip is a tapered capillary with a porous polymeric frit. They have been shown to be non-clogging, which is critical for multiplexing since one clogged tip can jeopardize all the multiplexed separations. To avoid interference of sprays, no two spray tips point directly at each other.

The use of the polymer fritted spray tips also maximizes the number of spray devices for Hadamard multiplexing. Around a 1-cm circle outside of the MS inlet, up to 23 spray devices can be accommodated. For a typical peak width of 30 s, a mass spectrometer that can scan at 1 s /full scan or faster will allow the Hadamard multiplexing to be implemented. The higher the scan rate is, the smaller will be the errors in the approximation introduced into the deconvolution. We are currently deconvoluting the gradient and the isocratic separations in the 3-nozzle experiment shown here. With more data on actual separations, we will be better able to assess the limitations of the proposed Hadamard multiplexing scheme in terms of overlapped peaks, peak density, etc.

Acknowledgements: This work was partially funded by an NIH SBIR grant. We acknowledge helpful discussions with Dr. Joel Kimmel of the U. of Colorado at Boulder.

References: 1) Bonneil E., Tessier C., Carrier A., Thibault P., Electrophoresis 2005 26, 4575-89.
2) Zare, R.N., Fernandez, F. M., and Kimmel, J.R., Angew. Chem. 2003, 42, 30-35.
3) Trapp, O., Angew. Chem. 2007, 46, 5609-5613.
4) McReynolds, J.A., Leyi, G., Barber-Singh J., Shippy, S.A., J. Separation Sci. 2005, 28, 128-126.
5) Harwit, M., Sloane, N.J.A., Hadamard Transform Optics, Academic Press, New York, 1979.