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Fast Hadamard Transform Capillary Electrophoresis for On-Line, Time-Resolved Chemical Monitoring

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We report a new approach for collecting and deconvoluting the data in Hadamard transform capillary electrophoresis, referred to as fast Hadamard transform capillary electrophoresis (fHTCE). Using fHTCE, total analysis times can be reduced by up to 48% per multiplexed separation compared to conventional Hadamard transform capillary electrophoresis (cHTCE) while providing comparable signal-to-noise ratio enhancements. In fHTCE, the sample is injected following a pseudorandom pulsing sequence derived from the first row of a simplex matrix (S-matrix) in contrast to cHTCE, which utilizes a sequence of twice the length. In addition to the temporal savings provided by fHTCE, a 50% reduction in sample consumption is also realized due to the decreased number of sample injections. We have applied fHTCE to the analysis of mixtures of neurotransmitters and related compounds to yield improved signal-to-noise ratios with a total analysis time under 10 s. In addition, we demonstrate the capability of fHTCE to perform time-resolved monitoring of changes in the concentration of model neurochemical compounds.

Capillary electrophoresis with laser-induced fluorescence detection (CE-LIF) is the most widely used separation technique for low-volume, high-speed, and high-sensitivity separations of biologically active compounds. The ease of automation and coupling of CE to a variety of sampling interfaces has allowed CE-LIF to be used for time-resolved, on-line analysis of chemical dynamics in a number of biological systems.[1−4] Despite the high sensitivity of CE-LIF, a number of important biomolecules elude detection using traditional CE-LIF schemes.[5,6] This problem is further exacerbated when on-line monitoring is performed as detection limits are inherently higher due to on-line reaction conditions.

A simple approach to improve signal-to-noise (S/N) ratios in CE, and thereby increase sensitivity, is to average multiple electropherograms obtained from the same sample; however, performing multiple separations in serial increases both the total analysis time and the sample consumption proportional to the number of electropherograms averaged. Variations in migration times and changes in the chemical composition of dynamic systems obtained over the long times required for multiple separations further limit this approach. A number of mathematical multiplexing approaches have been reported for CE separations that increase sensitivity and minimize the temporal limitations associated with averaging serial single separations. Examples of these approaches include multiplexed detectors,[7,8] Shah convolution Fourier multiplexing,[9,10] cross-correlation,[11,12] and Hadamard multiplexing.[8,13−15] The key requirement of multiplexing methods is increased signal throughput, which ultimately yields improved S/N ratios. Of these multiplexing methods, Hadamard multiplexing is unique in that it requires minimal instrumental modification on CE-LIF systems that are capable of on-line, sequential sample injection.

The Hadamard transform relies upon the injection of multiple, discrete sample plugs onto the same separation capillary in a well-defined order, requiring high injection precision throughout the time course of analysis. Reduced precision in sample injections leads to a number of deleterious effects. Most commonly, nonideal injections result in creation of ghost peaks that overlap analyte peaks, ultimately reducing S/N enhancements.[16,17] These demands are further compounded when multiplexed CE-LIF is applied to on-line measurement of fast chemical and biological processes

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since continuous separations may be performed over large time periods with the sample composition changing throughout the analysis.

Conventional electrokinetic or hydrodynamic sample injection is incompatible with Hadamard transformations as these methods are typically slow and require movement of the separation capillary. A variety of alternative on-line injection techniques have been utilized for Hadamard multiplexed CE, including electrokinetic injection using microfabricated crosses,[8,18] micromachined capillaries,[19] and optical gating.[15] Of these approaches, optical gating (OG-CE-LIF) provides the most flexibility for rapidly injecting discrete sample plugs with high mass and temporal reproducibility, coupled with short separation distances.[20, 21]

Due to the capability of rapid and continuous injection of discrete sample plugs, OG-CE-LIF is well suited for Hadamard multiplexing and shows great promise for enhancing the sensitivity of a number of key biological compounds.[8,13−15] Hadamard multiplexing is a matrix-based convolution method that encodes the sample injection process by injecting more than one sample plug per separation according to a gating sequence extracted from a cyclic **S**-matrix.[22] This **S**-matrix is derived from a Hadamard matrix,[22] $\mathbf{H}_n$, which is an $n \times n$ matrix composed of $+1$*s* and -1*s* that satisfies the requirement that

$$\mathbf{H}_n\mathbf{H}_n^{\mathrm{T}} = n\mathbf{I}_n \qquad (1)$$

in which $\mathbf{H}_n^{\mathrm{T}}$ is the transpose of $\mathbf{H}_n$ and $\mathbf{I}_n$ is an $n \times n$ identity matrix. In conventional HTCE (cHTCE), the injection sequence is derived from an **S**-matrix by placing two copies of the first row side-by-side and omitting the last element of this vector, resulting in a sequence with $(2n - 1)$ elements where n is the order of the **S**-matrix. When the injection element is 1, the shutter closes, resulting in injection of sample; whereas, when the element is 0, no sample is injected due to photobleaching of the sample through the open shutter. Analytes contained within the injected sample plugs separate within the capillary and are detected as a summation of the multiple peaks associated with their respective injection. The original electropherogram can be recovered by a simple multiplication of the multiplexed electropherogram with the inverse **S**-matrix, $[\mathbf{S}]^{-1}$, to achieve deconvolution. However, the multiplication of two matrices requires that they share at least one dimension; thus, n elements must be extracted from the raw data to enable multiplication. In cHTCE, the n elements are extracted from the nth to the $(2n - 1)$th experimental data points, discarding the information encoded in the other sample injections. Deconvolution of the multiplexed data yields an electropherogram with an improved S/N ratio.[22] The S/N gain depends on the order n of the injection matrix:

$$(n + 1)/2n^{1/2} \qquad (2)$$

Signal-to-noise enhancements as high as 18-fold have been observed using cHTCE,[14] though the collection of excess data in cHTCE increases the overall analysis time and sample consumption. Current cHTCE applications have been further limited by long injection and separation times,[8] thus reducing sample throughput.

Here, we report a new implementation of HTCE with optically gated sample injection, referred to as "fast HTCE" (fHTCE). With fHTCE, the time required to collect individual multiplexed separations can be reduced by as much as 33% while also reducing sample consumption by 50%. In a further refinement of the fHTCE approach referred to here as "reduced fHTCE" (rfHTCE), a 48% decrease in acquisition time when compared to cHTCE can be obtained. The application to fHTCE protocols to rapid CE separations decreased the overall analysis time for an individual mixture by a factor of 50 compared to previous cHTCE approaches.[15] We have applied the new fHTCE approaches to CE-LIF analysis of a mixture of five fluorescein isothiocyanate-labeled neurotransmitter amino acids and related compounds achieving complete multiplexed separation in 8.8 s with a 9-fold S/N enhancement. For separation times of 35 s, S/N enhancements of up to 15-fold were obtained.

EXPERIMENTAL SECTION

Chemicals. All solutions and chemicals were used as received. Fluorescein and fluorescein isothiocyanate (FITC) were obtained from Sigma Chemical Co. (St. Louis, MO). Phosphate buffer was prepared from mono- and dibasic sodium phosphate (EM Science, Gibbstown, NJ). FITC-labeled amino acids were prepared by reacting a 3:1 molar ratio of FITC to amino acid for 3 h at room temperature.

Instrumentation. The instrumental arrangement used for optically gated Hadamard transform CE-LIF is as follows. A 5-W multiline argon ion laser (Innova 70C, Coherent Inc., Santa Clara, CA) is passed through a prism (Melles Griot, Irvine, CA) to isolate the 488-nm line. A side reflected beam from the prism serves as the gating beam and is focused using a biconvex lens ($f = 2.5$ cm, Melles Griot) into the fused-silica separation capillary (10-μm i.d., 375-μm o.d., total length 20 cm, Polymicro Tech. L. L. C., Phoenix, AZ). The gating beam is modulated by a mechanical shutter (LS3, Uniblitz by Vincent Assoc., Rochester, NY), driven by a controller (VMM-T1, Uniblitz). Injection sequences, derived from **S**-matrices, constructed using the maximal length shift-register construction method,[22] are relayed to the shutter driver using a digital output board (PCI-DIO-32HS, National Instruments, Austin, TX). When the shutter is open, FITC-labeled analytes are photobleached. Analyte is introduced (injected) upon closure of the shutter, allowing fluorescent samples to migrate to the detectors. The main 488-nm line serves as the probe beam and is passed through a ND filter (ND = 1.0, Thorlabs, Inc., Newtown, NJ) to attenuate the beam before focusing using a biconvex lens ($f = 2.5$ cm, Melles Griot) positioned. The distance between the detection beam and the gating beam, which defines the separation distance, is 1.4 cm. Voltage is applied across the capillary with a high-voltage power supply (Glassman High Voltage, Inc., High Bridge, NJ). Fluorescence is collected by a microscope objective (20×, 0.40 NA, Melles Griot) and then passed through a band-pass filter (D525/25M, Chroma Technology, Rockingham, VT) preceded by a holographic notch filter (HNF-488.0-1.0, Kaiser Optical Systems, Inc., Ann Arbor, MI). Signal is detected using a

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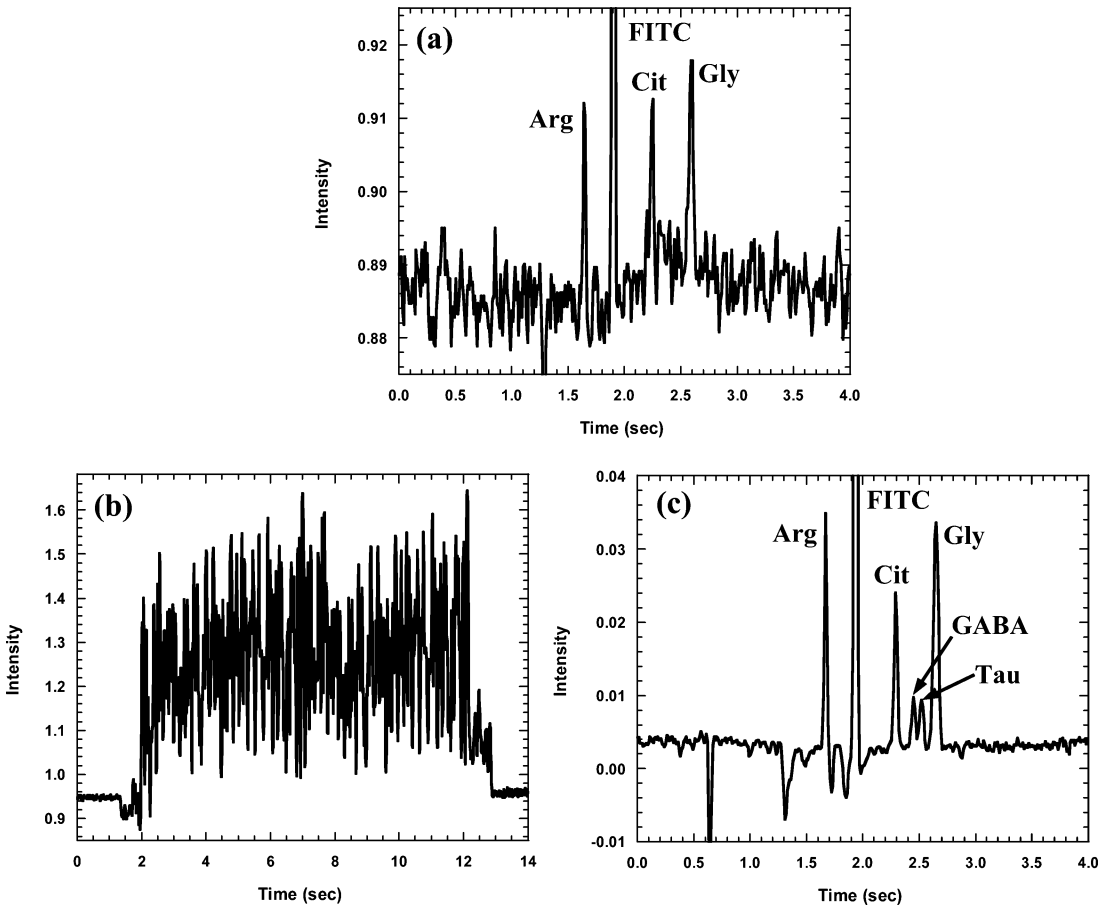

Figure 1. Analysis of a model neurochemical mixture using cHTCE with a 511-element **S**-matrix. (a) Single-injection electropherogram, (b) **S**-matrix encoded raw data, and (c) deconvoluted cHTCE electropherogram. Experimental conditions: 10 nM FITC-labeled amino acids, 10-ms injections, $E = 1.75$ kV/cm, gating beam 50 mW, and probe beam 12 mW.

photomultiplier tube (H957, Hamamatsu Photonics, Bridgewater, NJ), amplified by a current amplifier (model 428, Keithley Instruments, Cleveland, OH), and low-pass filtered (model 950, Frequency Devices, Inc., Haverhill, MA.). The signal is collected by a data acquisition board (PCI-MIO-16E-4, National Instruments) interfaced with a personal computer. Data are collected at a rate equal to the injection rate. Programs for data acquisition and subsequent deconvolution were written in-house using Labview 7.0 (National Instruments). The S/N enhancement is calculated by dividing the S/N ratio of the HTCE electropherogram by the S/N ratio calculated from a single injection electropherogram of the same sample.

Time-Resolved Chemical Monitoring. Temporal dynamics were monitored using fHTCE by interfacing the instrument described above to two flow channels. Two 1-mL airtight syringes (Hamilton, Reno, NE) controlled by a Harvard syringe pump (Harvard Apparatus, Inc. Holliston, MA) were used to introduce samples directly into the separation capillary. Each syringe was connected to a six-port injection valve (6UW, Valco Instruments, Houston, TX) through an 18-cm-long, 50-μm-i.d. capillary. The valve output (50-μm-i.d. capillary) was snugly coupled to the separation capillary (10-μm i.d.) using $^1/_{16}$-in.-i.d. Teflon tubing containing a hole at the junction of the two capillaries to release pressure at the interface. The Teflon tubing was submerged in grounded buffer to drop the electric field across only the separation capillary.

RESULTS AND DISCUSSION

As a benchmark test of our system, we first conducted experiments using conventional Hadamard transform CE with short injection times (10−100 ms) and short separation distances (1.4 cm). Previous applications of cHTCE relied upon injection times in excess of 250 ms with separation distances longer that 4 cm.[15,8] Figure 1 demonstrates the application of cHTCE to the separation of a model neurochemical mixture containing 10 nM FITC-labeled arginine (Arg), citrulline (Cit), γ-aminobutyric acid (GABA), taurine (Tau), and glycine (Gly) using 10-ms injection plugs. Under single-injection conditions (Figure 1a), GABA and Tau cannot be distinguished from the background noise. Application of cHTCE with a 511-element **S**-matrix and 10-ms injection plugs yields a multiplexed electropherogram (Figure 1b) that requires 12.9 s for detection of all the injected material. Deconvolution of this electropherogram (Figure 1c) provides a 9.6-fold S/N enhancement over the single-injection electropherogram (Figure 1a). Due to the smaller size of the sample plugs injected and the shorter separation lengths, the collection time was reduced 25−50-fold compared to previous applications of cHTCE,[8,23] though comparable S/N enhancements are maintained. Further, no reduction in separation efficiency is observed upon application of the Hadamard transform as more than 800 000 plates/m are obtained in both single-injection and cHTCE elec-

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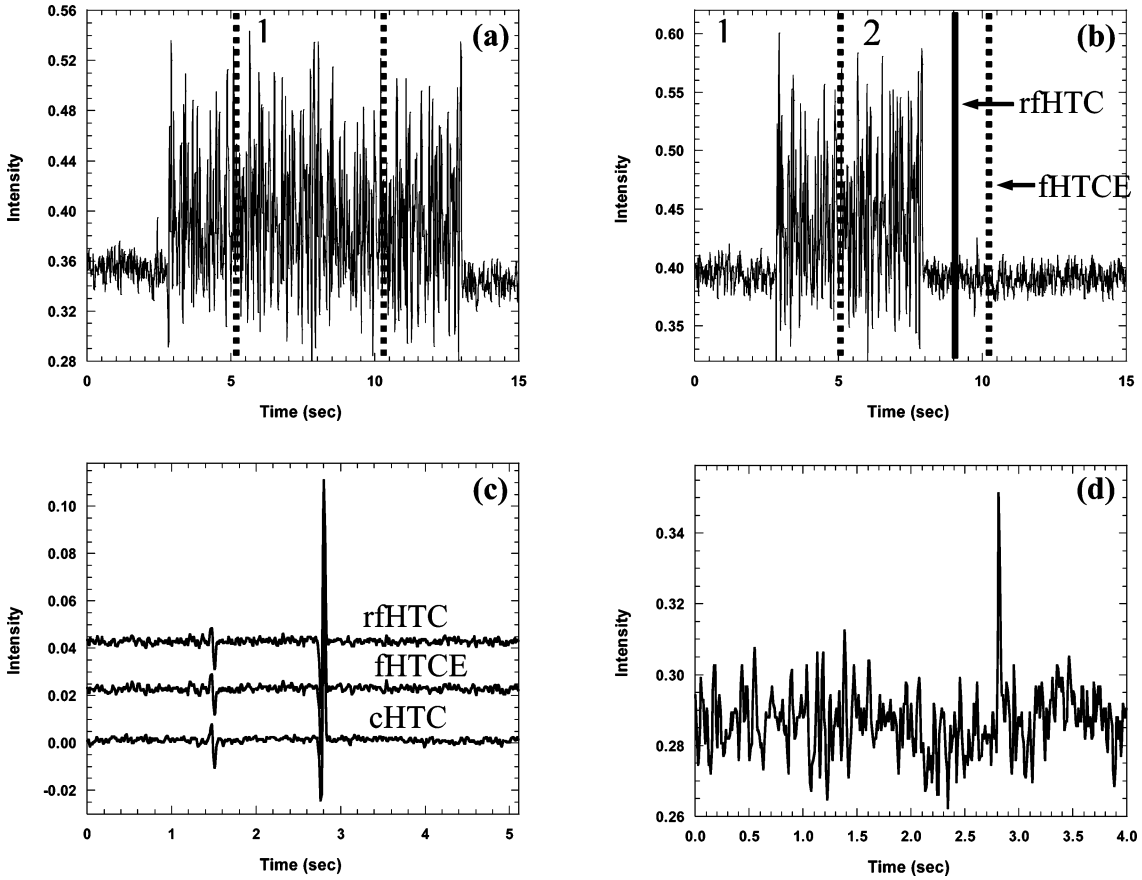

Figure 2. Comparison of HTCE injection protocols. Electropherograms obtained using a 511-element **S**-matrix with (a) cHTCE injection sequence and (b) fHTCE and rfHTCE injection sequences. (c) Deconvoluted electropherograms obtained using fHTCE, rfHTCE, and cHTCE and (d) a single-injection electropherogram. Solid line in (b) represents the collection time required for application of rfHTCE. Vertical dashed lines in (a) and (b) define the (*n*) elements utilized in the transform. Experimental conditions: 1 nM fluorescein, 10-ms injections, $E = 1.75$ kV/cm, gating beam 30 mW, and probe beam 10 mW.

tropherograms. While, the application of cHTCE increased the collection time from 2.8 to 12.9 s, obtaining similar enhancements by signal averaging would require a ~90-fold increase in total collection time compared to cHTCE.

To further improve the temporal resolution of HTCE for application in on-line monitoring, we have developed the fHTCE approach. In fHTCE, the first row of the **S**-matrix generated for a given matrix dimension is utilized to encode the injection sequence, similar to that described for HT-mass spectrometry.[24,25] Though only n injections are made, $2n$ data points are collected in order to provide the necessary data points for deconvolution. After $2n$ data points are collected, the n elements needed for deconvolution are generated by summing the 1st to nth and $(n + 1)$th to $2n$th data points.

Figure 2 shows the application of cHTCE and fHTCE utilizing a 511-element **S**-matrix with 10-ms injection plugs to a 1 nM fluorescein solution. Figure 2a shows the data collected using cHTCE, and Figure 2b shows data resulting from two implementations of fHTCE, cHTCE, and rfHTCE. In fHTCE, the n dimensional data set for transformation is extracted by summing domains 1 and 2 as defined by the dashed vertical lines (Figure 2b), whereas, for cHTCE, the n elements are acquired simply from domain 1 (Figure 2a). In this case, the total collection time required for cHTCE is 13 s, whereas fHTCE requires 10.22 s, a 21% reduction per electropherogram, resulting from the decreased injection sequence length. Although the fHTCE method halves the number of injections compared to cHTCE, a 50% reduction in collection time is not observed since deconvolution in fHTCE requires the collection of two n data domains that are summed prior to transformation. When expanded over long analysis times, the resultant decrease in data collection time yields significant enhancements in the number of data points that can be obtained and thus in the temporal resolution of the measurement. Further, the fHTCE approach utilizes 50% less sample per multiplexed separation, a significant improvement in sample-limited in vivo experiments.

When large matrices are used in combination with fHTCE, and particularly for analytes with fast migration times, the resulting $2n$ data domain contains a large amount of noninformative baseline information as seen in Figure 2b. To reduce the temporal impact of this data region on the total analysis time, and thereby increase sample throughput, we have developed an approach we term reduced fHTCE (rfHTCE), in which data collection ceases 1 s after the last injected material has been detected, thus allowing immediate application of the next injection sequence. The 1-s data segment collected is used to reconstruct the baseline data required to complete the second data domain for deconvolution. In Figure

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Table 1. Relationship between the Order of the S-Matrix, Injection Plug Length, S/N Enhancement, and Injection Method[a]

matrix size	injection sequence length	transformation method	S/N enhancement 10 ms*	20 ms*	50 ms*	100 ms*	theoretical (cHTCE)
255	255	fHTCE	5.4	5.5	6.1	6.2	
255	509	cHTCE	6.8	7.3	8.4	8.1	8.02
511	511	fHTCE	8.4	8.7	9.0	9.0	
511	1021	cHTCE	9.2	11.2	11.4	11.4	11.32
1023	1023	fHTCE	11.0	11.9	13.0	14.1	
1023	2045	cHTCE	13.1	14.6	16.2	16.4	16.01
2047	2047	fHTCE	15.5	17.6	19.5	19.5	
2047	4093	cHTCE	18.4	20.8	21.2	21.0	22.03

[a] The S/N enhancement is calculated as the ratio between the S/N values from electropherograms acquired using HTCE and the S/N of a single-injection electropherogram. (* ± 4%).

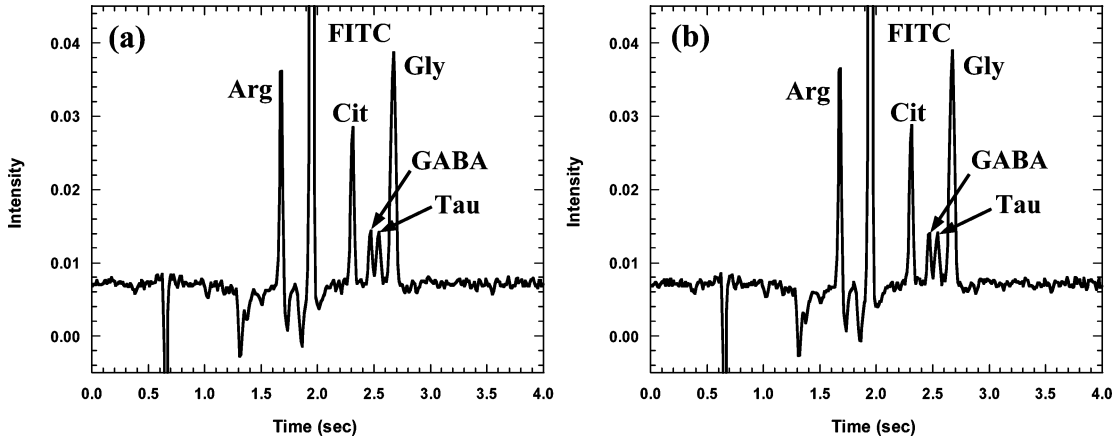

Figure 3. Analysis of model neurotransmitter mixture using fHTCE protocols. Application of a 511-element **S**-matrix using (a) fHTCE and (b) rfHTCE to the analysis of 10 nM FITC-labeled Arg, Cit, GABA, Tau, and Gly. Collection time and S/N enhancement for (a) 10.22 s/8.9 and (b) 8.8 s/9.0. Experimental conditions: 10-ms injections, $E = 1.75$ kV/cm, gating beam 50 mW, and probe beam 12 mW.

2b, the additional data are acquired from the information stored in the baseline segment between 8 and 9 s. Elements are randomly selected from the 1-s data set and used to construct the additional 1.22 s of necessary baseline data. The generated baseline data are then appended to the collected data in the second data domain and subsequently summed with the first n domain, as in fHTCE.

The reduction in total collection time required for rfHTCE is dependent on the analyte(s) migration time and on the order of the matrix used. When rfHTCE is applied to the sample and matrix shown in Figure 2, collection time is further decreased to 9 s, corresponding to 1 s after all the injected analyte has been detected, representing a 31% decrease compared to cHTCE. A maximum reduction of 48% in collection time (compared to cHTCE) can be realized through application of rfHTCE.

The deconvoluted electropherograms obtained using each method (Figure 2c) are nearly identical. When compared to a single-injection electropherogram (Figure 2d), S/N enhancements of 8.5 for fHTCE, 8.6 for rfHTCE, and 9.0 for cHTCE are observed. In addition, a few small ghost peaks are observed in all transformed electropherograms at ~1.5 s, likely due to imperfect laser gating that leads to misallocation of peak intensities.[16,17] The shutter utilized in this work exhibits bounce times between 5 and 15%. As seen in Table 1, longer injection times increase the S/N enhancement obtained, likely through minimization of this error, though at the expense of an increased total analysis time. Further, when shutter times longer than 50 ms are utilized, the ghost peaks are no longer observed. Thus, the appearance of these ghost peaks at fast gating times can be reduced, if not eliminated, through the use of more precise beam shutters/gates or by postprocessing techniques.[26]

Figure 3 shows the application of a 511-element **S**-matrix via (a) fHTCE and (b) rfHTCE to a solution containing 10 nM FITC-labeled Arg, Cit, GABA, Tau, and Gly, which produces similar S/N enhancements of 8.9 and 9.0, respectively. However, the application of rfHTCE decreases the collection time by 32% compared to cHTCE, whereas fHTCE reduces the collection time by 21%. Similar to Figure 1c, imperfect laser beam gating during sample injection results in the presence of a ghost peak at 0.65 s in the transformed electropherograms. These results suggest the potential of fHTCE and rfHTCE for time-resolved monitoring of low-abundance neurotransmitters, such as GABA and dopamine, that are difficult to detect in current CE-LIF monitoring applications.

The level of S/N enhancement is dependent on the order of the applied **S**-matrix as defined by eq 2. Table 1 summarizes the S/N enhancement as a function of matrix size, injection sequence length, and transformation method. The theoretical S/N enhancements for cHTCE are provided for comparison.[22] Using cHTCE with short separation distances and short injection times (10−

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100 ms), we observed S/N enhancements similar to those obtained using longer injection and separation times,[14] thus allowing significant reduction in total analysis time per sample. For the shortest (10−20 ms) injection times, the S/N enhancements are 15−20% lower than predicted by theory, possibly due to errors induced in the process of numerical calculations[14] and from imperfect gating.[17] The impact of these errors is reduced when injection times are increased to 50−100 ms. In contrast, S/N enhancements realized using fHTCE are ~20% lower for all injection times used here compared to cHTCE. Application of rfHTCE yielded S/N enhancements statistically similar to those obtained using fHTCE.

The S/N enhancement disparity between the cHTCE and fHTCE arises from increased noise generated by summing the two n domains needed for deconvolution in fHTCE, defined by

$$s_n = \sqrt{{s_{n1}}^2 + {s_{n2}}^2} \tag{3}$$

where s_{n1} and s_{n2} are the absolute standard deviation of domains 1 and 2, respectively. If both domains have similar absolute standard deviations, ~30% increase in the resultant total standard deviation, and a corresponding decrease in S/N enhancement, should be observed. Though undesirable, this slightly reduced S/N enhancement is tempered by a marked decrease in total analysis time and thereby an increase in the temporal resolution of the measurement. When fHTCE is coupled with rapid separations, sufficient temporal resolution may be obtained to allow on-line chemical monitoring of dynamic systems and thus may be useful for increasing the sensitivity of CE-based approaches used to monitor neurotransmitter and hormone dynamics in in vivo environments. Although eq 3 predicts a reduction in S/N enhancement of 30%, experimentally, S/N decreases of ≤20% were observed.

Time-Resolved HTCE. The reduced collection times afforded by fHTCE and rfHTCE substantially increase the overall data collection rate compared to cHTCE. The total analysis time for a single separation determines the maximum temporal resolution attainable as each individual separation can be thought of as one data point per analyte when on-line temporal monitoring is performed. Figure 4 demonstrates the different temporal resolutions that are achieved when a 2047-element **S**-matrix is sequentially applied for 320 s and subsequently deconvoluted by (a) cHTCE, (b) fHTCE, and (c) rfHTCE. In Figure 4a, seven electropherograms are obtained over 320 s. To ensure there is no overlap between the injection sequences for the application of cHTCE a 5-s delay is applied between each cHTCE cycle, thus increasing the cycle time to 45.93 s for each separation (data point). When fHTCE is utilized (Figure 4b), the cycle time is reduced to 40.94 s, allowing eight separations to be performed during the 320-s time period, though much of the data collected encodes baseline data in the second data domain. Application of rfHTCE (Figure 4c) allows removal of excess baseline data decreasing the cycle time to 24 s, 44% faster than cHTCE and 41% faster than fHTCE. This substantial time savings allows for 13 cycles to be acquired in the 320-s time period, nearly doubling the data collection rate. When shorter injection sequences are utilized, the temporal resolution increases proportionally.

Evaluation of the S/N ratios for the individual single-injection electropherograms in Figure 4a-c shows less than a 4% variation over the course of a 320-s analysis. Similar S/N variations have been observed for collection times up to 30 min in length, demonstrating the ability to continuously monitor the chemical evolution of time-dependent phenomena. The average S/N enhancements for multiple cycles compared to a single-injection electropherogram are (a) 19, (b) 16, and (c) 16.5. The small reduction in S/N enhancements resulting from the application of fHTCE and rfHTCE is balanced by the significant reduction in cycle times. Additionally, peak widths are comparable in Figure 4a−c with respect to a single-injection electropherogram regardless of the cycle number, thus maintaining the high separation efficiency associated with CE.

Both cHTCE and fHTCE require collection of two successive domains of data to acquire the n elements utilized for deconvolution. In both cases, transformation of the data yields only one data vector, n-elements long. Consequently, gaps are observed between the transformed electropherograms (Figure 4d−f). The gap in cHTCE can be calculated by summing the migration time of the slowest analyte with the time required to make $(2n - 1)$ injections and the delay time between sequences. For example, the application of cHTCE with a 5-s delay between injection sequences for the analysis of fluorescein (2.24-s migration time; Figure 4a), results in an observed gap between individual electropherograms of 25.46 s. In fHTCE, the time gap is calculated as the time required to acquire one n domain; thus, in Figure 4b, the time gap is 20.47 s wide. The gap in rfHTCE is calculated by adding 1 s to the migration time of the slowest analyte, being in this case equal to only 3.5 s (Figure 4c). Additionally, transformation of the acquired data yields a string of single-injection electropherograms in which the peak heights are the time average of the multiple single injections. The time domains of the transformed electropherograms in Figure 4d−f) are shifted to reflect this effect.

To demonstrate the capability of fHTCE for monitoring temporal dynamics, we have used on-line fHTCE to monitor a step change in the time-dependent concentration of a neurochemical test mixture. For this, a 5 nM solution of FITC-labeled GABA and the NO precursor Arg was allowed to continuously enter the separation capillary. Figure 5a shows a single injection separation of the mixture, where 5 nM FITC-labeled GABA was not detected. When fHTCE was used to analyze the same mixture, an 8.5-fold S/N enhancement was obtained, facilitating the detection of GABA (Figure 5b). Figure 5c shows the deconvoluted fHTCE peak height versus time obtained for a mixture of the neurotransmitter GABA and Arg. For multiplexing and transformation, a 511 **S**-matrix was applied for 24 consecutive fHTCE cycles, with each cycle requiring 10.22 s. After 20 s, the sample was switched to 10 nM FITC-labeled GABA and Arg for 100 s before switching back to the 5 nM solution. After switching solutions, a delay of ~30 s occurs before the concentration change is observed, due primarily to the volume of the connection capillary and associated flow rate. The rise time of the separation-based sensor is faster than one individual fHTCE separation, revealing the temporal resolution of the sensor to be limited by the separation time, ~10 s. Thus, these results represent the first application of HT for the on-line analysis of concentration dynamics of neurotransmitters. It should be noted

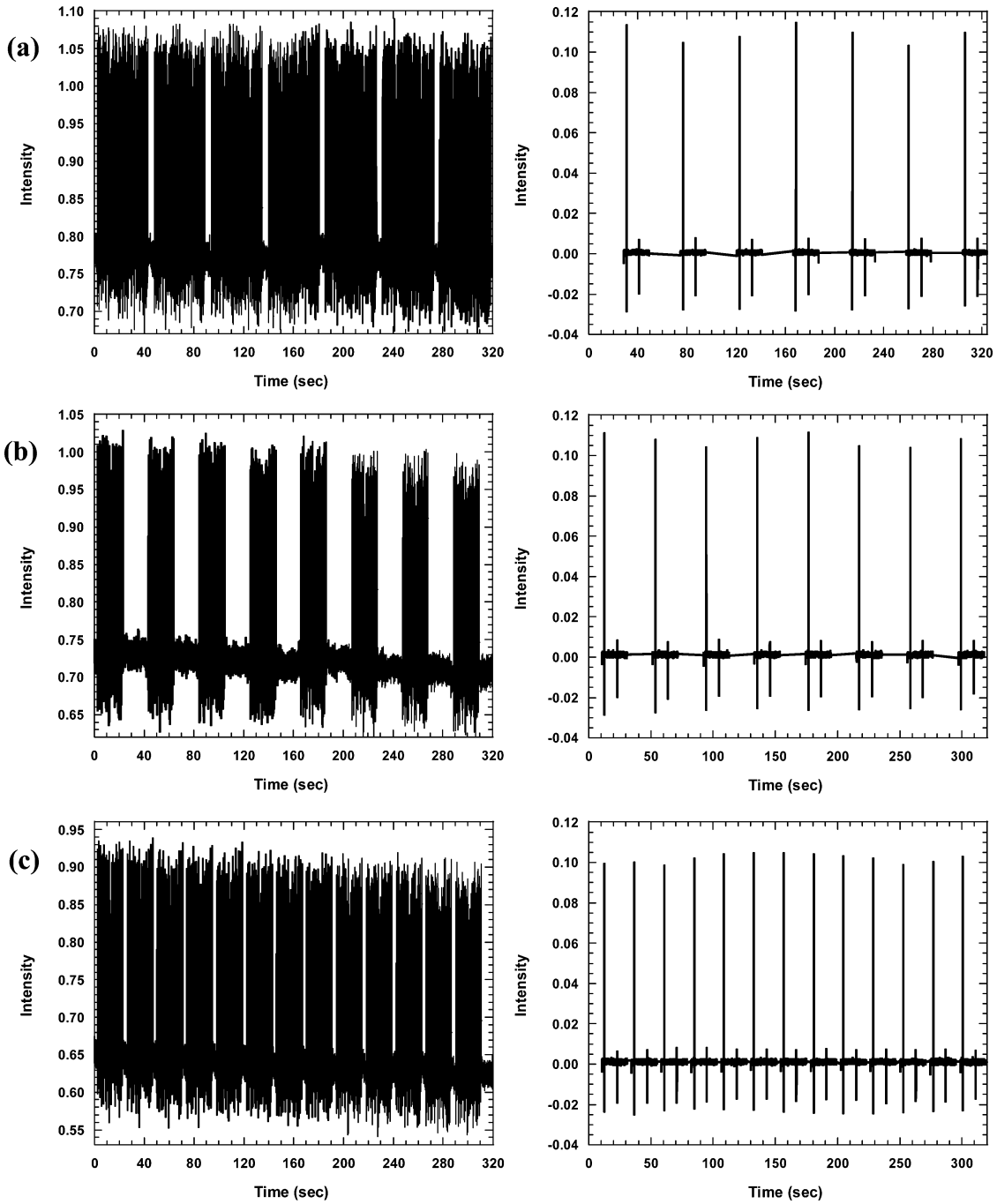

Figure 4. Sequential application and transformation of a 2047-element **S**-matrix for 320 s utilizing (a,d) cHTCE, (b,e) fHTCE, and (c,f) rfHTCE. Each cycle requires (a) 43.26, (b) 40.94, and (c) 24 s with an observed S/N enhancement of (a) 19, (b) 16, and (c) 16.5 compared to a single-injection electropherogram. Experimental conditions: 5 nM fluorescein, 10-ms injections, E = 1.75 kV/cm, gating beam 50 mW, and probe beam 13 mW.

that though the FITC labels utilized in this experiment are not amenable to on-line monitoring, the fHTCE approach demonstrated here could be readily extended to other fluorescent labels.

When applying fHTCE or rfHTCE for the measurement of concentration dynamics, the rate of change in the system determines the maximum matrix size that can be utilized. Utilization of large matrices such as a 2047 **S**-matrix with 10-ms injections increases the analysis time to between 20 and 40 s per multiplexed separation, whereas the 511 **S**-matrix used in Figure 5 requires only 5−10 s. When dynamic events change at a rate faster than the multiplexed separation time, the maximum and minimum of the change is lost in the transformation since the transformation is an average of each single injection event encoded by the matrix. Though utilization of large matrices is advantageous due to the increased S/N enhancement (Table 1), the data generated may not truly reflect the concentration dynamics within the system if an inappropriate matrix is chosen. Though it is possible to decrease the total analysis time by performing faster injections, the effects of shuttering errors are increased, reducing the observed S/N enhancement. Conversely, for fHTCE, the smallest **S**-matrix that can be used, and thus the fastest overall analysis time, is determined by the single-injection separation time.

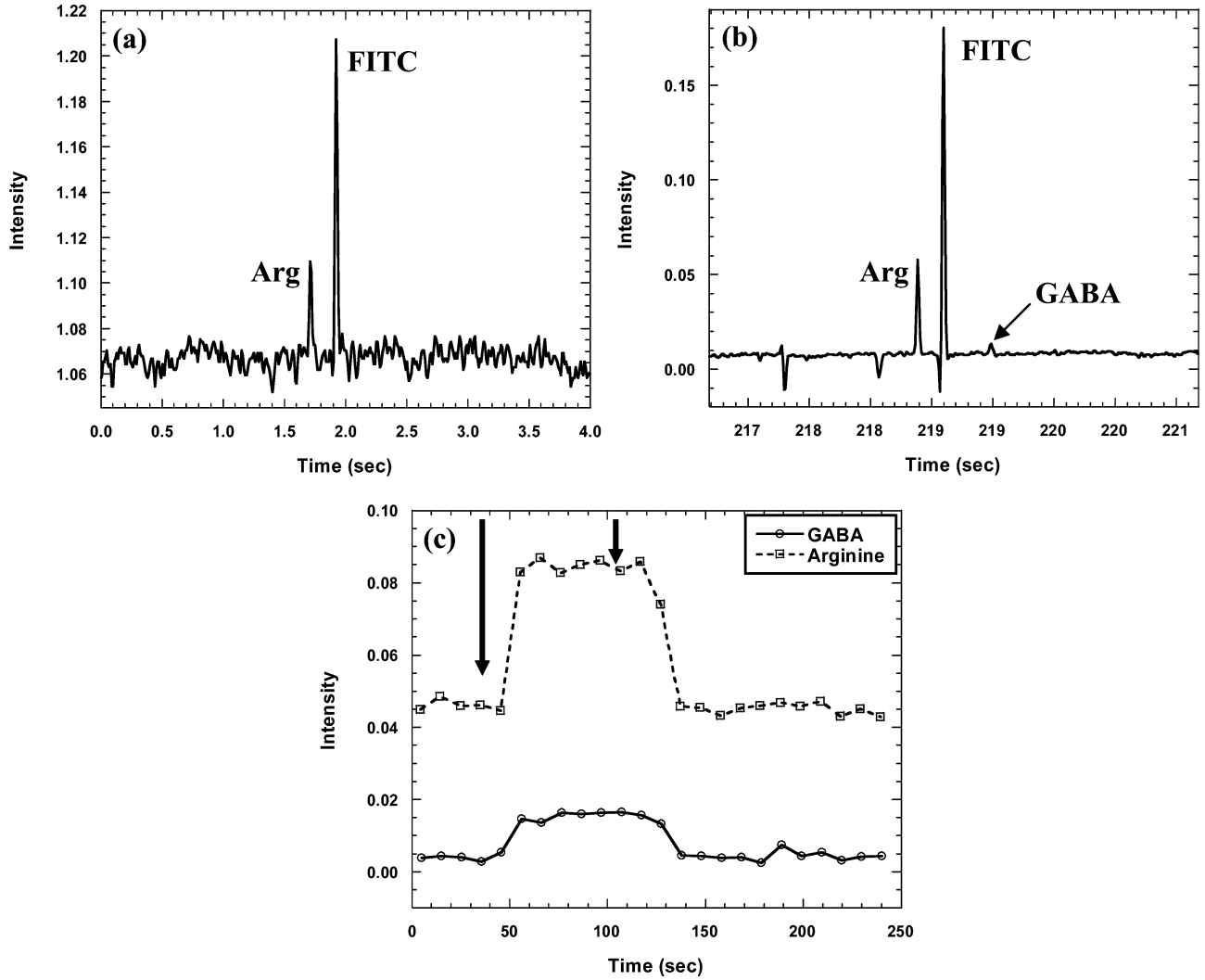

Figure 5. Temporal monitoring of a model neurochemical mixture using fHTCE. (a) Single-injection electropherograms of a 5 nM mixture of FITC-labeled GABA and Arg. (b) fHTCE separation of the mixture used in (a). (c) Time-resolved analysis of of FITC-labeled Arg (squares) and GABA (circles) using a 511-element **S**-matrix. Peak heights obtained from deconvoluted fHTCE separations are plotted as a function of time. The sample was switched from 5 (first arrow) to 10 nM and back to 5 nM (second arrow) as indicated. Experimental conditions: 10-ms injections, $E = 1.75$ kV/cm, gating beam 45 mW, and probe beam 12 mW.

Specifically, a single separation must occur with the first (n) elements. Thus, appropriate injection time and matrix size must be identified in advance for a given series of analytes. The opposite effect is observed for larger matrices.

CONCLUSIONS

Matrix-based multiplexing using Hadamard transformation provides a simple means to realize improved sensitivity in CE. To reduce the temporal impact of HTCE, two new Hadamard techniques were developed. The new HTCE methods, fHTCE and rfHTCE, described here yield transformed electropherograms similar to cHTCE, but with substantially reduced total sample volumes and total analysis times. When sequentially applied, rfHTCE nearly doubles data collection rates compared to cHTCE, a key step in the utilization of HTCE for monitoring fast temporal dynamics. The improved temporal resolution that is demonstrated in this paper makes fHTCE an attractive technique for application in the measurement of concentration dynamics. The application of multiplexing approaches to complex solutions containing analytes with large variations in concentration may lead to a reduced dynamic range due to peak broadening and overlap, though this affect is minimized in fHTCE due to the fast separation and high resolution obtainable.

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