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Investigation of Chemometric Instrumental Transfer Methods for High-Resolution NMR

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The implementation of direct standardization (DS), piecewise direct standardization (PDS), and double-window piecewise direct standardization (DWPDS) instrumental transfer techniques for high-resolution ^{1}H NMR spectral data was explored. The ability to transfer a multivariate calibration model developed for a "master or target" NMR instrument configuration to seven different ("secondary") NMR instrument configurations was measured. Partial least-squares (PLS) calibration of glucose, glycine, and citrate metabolite relative concentrations in model mixtures following mapping of the secondary instrumental configurations using DS, PDS, or DWPDS instrumental transfer allowed the performance of the different transfer methods to be assessed. Results from these studies suggest that DS and PDS transfer techniques produce similar improvements in the error of prediction compared to each other and provide a significant improvement over standard spectral preprocessing techniques including reference deconvolution and spectral binning. The DS instrumental transfer method produced the largest percent improvement in the predictions of concentrations for these model mixtures but, in general, required that additional transfer calibration standards be used. Limitations of the different instrumental transfer methods with respect to sample subset selection are also discussed.

High-resolution nuclear magnetic resonance (NMR) spectroscopy is a powerful technique for the investigation of complex mixtures and has found application in metabonomic, environmental, and pharmaceutical research. The complexity and size of NMR data sets in these fields has resulted in multivariate or chemometric analysis becoming routine within the NMR community.[1−3] It is also becoming increasingly more common to combine NMR spectral data from different sources into large databases prior to chemometric analysis. These sources may include (1) NMR data from different laboratories, (2) data within the same laboratory but obtained on different NMR instruments (including different fields), (3) data from the same NMR instrument over extended time periods, or (4) data obtained using different pulse sequences and solvent suppression techniques. The impact and magnitude of spectral variations related to different instrumental sources within combined data sets is poorly understood. For example, changes in the high-resolution ^{1}H NMR spectra associated with the use of different NMR instruments have been explored by Potts et al.,[4] who found that instrumental variations can compete with the physiological spectral changes occurring during metabonomic studies. Nonreproducible baselines associated with water solvent suppression were found to introduce significant error. Chemical shift variations, including magnetic field dependent shifts in strongly *J*-coupled multiplet systems, were also shown to contribute to the instrument-to-instrument spectral differences. These chemical shift variations were reduced by binning or integral averaging over the citrate region.[4] Similar NMR instrumental variations were also observed for principal component analysis (PCA) of ^{1}H NMR rat plasma metabonomic studies.[5] Saude et al.[6] demonstrated that spectral variations in quantitative ^{1}H NMR of synthetic urine samples were introduced by a variety of factors, including pulse sequence employed, water suppression method, differences in the spin−lattice T_1 relaxation rates of the metabolites, along with NMR hardware issues including the type of low-pass filters employed in the instrumental configuration. Spin−lattice relaxation effects and variation in signal amplitude due to *J*-coupling evolution during the CPMG (Carr−Purcell−Meiboom−Gill) pulse sequence used for protein background signal suppression have also been reported.[7] In contrast, Keun

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et al.[8] demonstrated that the PCA analysis of ^{1}H NMR spectral data of rat urine samples following hydrazine dosage at two different NMR frequencies was very reproducible, with observed instrument-correlated changes being smaller than the physiological spectral variations. Similarly, the analysis of samples produced from different collaborative centers has shown consistent classification when obtained on the same instrument under the same operating conditions.[9]

Variation of the spectral response between different NMR instruments and/or probes makes comparison of combined data sets challenging. In addition, changes in a given NMR instrument's response over extended time periods may also make classification and quantification of different chemical species difficult. The increased pooling of NMR spectral databases and collaboration of different laboratories with their own NMR instrumentation suggest that a standard operating procedure for NMR data acquisition, calibration, and instrumental response corrections is needed. The transfer of calibration models between different NMR instruments or configurations is explored in this paper.

There are several approaches to correct for different spectral responses between instruments, commonly referred to as instrumental transfer or standardization. One of the most basic approaches is to use line shape deconvolution to correct for spectral distortions resulting from magnetic field inhomogeneities. These inhomogeneities can arise from imperfect shimming, static magnetic field instabilities, homogeneity variation across the sample detection volume, pulse phase and amplitude variations, magnetic susceptibility discontinuities due to sample configuration, probe design and condition, and field homogeneity modulation due to spinning. In general, these inhomogeneity distortions affect all NMR resonances equally and can be removed or reduced using reference deconvolution. This correction method deconvolutes the experimental spectra with the reference signal's line shape and then reconvolutes the spectra with the ideal reference signal line shape. Deconvolution can be considered a simplistic method of instrumental transfer, but it does not address frequency-dependent variations between different instruments. Details and examples of reference deconvolution for high-resolution NMR spectra have been described extensively.[10−20]

Another method of instrumental transfer is to add calibration spectra from a secondary instrument to the original calibration set and then recalculate the predictive model. This type of multi-instrument calibration model development is commonly referred to as hybrid calibration (HC).[21] The HC method can be extended to collecting spectra on multiple instrumental configurations and then attempting to build a calibration model that simultaneously performs well for each configuration. A variation of this is to use combined data sets from multiple instruments, then identify and remove spectral differences between the different configurations that do not contribute to either sample classification or the model calibration. Orthogonal signal correction (OSC) is an example of this and has been used to improve the performance of near-infrared (NIR) instrumental transfer.[22] OSC has been applied to ^{1}H NMR metabonomic data to eliminate spectral components due to instrumental variations (on a single instrumental configuration) or physiological changes not associated with classification[5] but has not been directly applied to NMR instrumental transfer. In the OSC studies of Beckwith-Hall the initial PCA model showed classification based on the NMR instrument identity. Removing two orthogonal components via OSC removed NMR instrument identity clustering in the PCA. This same study demonstrated that OSC can remove time-dependent experimental and physiological spectral changes. Instrumental transfer involving the mapping of one instrumental response onto other instrumental configurations using a finite impulse response (FIR) filtering of the spectra has also been described[23] but has not been demonstrated for NMR.

A limitation of HC and OSC methods is that the addition of more instruments (or instrumental configurations) requires that multivariate models be recalculated. In the case of OSC, the orthogonal components that do not describe the classification or calibration are removed from all the configurations, and the resulting OSC-filtered data is used in the subsequent model development. If an additional instrument configuration is added at a later time, all the data are combined, followed by subsequent OSC analysis and filtering. Unfortunately, this filtering invalidates any previously developed calibration or identification models and might prove costly or time-consuming if new calibration models must be evaluated for each new instrumental configuration. Because of the multiple NMR instrument configurations analyzed in this manuscript, the use of OSC filtering is not presented but will be discussed in a later communication.

The most common instrumental transfer methods involve the direct transfer of experimental spectra from a new (secondary) instrumental configuration to the original master (primary or target) instrument using either direct standardization (DS) or piecewise direct standardization (PDS) with additive background correction.[24−30] The DS and PDS instrumental transfer methods

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have been extended to include double-window piecewise direct standardization (DWPDS).[31] The use of DS and PDS methods has been demonstrated for low-resolution (20 MHz) process NMR spectroscopic data sets.[32] By using the ^{1}H free induction decay (FID) to develop a PLS model for prediction of high-load melt index values, the impact of probe ring-down, pulse widths, and temperature offsets was addressed. For these time-domain NMR studies, the PDS method was found to be the most successful instrumental transfer method. These initial low-resolution results demonstrate that instrumental transfer techniques can be applied to NMR spectroscopy and provide the impetus to extend the techniques to high-resolution NMR spectral data sets.

In this paper, the performance of DS, PDS, and DWPDS methods for the transfer of a partial least-squares (PLS) calibration model of metabolite concentrations in model mixtures is explored. The optimization of the instrumental transfer methods with respect to the number of transfer calibration standards and filter window size is addressed. In addition, the impacts of phasing, binning, and spectral deconvolution on the observed prediction errors between different NMR instrument configurations are evaluated.

METHODS

NMR Data Sets. A set of 15 standard samples composed of a simple mixture (0−50 mM) containing the metabolites citrate, glucose, and glycine in D_2O (pH = 7.0, 10 mM phosphate buffer) with 5 mM 3-trimethylsilylpropionate (TSP) were prepared, sealed in matched tubes, and used for all experiments described. The mixture concentrations are provided in the Supporting Information. No experimental design was used in preparing these samples. NMR data were collected on two different NMR instruments, a Bruker Avance 600 and a Varian Unity Plus 600, using either a 5 mm broad-band (BB) X(^{1}H) observe probe (Bruker), a ^{1}H(X) inverse (INV) probe (Bruker), or two vintages of an HCN probe (Varian). For each instrument and probe, NMR data using both a single-pulse 1D direct and a 1D nuclear Overhauser enhancement spectroscopy (NOESY) (100 ms) sequence was obtained to give data sets for eight different configurations. These instrumental configurations will be designated as 1D BB Bruker, NOESY BB Bruker, 1D INV Bruker, NOESY INV Bruker, 1D HCN(a) Varian, NOESY HCN(a) Varian, 1D HCN(b) Varian, and NOESY HCN(b) Varian. A combined data set composed of 120 different ^{1}H NMR spectra (15 samples × 8 configurations) was obtained. Experimental conditions between the different NMR instruments were matched as closely as possible using a spectral width of 7183 Hz, 32K spectral points, zero filling to 64K, 9 μs $\pi/2$ pulse, 1 s recycle delay, 64 scan averages, with no water presaturation employed. To ensure similar data treatment between the different instrument platforms, the NMR data sets were all transformed, phased, referenced, and baseline-corrected in the CHENOMX NMR Suite 5.0 (Edmonton, Canada). The chemical shifts were referenced to internal TSP, $\delta = -0.016$ ppm. The shift of TSP is pH-dependent as incorporated into CHENOMX NMR Suite 5.0. In addition, spectral binning and line shape deconvolution[20] were performed in the CHENOMX Suite. To study the impact of phasing on the model transfer, a single instrumental configuration data set was phased using the automatic baseline routines ABS and ABSD in the Bruker TOPSPIN software, and manually by two different operators, to give a total of four different data sets for analysis of phasing effects. The data sets were integral normalized to the total spectral intensity and then mean-centered. Of the 15 standard mixtures, three represent pure component spectra and were removed during model development to reduce extensive biasing and leveraging. This reduction means that 12 samples were used in the calibration model development for a total of 96 different NMR spectra in the data set (12 samples × 8 configurations). A PLS calibration model (using the SIMPLS algorithm) was developed for the relative metabolite concentrations on the target instrument (1D BB Bruker) using the 12 calibration samples with three factors on mean-centered data. This model was then used to predict the metabolite concentrations on the remaining seven instrumental configurations for the same calibration samples both before and after instrumental transfer. In order to reduce possible variations between the calibration samples an independent calibration sample set was not utilized on the secondary instruments to evaluate the PLS model.

Computational Details. The analysis of the data and development of the calibration models were performed in MATLAB 2007b (The Mathworks, Inc.), whereas the different instrumental transfer algorithms employed were implemented using the PLS Toolbox 4.1 (Eigenvector Research, Inc.). The root-mean-square errors of calibration ($RMSEC_1$) on the primary instrument and the root-mean-square errors of prediction ($RMSEP_j$) of the jth secondary instrumental configuration are defined by

$$\mathrm{RMSEP}_j\ [\text{or } \mathrm{RMSEC}_1] = \sqrt{\frac{\sum_{p=1}^{n} (\hat{y}_p(j) - y_p)^2}{n}} \qquad (1)$$

where n is the number of calibration samples, $\hat{y}_p(j)$ are the predicted concentration values for the pth standard sample on the jth instrumental configuration, and y_p is the true concentration. The improvement in prediction for a given instrumental transfer method on the jth instrumental configuration summing over the m different components is given by

$$\%\ \text{improvement (method},j) = \frac{1}{\text{ncomp}} \sum_{m=1}^{\text{ncomp}} \frac{(\mathrm{RMSEP}_{\text{method},j,m} - \mathrm{RMSEP}_{\mathrm{Raw},j,m})}{\mathrm{RMSEC}_{\mathrm{Raw},1,m}} \times 100 \qquad (2)$$

where $\mathrm{RMSEP}_{\mathrm{Raw},j,m}$ is the error for the mth component, on the jth instrument using the raw unmodified data with no binning and prior to instrumental transfer.

Direct Standardization. Throughout this paper scalars are represented by italic lowercase letters, column vectors by boldface lowercase letters, and matrices by boldface capital letters. Mean-centered vectors and matrices are denoted by a bar over the letter. Instrumental transfer using the DS method correlates the spectra (or FIDs) between different NMR instruments by modeling the responses as[24−26,29]

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$$\mathbf{S_1} = \mathbf{S}_j\mathbf{F_b} + \mathbf{1b}_{j\mathbf{s}}^{\mathrm{T}} \quad (3)$$

where the matrix $\mathbf{S_1}$ is the response of the target NMR instrument on which the calibration model was developed, and $\mathbf{S}_j$ is the response matrix of instrumental configuration j, $\mathbf{F_b}$ is the ($\nu \times \nu$) transformation matrix, and $\mathbf{b}_{j\mathbf{s}}^{\mathrm{T}}$ is the background correction vector ($1 \times \nu$) for the jth configuration. The response matrix $\mathbf{S}$ ($n \times \nu$) is composed of n samples and ν frequencies. In the present case, the $\mathbf{S}_j$ matrices represent the spectral response of different instrument configurations (different instruments, different probes, different pulse sequences, or data over extended periods of time) that we want to transform into the target instrument. Once the data is transformed, the $\mathbf{S_1}$ model can be applied. For mean-centered spectral data ($\bar{\mathbf{S}}_j$), eq 3 becomes

$$\bar{\mathbf{S}}_\mathbf{1} = \bar{\mathbf{S}}_j\mathbf{F_b} \quad (4)$$

For the DS method the transfer matrix is calculated using

$$\mathbf{F_b} = \bar{\mathbf{S}}_j^{+}\bar{\mathbf{S}}_\mathbf{1} \quad (5)$$

where $\bar{\mathbf{S}}_j^{+}$ is the pseudoinverse of $\bar{\mathbf{S}}_j$. Calculation of the pseudoinverse employs singular value decomposition (SVD) where the response matrix is decomposed into three matrices $\mathbf{U}$, $\mathbf{\Sigma}$, and $\mathbf{V}$ using

$$\bar{\mathbf{S}}_j = \mathbf{U}\Sigma\mathbf{V}^{\mathrm{T}} \quad (6)$$

The pseudoinverse is then given by

$$\bar{\mathbf{S}}_j^{+} = \mathbf{V}\Sigma^{-1}\mathbf{U}^{\mathrm{T}} \quad (7)$$

The additive background term is then estimated as

$$\mathbf{b}_{j\mathbf{s}} = \mathbf{s}_{\mathbf{1}m} - \mathbf{F}_\mathbf{b}^{\mathrm{T}}\mathbf{s}_{j\mathbf{m}} \quad (8)$$

where $\mathbf{s}_{j\mathbf{m}}$ are the mean vectors of the response matrices $\mathbf{S}_j$. For NMR, the spectral baselines are commonly corrected during preprocessing such that the background $\mathbf{b}_{j\mathbf{s}}$ should be vanishingly small and will have no impact on analysis employing mean-centered data. If the baseline is highly structured (i.e., baseline roll) and not corrected during routine preprocessing prior to instrumental transfer, then the additive background term will need to be determined.

Piecewise Direct Standardization. For the DS method described above, the entire spectrum of the secondary instrument is used for the calculation of the transfer matrix $\mathbf{F_b}$ at each frequency. For NMR data sets (as well as other types of spectroscopies), the spectral changes observed between instruments will only involve small frequency changes such that determination of $\mathbf{F_b}$ can be restricted to nearby frequencies within the spectrum. Using PDS,[24−26,29] the spectral response on the primary instrument at the νth frequency ($\mathbf{r}_{1,\nu}$) can be related to the spectral response on the jth instrumental configuration at nearby wavelengths with a window width of $2k + 1$ ($\mathbf{r}_{j,\nu-k}$, $\mathbf{r}_{j,\nu-k+1}$, ... , $\mathbf{r}_{j,\nu+k-1}$,$\mathbf{r}_{j,\nu+k}$). These measurements for the νth frequency can be placed into a matrix to form

$$\mathbf{X}_\nu = [\mathbf{r}_{j,\nu-k}, \mathbf{r}_{j,\nu-k+1}, ..., \mathbf{r}_{j,\nu+k-1}, \mathbf{r}_{j,\nu+k}] \quad (9)$$

A regression vector at each frequency can then be calculated using

$$\mathbf{b}_\nu = \mathbf{X}_\nu^{+}\mathbf{s}_{\mathbf{1},\nu} \quad (10)$$

The transformation $\mathbf{F_b}$ is then a banded diagonal matrix given by

$$\mathbf{F_b} = \mathrm{diag}(\mathbf{b}_1^{\mathrm{T}}, \mathbf{b}_2^{\mathrm{T}}, \mathbf{b}_3^{\mathrm{T}}, ..., \mathbf{b}_\nu^{\mathrm{T}}) \quad (11)$$

where most of the off-diagonal elements in the transformation matrix are zero.

Double-Window Piecewise Standardization. The PDS method can be extended through the use of a double-window (DW) PDS, where the first window length is defined by $2k + 1$ frequencies and a second window of width $2m + 1$ samples are used in the calculation of $\mathbf{F_b}$. This method is detailed extensively in the PLS 4.1 Toolbox software (Eigenvector Research, Inc.) and will not be reproduced here.

Selection of Standardization Subset. The subset of standard samples used for the instrumental transfer must contain enough spectral information to fully describe the differences between the two instrumental configurations and span the model of interest. In this study, the transfer calibration samples were identified by calculation of the hat matrix $\mathbf{H}$ using[24]

$$\mathbf{H} = \bar{\mathbf{S}}_\mathbf{1}\bar{\mathbf{S}}_\mathbf{1}^{+} \quad (12)$$

The diagonal elements of this matrix contain the leverage of the samples with respect to their influence on the calibration model and were used to identify the ranking of the calibration standard samples. The calculation of $\mathbf{H}$ used the combined PLS model for all three metabolites in the mixture (glucose, glycine, and citrate). Selection of the transfer calibration samples based on the $\mathbf{H}$ leverage determined using only the glucose calibration gave similar ordering.

RESULTS AND DISCUSSION

Figure 1 shows the high-resolution solution ^{1}H NMR spectra for the 15 different mixtures obtained using the broad-band probe on a Bruker Avance 600 instrument. Similar spectra were collected for the other instrument, probe, and pulse sequence configurations. These NMR spectra are composed of signals from glycine, glucose, and citrate. The data at 0.001 ppm resolution and following 0.04 ppm binning are also shown in Figure 1. Only the portion of the NMR spectra between ~+4 ppm and +2.2 ppm was used in the model development described below. This spectral range does not contain the water region (~4.8 ppm), eliminating the interference of exchangeable protons and the large dominant residual water resonance. Previous studies have shown spectral variations result from the use of different water saturation sequences including the optimization of these sequences.[4] The ability of instrumental transfer methods to correct spectral differences resulting from water presaturation was not

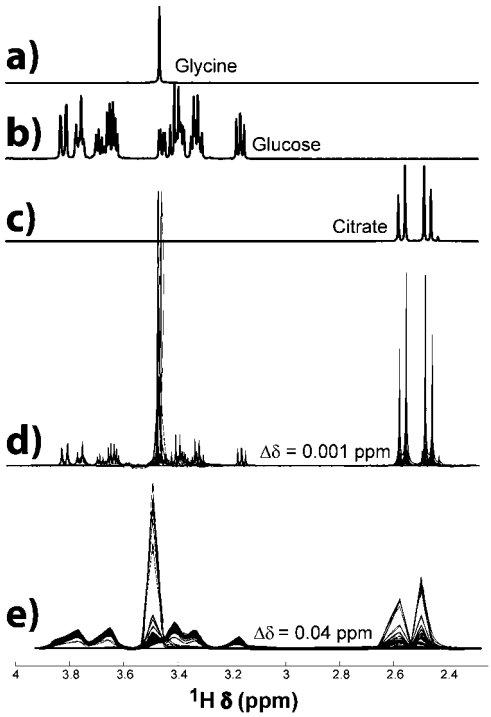

Figure 1. High-resolution ^{1}H NMR spectra of the individual components used to prepare the transfer calibration standard samples: (a) glycine, (b) glucose, and (c) citrate. The overlapping NMR data set of the 15 different standard mixtures with (d) a spectral resolution of $\Delta\delta = 0.001$ ppm and (e) following a $\Delta\delta = 0.04$ ppm binning.

pursued in the current study and will be the focus of future analysis. The metabolites chosen did provide examples where the NMR spectral features were isolated and well-resolved (i.e., the citrate multiplet at $\delta \sim +2.55$ ppm), while other signals contain significant overlap (i.e., the complex set of glucose multiplets and the glycine singlet at $\sim$+3.5 ppm).

To address the performance of the instrumental transfer methods, the ^{1}H NMR spectra obtained using the eight different instrumental configurations (see the Methods section) were used for the prediction of the relative glucose, glycine, and citrate concentration within the mixtures. For our analyses, the data set obtained on the Bruker Avance 600 instrument using a 5 mm broad-band probe with a simple single-pulse 1D excitation (1D BB Bruker) was chosen as the master (target or primary) instrument to which the other instrumental data sets were transferred using different protocols.

Predictions Without Instrumental Transfer. On the master NMR instrument RMSEC values of 0.0099, 0.0058, and 0.0075 were obtained for the relative glucose, glycine, and citrate concentrations, respectively (see Table 1). Figure 2 shows the glucose predictions for the remaining seven instrumental configurations, with the glycine and citrate predictions displayed in Figures S1 and S2 (Supporting Information). Using the PLS model developed for the target instrument (1D BB Bruker), the corresponding RMSEP for the other instrumental configurations are given in Table 1 (row 1, analysis method). It is clear that the predictive performance of the relative concentration model does not transfer well to different instrumental configurations. The exception to this trend was the NOESY data set for the BB probe (NOESY BB Bruker) that are spectra obtained on the same instrument and probe used to develop the calibration model, but with a different pulse sequence. Closer inspection of Figure 2 shows that the predictions from the single-pulse experiments (1D) and the NOESY experiments always cluster together for a particular instrument and probe. This demonstrates that the impact of using a 1D Bloch decay experiment versus a 100 ms NOESY experiment is small and does not greatly influence the predictive model transfer between these configurations. PLS models were also developed for each instrument configuration individually and then used to predict the relative concentrations on the remaining instruments. These results are summarized in Supporting Information Table S1.

Inspection of Figure 2 reveals that glucose concentrations are overpredicted ($\sim$20%) for low relative concentrations on all of the secondary instrumental configurations, including the data sets obtained from the same instrument console (Bruker) but with an inverse probe. This suggests that the issues in the spectral response were not simply vendor console related. At high glucose concentrations the PLS predictions underestimate the concentration (up to 10%) with the Varian data sets showing the largest deviation from the developed model. These observed RMSEP errors result from a combination of line width changes and reduced resolution of the glycine singlet from the glucose multiplets. Note that relative concentrations were used for the PLS model development, with an error in the measurement of any single component impacting the other calculated concentrations. This is also reflected in the largest error occurring at high glycine and citrate relative concentrations (Supporting Information Figures S1 and S2) mirroring the glucose results in Figure 2. The consistently larger citrate RMSEP (in comparison to glycine) may also reflect the limited number of samples containing citrate in the PLS calibration samples. There are several spectral variations that could impact these concentration predictions including changes in resolution (binning), line shape and line width changes between the different instrumental configurations, variations in spectral preprocessing including baseline and phase corrections, along with differences in pulse excitation efficiency. In the next section the impacts of these different variations are addressed, followed by the assessment of methods for improving the performance of model transfer between different NMR instrument configurations.

Impact of Phasing. Differences in the quality of spectral phasing are one of the simplest variations that might be encountered when comparing NMR data between different instrumental configurations. The data may be processed by automated phasing routines or manually by different operators prior to data set combination. To address the magnitude of changes in RMSEP (in the current data set) as a function of phasing, a single NMR data set was manually phased by two different operators and automatically using two different phasing routines available in the Bruker TOPSPIN package. It was observed that the largest differences in the spectral phasing for the different operators and automatic routines involved large dominant resonances (i.e., H_2O) that in some situations may subtly impact the instrumental transfer performance. In the present study, the developed PLS models excluded the H_2O resonance, eliminating any large phasing errors associated with this major resonance. The resulting RMSEP for the glucose, glycine, and citrate concentrations as a function of the phasing protocol are included in

Table 1. RMSEP Values for Prediction of Glucose, Glycine, and Citrate Concentration, and Percent Improvement for the Different NMR Instrumental Configurations as a Function of Transfer Method Employed

Analysis Method	RMSEP Data Set Prediction[a]							
	#1 1D BB Bruker[b]	#2 1D INV Bruker	#3 1D HCN(a) Varian	#4 1D HCN(b) Varian	#5 NOESY BB Bruker	#6 NOESY INV Bruker	#7 NOESY HCN(a) Varian	#8 NOESY HCN(b) Varian
Full Data No Transfer	0.0099 0.0058 0.0075	0.0647 0.0244 0.0556	0.0876 0.0227 0.0791	0.0941 0.0264 0.0894	0.0162 0.0135 0.0090	0.0678 0.0241 0.0583	0.0912 0.0228 0.0818	0.0971 0.0264 0.0920
Spectral Decovolution No Transfer	0.0090 0.0050 0.0064	0.0725 0.0219 0.0609	0.0855 0.0196 0.0726	0.0819 0.0252 0.0727	0.0137 0.0104 0.0084	0.0753 0.0212 0.0638	0.0899 0.0196 0.0766	0.0879 0.0253 0.0788
% Improvement	+12.5%	-3.8%	+8.1%	+12.1%	+15.0%	-2.8%	+7.3%	+9.3%
Spectral Deconvolution + (DS)[c]	N/A	0.0141 0.0111 0.0046	0.0142 0.0090 0.0097	0.0110 0.0125 0.0065	0.0080 0.0054 0.0055	0.0134 0.0106 0.0047	0.0184 0.0095 0.0150	0.0262 0.0129 0.0199
% Improvement	--[b]	+74.8%	+77.3%	+77.9%	+49.8%	+76.1%	+73.3%	67.5%
Spectral Deconvolution + PDS[d]	N/A	0.0225 (0.0175) 0.0135 (0.0094) 0.0174 (0.0103)	0.0450 (0.0333) 0.0151 (0.0120) 0.0327 (0.0249)	0.0421 (0.0400) 0.0138 (0.0130) 0.0303 (0.0289)	0.0134 (0.0080) 0.0124 (0.0067) 0.0078 (0.0058)	0.0225 (0.0190) 0.0128 (0.0098) 0.0176 (0.0112)	0.0484 (0.0354) 0.0159 (0.0121) 0.0355 (0.0275)	0.0436 (0.0394) 0.0129 (0.0122) 0.0343 (0.0303)
% Improvement	--[b]	59.5% (72.0%)	46.9% (59.2%)	56.4% (58.6%)	12.9% (45.5%)	61.2% (70.7%)	44.6% (58.2%)	56.3% (60.1%)
Spectral Deconvolution + DWPDS[e]	N/A	0.0180 0.0090 0.0104	0.0403 0.0132 0.0317	0.0402 0.0136 0.0317	0.0073 0.0073 0.0059	0.0190 0.0092 0.0115	0.0448 0.0140 0.0348	0.0436 0.0137 0.0330
% Improvement	--[b]	72.2%	52.0%	56.8%	45.1%	71.4%	49.0%	55.8%

[a] RMSEP = root-mean-square error of prediction. The three vertically listed errors in a group correspond to the error for glucose, glycine and citrate, respectively. [b] RMSEC = root-mean-square error of calibration. The PLS model was developed for this target instrument (green) using 12 calibration samples. No % improvement is calculated for this sample using DS, PDS and DWPDS since this is the target instrumental configuration. The NOESY related configuration is denoted by the grey column. [c] DS = direct standardization. Instrumental calibration RMSEP calculated using 5 transfer calibration samples. [d] PDS = Piece-Wise Direct Standardization. Instrumental calibration RMSEP calculated using 4 transfer calibration samples and 1 calibration frequency or (7 calibration frequencies). [e] DWPDS = Double-Window Piece-Wise Direct Standardization. Instrumental calibration RMSEP calculated using 4 transfer calibration samples and window sizes of 7 and 1.

Table S2 (Supporting Information). This result shows that the impact of phasing in the present example is very small and that phasing variations do not account for the large RMSEP differences between instrumental configurations observed in Figure 2 (and Supporting Information Figures S1 and S2). In some cases involving large solvent peaks, phasing may play a more significant role in the instrumental variations and will need to be considered.

Impact of Binning. Binning is commonly utilized in the preprocessing of NMR spectral data prior to chemometric analysis to help reduce small spectral shifts and minor line shape variations. PLS models for concentration were developed as a function of binning size to address if the RMSEP between the different instrumental configurations might be improved by increasing the bin size. These results are summarized in Supporting Information Table S3 and Figure S3. For the PLS model predictions of glucose, glycine, and citrate concentrations, no significant changes in RMSEC were observed with increased binning size. For the other instrumental configurations, there were modest improvements in the RMSEP (Supporting Information Table S3) ranging between ~5% and 30% as a function of binning size. There does not appear to be a unique binning size for optimal improvement in RMSEP between the different instrumental configurations making it difficult to select a binning size for predictive purposes. For these particular model mixtures use of 0.005 ppm binning would have produced between 6% and 28% improvement in RMSEP. The RMSEP of the different instrumental configurations still continue to be 3−10 times larger than that of the target instrument for which the PLS model was developed (1D BB Bruker). The exception to this is the NOESY BB Bruker configuration, again demonstrating that the pulse sequence (1D vs NOESY) has a minimal impact on the instrumental transfer.

In metabonomic studies, binning helps remove small chemical shift variation due to changes in pH, concentration, temperature, and ionic strength. Peak alignment or spectral registration techniques have also been employed to eliminate these chemical shift variations.[33−36] In this paper such chemical shift perturbations were expected to be small since identical standard samples

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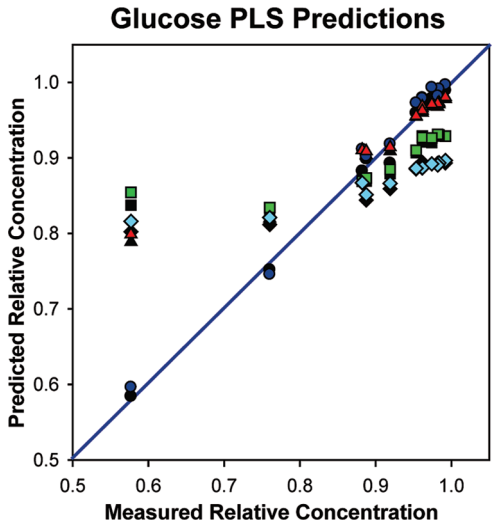

Figure 2. Correlation between the ^{1}H NMR PLS predicted relative concentration of glucose and the measured relative concentration of glucose, for the standard mixtures using eight different instrumental configurations. The PLS model was developed for the relative concentration of glucose, glycine, and citrate simultaneously using the unbinned 1D ^{1}H NMR spectra data set from the Bruker instrument with a broad-band probe, 1D BB Bruker (black circles). This model was subsequently used for the prediction of metabolite concentrations on the remaining configurations prior to any instrumental transfer modification, NOESY BB Bruker (blue circles), 1D INV Bruker (black triangles), NOESY INV Bruker (red triangles), 1D HCN(a) Varian (black squares), NOESY HCN(a) Varian (green squares), 1D HCN(b) Varian (black diamonds) and NOESY HCN(b) Varian (light-blue diamonds). The results for a given NMR probe utilize the same symbols, with the NOESY results being colored.

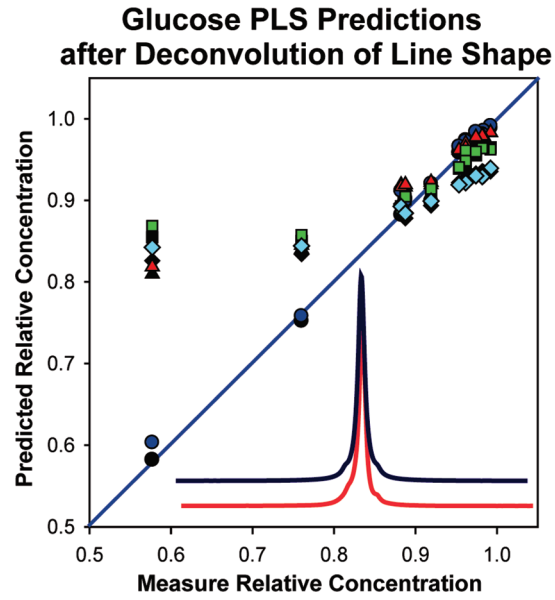

Figure 3. Correlation between the ^{1}H NMR PLS predicted relative concentration of glucose and the measured relative concentration of glucose, for the standard mixtures using eight different instrumental configurations following line shape deconvolution. The PLS model was developed for the relative concentration of glucose, glycine, and citrate simultaneously using the unbinned 1D ^{1}H NMR spectra data set from the Bruker instrument with a broad-band probe, 1D BB Bruker (black circles). This model was subsequently used for the prediction of metabolite concentrations in the remaining data sets, NOESY BB Bruker (blue circles), 1D INV Bruker (black triangles), NOESY INV Bruker (red triangles), 1D HCN(a) Varian (black squares), NOESY HCN(a) Varian (green squares), 1D HCN(b) Varian (black diamonds) and NOESY HCN(b) Varian (light-blue diamonds). The inset shows an example of the slight asymmetric line shape observed in the TSP resonance (red) that has undergone line shape deconvolution (dark blue).

were used in the analysis. It should be noted that the instrumental transfer methods, that are the focus of this paper, attempt to remove instrumental variations through transfer calibration samples and that sample-to-sample variations such as pH-dependent chemical shift changes are not directly addressed by the instrumental transfer methods. These results show that, although a judicious use of binning could improve the RMSEP, the small chemical shift variations addressed by binning were not responsible for the poor instrumental transfer predictions shown in Figure 2 (and Supporting Information Figures S1 and S2).

Impact of Deconvolution. The impact of line shape deconvolution on the prediction of concentration was also investigated by reprocessing the data sets with the CHENOMX deconvolution algorithm. Using the TSP resonance as the internal line shape reference, the asymmetric non-Lorentzian line shapes of the other resonances were corrected. A default assessment of the line broadening based on the TSP line width for each individual spectrum was used for deconvolution and ranged from 1.0 to 5.0 Hz. By targeting the deconvolution to the observed TSP line width, the deconvolution procedure removed asymmetries present within the spectrum but did not remove the large differences in line width observed between the different NMR instrument configurations. Figure 3 shows the resulting PLS predictions for the relative concentration of glucose following line shape deconvolution of the entire data set. Comparison of this prediction to the results in Figure 2 shows only a minor improvement (in particular at the high glucose concentration range), but the change is still small compared to the larger deviations observed between different instrument configurations and the original PLS model. The RMSEP following spectral deconvolution are summarized in Table 1 (row 2, analysis method) and Table S4 (Supporting Information). Between 7% and 15% improvement in RMSEP was observed, but for some configurations (1D INV Bruker and NOESY INV Bruker) there was actually a decrease in RMSEP performance.

Recall that for each individual sample the shimming was optimized and thereby may introduce a sample-to-sample variation within an instrumental configuration. If all of the samples in a given instrumental configuration are not shimmed exactly the same, then the relationship on which the instrumental transfer function was developed (eq 3) no longer strictly holds. As noted above, the target line width was chosen to match the experimental TSP line width, and this effectively removes the asymmetries and distortions of the line shapes due to poor shimming between different instrumental configurations, allowing the instrumental transfer method to be employed. To reduce the impact of small line shape asymmetries and distortions unbinned but line shape deconvoluted spectral data sets were used during the remainder of the instrumental transfer analysis discussed below.

By not choosing the same target line width during deconvolution of the spectra on all the different instrumental configurations, the impact of line width changes on the predicted performance has been maintained. In addition, it is always possible to add future additional instrumental configurations such that there

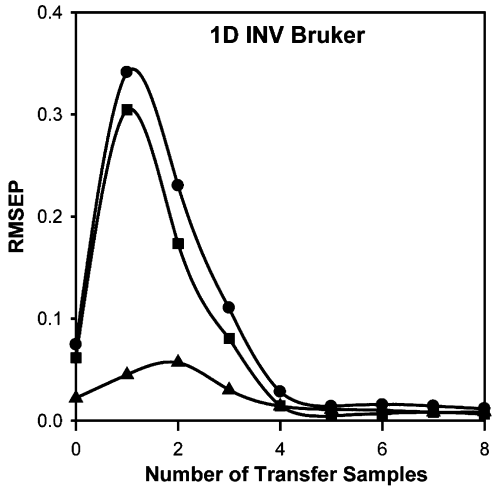

Figure 4. Variation of RMSEP for the relative concentration of glucose (●), glycine (■), and citrate (▲) using direct standardization (DS) instrumental transfer on the 1D INV Bruker instrument configuration as a function of number of transfer calibration samples.

is no way to know the most appropriate target line width a priori. Analysis using spectral data that was deconvoluted to match the smallest line width observed in all of the instrumental configurations resulted in dramatically increased RMSEP values. This was particularly true for configurations (i.e., 1D HCN(b) Varian) with large experimental line widths (~5 Hz) where the signal-to-noise ratio (S/N) of the deconvoluted spectra dropped dramatically when the smaller target line widths were imposed. This reduction in S/N performance as a function of the target reference deconvolution line width has been noted before.[19]

DS Method. The use of the DS method to transfer NMR spectra between different instrumental configurations was explored first. This method has been used for NIR and IR data, with only limited application to NMR data.[32] The calculation of the transfer function $\mathbf{F_b}$ (see details in the Methods) is based on using a subset of standards and is therefore a function of the number of transfer calibration samples utilized. The DS method is considered to have converged when the RMSEP reaches a minimum with increasing number of transfer calibration samples. One of the goals of this study was to address how many transfer samples are needed to obtain convergence in high-resolution NMR data sets. The RMSEPs for metabolite concentrations based on the PLS model developed using the target configuration (1D BB Bruker) were evaluated for increasing number of calibration samples (on unbinned, but deconvoluted, data sets). As an example, Figure 4 shows the variation in the RMSEP of the glucose, glycine, and citrate concentrations as a function of the number of calibration samples used for instrumental transfer of the secondary 1D INV Bruker configuration. The ranking (eq 12) of the transfer calibration samples chosen was based on the highest leverage for the entire PLS calibration. There is an initial large increase in RMSEP for DS when only a single sample is used for the estimation of $\mathbf{F_b}$. The RMSEP then decreases quickly and reaches a plateau or intermediate minimum when more than four transfer calibration samples were used. Table 1 shows the RMSEP observed for the other NMR instrumental configurations following DS instrumental transfer. By using five transfer calibration samples there is a decrease in the RMSEP error, producing between 50% and 75% improvement (Table 1). This improvement in the prediction error is significantly larger than that observed for simple binning (Supporting Information Table S3) or simple line shape deconvolution (Table 1) discussed above. Following this intermediate minimum in RMSEP there is a continued gradual decrease with increasing number of transfer calibration samples.

Similar results (Figure S4, Supporting Information) were observed for the RMSEP behavior in the other instrumental configurations following DS instrumental transfer. There are a few instrumental configurations where the initial intermediate minimum in RMSEP was not reached until more than five transfer calibration samples were utilized, specifically the 1D HCN(b) Varian and the NOESY BB Bruker instrumental configurations. This number of required transfer calibration samples (either four or five) is significantly smaller than the 14 up to 56 transfer calibration samples required for instrumental transfer of the time-domain ^{1}H process NMR previously reported[32] and reflects the high-resolution nature of the current data set. In summary, DS instrumental transfer provided a large reduction in RMSEP for the PLS concentration models across the different instrumental configuration, with an intermediate minimum being observed for four or five transfer calibration samples. Further improvements in the model transfer can be obtained by including additional transfer calibration samples, but the improvements are very small compared to the initial RMSEP reduction observed for either four or five transfer calibration samples.

PDS Method. For the PDS instrumental transfer method, the RMSEP is a function of both the number of transfer calibration samples used and the number of adjacent instrumental frequencies employed in the estimation of $\mathbf{F_b}$. The variation of the RMSEP following instrumental transfer of the 1D INV Bruker instrumental configuration is shown in Figure 5. Similar results were obtained for the remaining NMR instrument configurations. (An example for the Varian 1D HCN(b) configuration is shown in Figure S5 in the Supporting Information.) Consistent with results obtained using the DS method, there is an initial increase in RMSEP for instrumental calibrations using only one transfer calibration sample, followed by a drop in RMSEP for additional transfer calibration samples. For PDS an intermediate minimum was observed for two or three transfer calibration samples, resulting in a 45% to 60% improvement in RMSEP (Table 1). Again this intermediate minimum was followed by a gradual decrease in RMSEP (5−10%) with increasing number of transfer calibration samples. Variation of RMSEP with the number of instrumental frequencies was also observed using the PDS method but was small compared to the variation due to number of transfer calibration samples. In several of the configurations tested, there is an additional decrease in RMSEP with increasing number of instrumental frequencies (Figure 5). For example, the RMSEP for the prediction of glycine (Figure 5e) decreases noticeably for predictions increasing from one to seven instrumental frequencies, but for glucose (Figure 5d) or for citrate (Figure 5f), the decrease is less pronounced. The improvement in RMSEP with increasing number of filter frequencies was more pronounced for the prediction of some metabolites, suggesting subtle chemical shift changes are occurring in these standard samples. When very large numbers of filter frequencies are employed with PDS, this method converges to

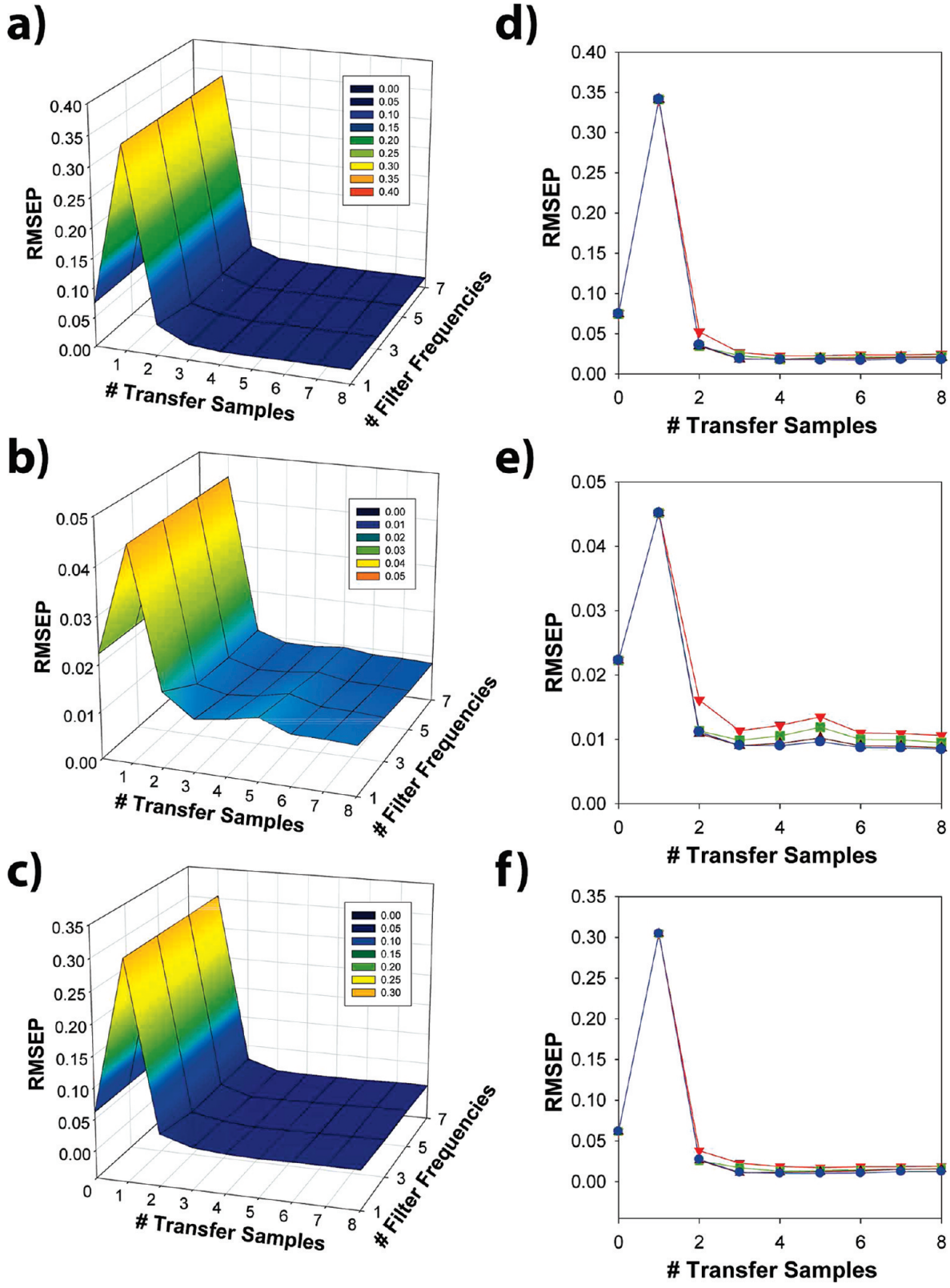

Figure 5. Variation of RMSEP as a function of the number of transfer calibration samples and number of instrumental frequencies used in the piecewise direct standardization (PDS) instrumental transfer on the 1D INV Bruker configuration for the relative concentrations of (a) glucose, (b) glycine, and (c) citrate. In panels d−f the RMSEPs for different number of instrumental frequencies are shown for (▼) one, (■) three, (▲) five, and (●) seven instrumental frequencies. These results can be compared to the RMSEP variation obtained using the DS method shown in Figure 4.

the DS results (DS uses all frequencies to develop the transfer function). The insensitivity of the RMSEP to the number of instrumental filter frequencies shows that the chemical shift variations are not the dominant source of error between these different instrumental

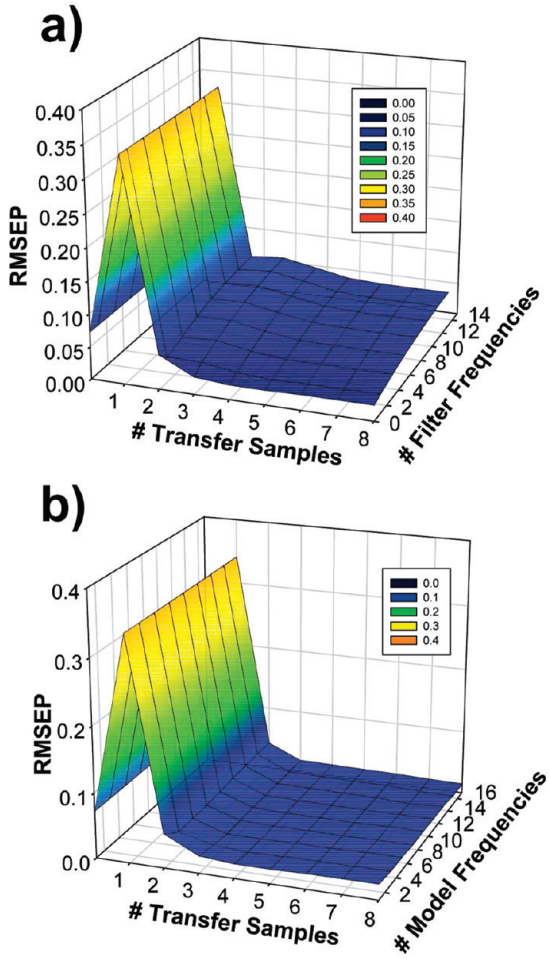

Figure 6. Variation of RMSEP for the prediction of glucose concentration for the 1D INV Bruker configuration following double-window piecewise direct standardization (DWPDS) method as a function of transfer calibration sample number along with (a) the number of filter frequencies and (b) the number of filter samples.

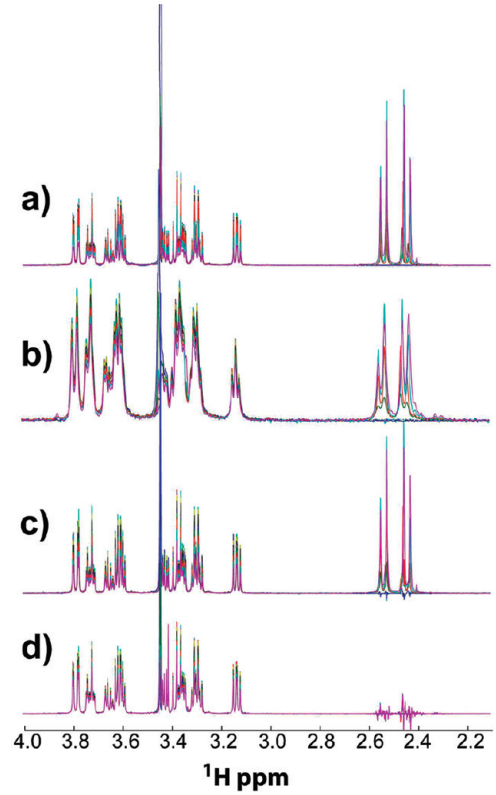

Figure 7. Representative ^{1}H NMR spectral data sets for different instrumental configurations: (a) 1D BB Bruker and (b) 1D HCN(b) Varian prior to instrumental transfer. The (c) corrected spectral data set for the Varian 1D HCN(b) configuration following direct standardization (DS) instrumental transfer using four transfer calibration samples and (d) following DS using a subset of transfer calibration standard samples in which the citrate metabolite was not present. The Bruker data set (a) was used to develop the PLS model and was the target configuration. Note the improved spectral resolution of the corrected spectra in panel c in comparison to the original in panel b and the close similarity to the spectral data set in panel a. The loss of the citrate signal for instrumental transfers using a limited subset of standard samples is clearly seen in panel d.

configurations. Table 1 summarizes the RMSEP obtained using the PDS method for calibrations using three transfer calibration samples and one instrumental frequency and for calibrations using four transfer calibration samples and seven instrumental frequencies. The percent improvements in RMSEP range from 45% to 75% using the PDS method and are comparable to the results of the DS method. For example, one can compare the results for the PDS transfer results of the 1D INV Bruker configuration (Figure 5) to the results of the DS transfer (Figure 4), where the PDS method requires fewer number of transfer calibration samples (two or three vs four or five in DS). This reduction in the calibration sample subset could prove beneficial for instances where a limited number of calibration samples are available for implementing the instrumental transfer.

DWPDS. For the DWPDS method, the instrumental transfer is now a function of three variables: the number of transfer calibration samples, the number of filter frequencies, and the number of filter samples used in the calibration. Figure 6 shows an example of the RMSEP variation as a function of the number of transfer calibration samples, and the number of filter frequencies (Figure 6a), and the number of filter samples (Figure 6b). These responses are reminiscent to those observed for the PDS method (Figure 5). Even with the additional parameter used in the development of the transfer function the percent improvement is almost the same as in the PDS method (see Table 1). The calculation of the DWPDS transfer functions also took considerably longer than the PDS method. On the basis of these results, there is no clear reason to utilize the DWPDS method over the PDS method in the instrumental transfer of high-resolution NMR spectral data.

Transfer Calibration Sample Subset. The power and capability of transferring data between different NMR instrument configurations is shown in Figure 7. The spectral data from the target or primary instrument (1D BB Bruker, Figure 7a) is composed of sharp, well-resolved spectral lines. In contrast, Figure 7b shows the spectral data for the exact same sample set on a secondary instrumental configuration (1D HCN(b) Varian). For this configuration there is significant broadening of the resonances. Following instrumental transfer using the DS method, the spectral data set from this secondary configuration is now also composed of sharp well-resolved resonances (Figure 7c) and is almost identical to the spectral data set of the target instrument.

The performance of these instrumental transfer methods (DS, PDS, DWPDS) ultimately depends on the number and information content of the transfer samples used for calibration. In particular,

does the transfer calibration sample subset span the spectral space of the data set that instrumental transfer is being employed on? For the model mixtures investigated here with a limited number of components, it is simple for a limited number of transfer calibration samples to fully represent the spectral response of the entire data set. This assumption will not necessarily hold for more complex mixtures (biofluids or metabonomics) where hundreds of different compounds may be present. Two questions immediately arise: (1) Are the chosen transfer calibration samples sufficient to model all of the resonances of interest for the entire set of compounds present? (2) What happens if the transfer calibration sample subset does not contain a specific compound?" These questions are partly addressed in Figure 7d, where the transfer calibration samples selected were chosen such that the metabolite citrate was not present in this subset. It should be noted that this was a totally artificial selection process. The leverage matrix (eq 12) used for transfer sample selection ranks the citrate containing samples very high, but in this case the ranking was ignored with the forced selection provided as an example. This "poor" selection of transfer calibration samples results in transformations in which no citrate resonances (at ~2.5 ppm) are present during the calculation of $\mathbf{F_b}$, such that the DS transfer method (or PDS/DWPDS) attempts to suppress signals in this spectral region in the remainder of the data set. This example shows the importance of the transfer calibration sample subset providing a complete spectral representation of the entire data set using the existing DS, PDS, and DWPDS instrumental transfer methods and shows a possible limitation of instrumental transfer methods as applied to complex mixtures. We are currently developing filtering techniques for these instrumental transfer methods to overcome this limitation (Alam, unpublished).

CONCLUSIONS

It has been demonstrated that the DS, PDS, and DWPDS instrumental transfer methods can be used on high-resolution NMR spectral data sets. All of these transfer methods provided significant improvement for the PLS prediction of metabolite concentrations in model mixtures between different instrumental configurations. In addition, all of these instrumental transfer methods improved the RMSEP in comparison to simple binning or line shape deconvolution techniques. The DS instrumental transfer method gave the largest percent improvement in the concentration predictions but also required a larger number of transfer calibration samples than the PDS method. The PDS and DWPDS methods gave similar improvement in the prediction of concentrations but required fewer transfer calibration samples. For simple mixtures and other limited examples, the instrumental transfer methods perform well and allow the transfer of calibration models between different NMR instruments. It was demonstrated that there are limitations imposed on the selection and number of transfer calibration standards. This limitation is a major issue for complex mixtures and currently precludes the direct application of instrumental transfer methods to biofluid/metabonomic studies.

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SUPPORTING INFORMATION AVAILABLE

Additional details of the mixture compositions, results of the binning and phasing predictions, and additional examples of the PLS predictions as a function of the variables in the instrumental transfer methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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