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Software Description

MCR-ALS GUI 2.0: New features and applications

Joaquim Jaumot [a,*], Anna de Juan [b], Romà Tauler [a]

[a] Department of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, Barcelona 08034, Spain
[b] Department of Analytical Chemistry, Universitat de Barcelona, Diagonal 645, Barcelona 08028, Spain

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ABSTRACT

An updated version of the graphical user-friendly interface related to the Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) algorithm is presented. This GUI works under MATLAB® environment and includes recently published advances of this algorithm linked to the implementation of additional constraints, such as kinetic hard-modeling and correlation (calibration), as well as constraints linked to model structure for multiset and multi-way data analysis, such as the possibility to use fully or partially multilinear models (trilinear or quadrilinear) to describe the data set.

In addition, a step has been included to allow the preliminary subspace maximum likelihood projection to decrease noise propagation effects in case of large non-homoscedastic uncertainties, and the possibility of direct selection of number of components and of initial estimates.

Finally, a number of options to present and handle the output information have been added, such as the display of data fitting evolution, improvement in the display of loading profiles in different modes for multi-way data, refolding MCR scores into 2D distribution maps for hyperspectral images and the internal connection to the MCR-Bands GUI, previously designed for the assessment of the extent and location of ambiguities in the MCR resolved profiles. Different examples of use of this updated interface are given in this work.

1. Introduction

Ten years ago we presented the first version of the MCR-ALS graphical user-friendly interface [1] which has powered the use of the MCR-ALS algorithm in a simpler manner than in the previously existing command line routines. Since then, the recognition and use of the MCR-ALS method have significantly increased [2] and, nowadays, it is widely used for the analysis of very diverse kinds of data. In this work, an updated version of the graphical user friendly interface for the MCR-ALS software is presented, which includes in a single program the latest developments implemented in the ALS optimization algorithm and, also, some comments and suggestions made by users during this time.

Scheme 1 shows the flowchart of an MCR-ALS analysis of a general data set.

First, tools to estimate the number of components to use in the optimization and to generate initial estimates have been incorporated in the toolbox. The selection of the appropriate number of components can be performed manually or by assessing the results obtained using a Singular Value Decomposition [3] algorithm. The generation of initial estimates is also possible manually or by means of using the Evolving Factor Analysis [4] method or by a purest variable detection method similar to the one used by the SIMPLISMA algorithm [5,6].

The choice of constraints is now presented in two clearly separated windows: one related to row mode profiles (**C** matrix) and another to column mode profiles ($\mathbf{S}^T$ matrix). Now, the possible diverse application of constraints in the **C** and $\mathbf{S}^T$ submatrices related to multiset structures is more straightforwardly defined. As in the previous version, constraints can also vary component-wise. Note that, by convention, row mode profiles are associated with concentration profiles and column mode profiles with instrumental responses. This is the reason why there are options for row mode constraints, which are specifically designed for concentration profiles, absent in the menu of column mode constraints. Therefore, the user should orient the original data set **D** in the appropriate way to make the most efficient use of the constraints implemented.

Two new constraints related to row mode profiles (**C** matrix), have been included: kinetic hard-modeling and correlation. Although MCR-ALS is generally considered a soft-modeling method, since natural 'soft' constraints do not force the data to follow a deterministic model, the kinetic hard-modeling constraint allows using kinetic models to fit the shape of all or some concentration profiles in the data set. In this way, MCR-ALS can become a hybrid 'hard-soft' modeling algorithm [7]. Using this new approach, part (or all) of the concentration profiles of the different MCR-ALS resolved species will follow a kinetic model proposed by the user. In a multiset context, all or some experiments can be fitted by a global kinetic model, individual models per each experiment can be used and combination of hard-modeled with other soft-modeled experiments in the same multiset structure can be accepted. Inclusion of

* Corresponding author. Tel.: +34 934006100 1643.
E-mail address: joaquim.jaumot@idaea.csic.es (J. Jaumot).

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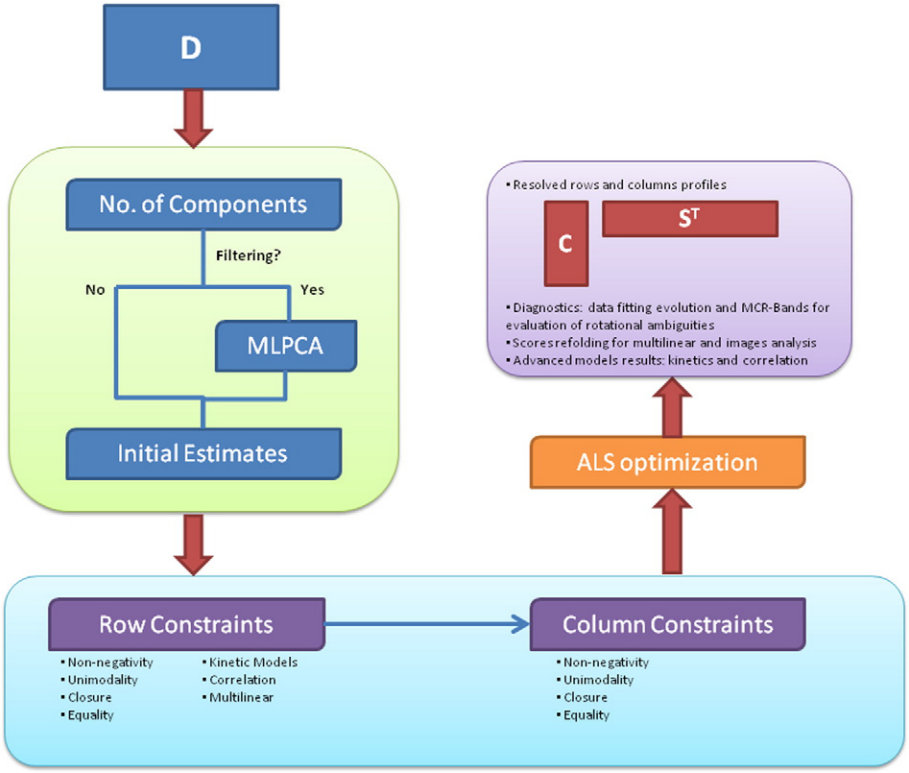

Scheme 1. Flowchart of the analysis using the MCR-ALS GUI.

non-absorbing species is also possible. Other types of deterministic models [8–10] could be possible but not currently available in this GUI.

The new correlation (calibration) constraint has been included to obtain quantitative information in real concentration units for first order data, where sets of single vectors (spectra) per sample are grouped in the same data matrix and are simultaneously analyzed [11]. In addition to the qualitative and relative quantitative information obtained by the MCR-ALS algorithm under natural constraints when applied to a single data matrix, the use of this new constraint provides quantitative information in real concentration units. With this correlation constraint, an internal calibration model is built relating known concentration values of calibration samples to the ones provided by the ALS optimization procedure. This model rescales the concentration values found by the resolution algorithm (in calibration and test samples) to the real concentration units during the iterative optimization. As recently shown in an exhaustive work by Ahmadi and Abdollahi [12], quantitative results obtained by soft-modeling methods such as MCR-ALS could have quite large deviations from the true solutions due to rotational ambiguities. For this reason, the application of the correlation constraint could help to overcome these difficulties as the imposition of an internal calibration model will dramatically reduce the rotational ambiguities.

Recent works have shown that, when dealing with low signal-to-noise ratio data and non-homoscedastic noise, a preliminary step of MLPCA filtering can help to obtain more reliable results [13]. This is especially important with data sets, such as environmental data tables or metabolomics/genomics data. MLPCA is an analogous methodology to PCA but incorporates known measurement error information into the bilinear decomposition process in order to develop optimal PCA models in a maximum likelihood sense.

An important incorporation to the GUI is the inclusion of new options of model constraints, related to multiset/multi-way structures. Thus, multilinear models of high order (trilinear or quadrilinear) can be used to define the profiles of the original data set. As usual, this definition of the model can be done component-wise and provide partially multilinear models, an asset specific to the MCR-ALS algorithm, absent in classical multi-way data analysis methods.

In addition, other minor changes have been implemented to facilitate the selection of constraints, such as working with multiple experiments and techniques and to display and interpret all the output information provided by the algorithm. A summary of the features included in the new version of the MCR-ALS GUI is listed in Table 1.

Finally, a variety of examples is given in this manuscript to show the new capabilities of the GUI and how they can be applied in examples commonly found in the resolution field. In order to facilitate the use of the new GUI, all the discussed examples in the manuscript are accompanied by a set of videos showing step by step the application of the method in multiple situations which can be of great help for users and that are given as Supplementary material.

Table 1
Summary of new options and constraints included in this version of the MCR-ALS GUI.

Novelty	Novelty details	References
Input information		[3–5]
Rows/columns constraints input		[14]
Hybrid kinetic hard-modeling		[7,15,16]
Correlation constraint		[11,17,18]
MLPCA filtering		[13,19]
Multi-way model constraints	Trilinear, quadrilinear	[20,21]
MCR-Bands connection		[22,23]
Output information		
General	Evolution R2, LOF, profiles	[1]
Kinetic analysis	Reaction rates values Fitting information	
Calibration analysis	Regression fitting Information	
Image analysis	Distribution maps refolding Image quantitation	[24,25]
Multilinear analysis	Trilinear–quadrilinear scores refolding	[21]

2. MCR-ALS basic model and equations

Multivariate Curve Resolution methods are focused on extracting relevant information of the pure components in a mixture system through a bilinear model decomposition of the experimental data matrix **D** into the product of matrices **C** and $\mathbf{S}^T$ that contain pure profiles of components linked to the row mode (usually concentrations or peak profiles) and the column mode (usually spectra), respectively [26,27]. This bilinear model can be written as:

$$\mathbf{D} = \mathbf{C}\mathbf{S}^T + \mathbf{E} \tag{1}$$

where **E** is the matrix of residuals not explained by the resolved components.

Multiset analysis can be done by MCR-ALS when applied simultaneously to several data sets organized in a single structure. Thus, the extension of Eq. (1) allows the simultaneous MCR-ALS analysis of multiple independent experiments run under different experimental conditions by the same instrumental technique.

$$\begin{bmatrix}\mathbf{D}_1\\ \vdots\\ \mathbf{D}_n\end{bmatrix} = \begin{bmatrix}\mathbf{C}_1\\ \vdots\\ \mathbf{C}_n\end{bmatrix}\mathbf{S}^T + \begin{bmatrix}\mathbf{E}_1\\ \vdots\\ \mathbf{E}_n\end{bmatrix} \tag{2}$$

This data arrangement gives rise to a column-wise augmented matrix, where the resolved pure spectra are common to all experiments and the concentration profiles can be different from experiment to experiment. In this case, a common pure spectra matrix $\mathbf{S}^T$ and several different matrices containing independent concentration profiles ($\mathbf{C}_1$, …, $\mathbf{C}_n$) are resolved.

Furthermore, if a single experiment is monitored by more than one instrumental technique, Eq. (1) is extended to a row-wise augmented data matrix where individual data matrices related to each technique are one besides each other:

$$[\mathbf{D}_A \quad \cdots \quad \mathbf{D}_m] = \mathbf{C}\left[\mathbf{S}_A^T \quad \cdots \quad \mathbf{S}_m^T\right] + [\mathbf{E}_1 \quad \cdots \quad \mathbf{E}_m]. \tag{3}$$

In this case, there is a single matrix of concentration profiles **C** and several matrices ($\mathbf{S}_A^T$, …, $\mathbf{S}_m^T$) containing the pure response for each instrumental technique.

Finally, simultaneous row- and column-wise augmentation strategies can be designed when different chemical systems are monitored by means of different instrumental techniques at different experimental conditions:

$$\begin{bmatrix}\mathbf{D}_{1,A} & \cdots & \mathbf{D}_{1,m}\\ \vdots & \ddots & \vdots\\ \mathbf{D}_{n,A} & \cdots & \mathbf{D}_{n,m}\end{bmatrix} = \begin{bmatrix}\mathbf{C}_1\\ \vdots\\ \mathbf{C}_n\end{bmatrix}\left[\mathbf{S}_A^T \quad \cdots \quad \mathbf{S}_m^T\right] + \begin{bmatrix}\mathbf{E}_{1,A} & \cdots & \mathbf{E}_{1,m}\\ \vdots & \ddots & \vdots\\ \mathbf{E}_{n,A} & \cdots & \mathbf{E}_{n,m}\end{bmatrix}. \tag{4}$$

In all these cases, the resolved concentration profiles and pure spectra are, respectively, in a column-wise augmented matrix formed by different **C** submatrices and in a row-wise augmented matrix formed by the different $\mathbf{S}^T$ submatrices.

The ALS optimization is based on the application of constraints during the resolution. Both natural constraints (non-negativity, unimodality, closure…) and more advanced constraints such as multilinear, kinetic or correlation constraints can be chosen.

The quality and reliability of the MCR-ALS solution may be assessed using the explained data variance (Eq. (5)) and the lack of data fit (Eq. (6)) parameters that allow assessing the dissimilarity among the experimental data matrix (**D**) and the data modeled by MCR-ALS. The equations defining these two parameters are:

$$R^2 = 100\sqrt{\frac{\sum_{i,j} d_{ij}^2 - \sum_{i,j} e_{ij}^2}{\sum_{i,j} d_{ij}^2}} \tag{5}$$

$$\text{lack of fit}(\%) = 100\sqrt{\frac{\sum_{i,j} e_{ij}^2}{\sum_{i,j} d_{ij}^2}} \tag{6}$$

where d_{ij} is an element of the experimental data matrix and e_{ij} is the related residual value obtained from the difference between the experimental data (matrix **D**) and the reproduced data ($\mathbf{C}\mathbf{S}^T$ matrix product obtained by MCR-ALS).

3. Software

The GUI updated version for the MCR-ALS algorithm consists of a series of MATLAB® files developed under its release 2013a. The main MCR routine is named *mcr_main* and it calls all the necessary auxiliary routines. The MCR-ALS code, related tutorials and data sets for practicing are available at the Multivariate Curve Resolution web page: http://www.mcrals.info. A compiled version of the interface is available from the authors upon request.

4. Data examples

Six example data sets are used in this tutorial to show the different possibilities of the presented graphical user-friendly interface.

4.1. Chromatographic data set

This data set is constituted by four HPLC-DAD runs (sized 51 rows by 96 columns). The first and fourth HPLC runs have four overlapped compounds while the second and third runs have three overlapped compounds. First, the resolution of an individual HPLC run (variable called **chrom_data** that contains the first HPLC run) is considered. In addition, the four chromatographic runs are concatenated to form a column-wise augmented data matrix (sized 204 rows by 96 columns) in a variable called **augmented_data**. Additional variables for applying selectivity and local rank constraints (**csel_matrix**) [27] and information on the presence/absence of compounds in the column-wise augmented matrix (**isp_matrix**) are also included. All these variables are in a MATLAB file called *multiset.mat*. This data set is similar to that used in the description of the first version of the MCR-ALS GUI and in the MCR-Bands software [1,22].

4.2. Kinetic modeling data set

This data set is an example of a spectroscopically monitored kinetic reaction. The mechanism of the considered reaction is a two-step consecutive reaction (A → B and B → C) with an initial concentration of species A of $1 \cdot 10^{-3}$ M. Data matrix has been simulated from a set of concentration profiles (**C**) following a reaction mechanism with two steps and rates $k_1 = 2.0\ s^{-1}$ and $k_2 = 0.2\ s^{-1}$. Three NIR spectra ($\mathbf{S}^T$) with a considerable overlapping (the lowest value of the correlation coefficient between them is 0.85) were used as pure species spectra of the three assumed components A, B and C. Finally, experimental error (**E**) has been simulated by adding normally distributed random numbers with a mean of zero and 1.0% of the maximum of absorbance standard deviation of the $\mathbf{C}\mathbf{S}^T$ product, giving a noisy simulated data matrix, **D** ($\mathbf{D} = \mathbf{C}\mathbf{S}^T + \mathbf{E}$). The whole spectroscopically monitored kinetic process is given in matrix **D** (300,100) and saved in a variable called **kin_data**. This variable together with time (**times**) and wavelength (**waves**) variables is in the MATLAB file called *kinetic.mat*.

4.3. Calibration data set

This spectroscopic data set example has been built using a concentration matrix **C** (35,3) of 3 components in 35 samples and a pure spectra matrix, $\mathbf{S}^T$ (3,22), corresponding to the three pure UV–Vis spectra of

these three components with medium to high overlapping degree (correlation coefficient ranging from 0.70 to 0.87). Experimental error (**E**) was simulated by adding normally distributed random numbers with a mean of zero and 1.0% of the maximum of absorbance standard deviation of the $\mathbf{CS}^T$ product, giving the data matrix $\mathbf{D} = \mathbf{CS}^T + \mathbf{E}$, called **correl_data**. Calibration matrix **Cknown** contains the concentration values of the first species in the first 28 samples used for calibration and the rest of concentration values in the last 7 samples of the first species used for validation and all the other values of the second and third species are coded as not-a-number (NaN, following MATLAB notation). In addition, a variable with the concentration of the first component in the validation samples (**Cval**) is also included. Finally, a variable with spectral initial estimates **Sint** is included to be used in ALS optimization. All these variables are in a MATLAB file called *correlation.mat*.

4.4. Spectroscopic image

This example consists of the image of the surface layer of an emulsion scanned by Raman spectroscopy, which has been described in a previous work [28]. Data were baseline corrected and presented in a cube (variable **imageC**) of size 60 pixels × 60 pixels × 253 wavelengths. The data matrix obtained after the unfolding of the data cube (variable **imageM**) of size 3600 rows × 253 columns is also provided. These variables are in a MATLAB file called *image.mat*.

4.5. Multilinear fluorescence spectra

This data set consists of Excitation–Emission Fluorescence (EEM) spectra of mixtures of three fluorescent dyes (acridine orange, acridine yellow and coumarin 6) and it is used to demonstrate the capabilities of the MCR-ALS method to deal with multilinear (in this case, trilinear) data. The data set was simulated considering variable concentrations of the dyes in 20 samples (variable **concs**), excitation spectra with medium overlapping degree (variable **excitation**), emission spectra with high overlapping degree (variable **emission**) and random noise scaled to 1.0% of the maximum of the EEM spectral signal for each sample. A data cube of size 301 excitation wavelengths, 321 emission wavelengths and 20 samples was obtained and stored in the variable **EEMCube**. In order to facilitate the analysis, the data cube was unfolded and stored in the variable **EEMMatrix** of size 6020 rows (samples × excitation wavelengths) by 321 columns (emission wavelengths). This variable together with excitation (**exc_axis**) and emission wavelength (**em_axis**) variables is in the MATLAB file called *fluorData.mat*.

4.6. Environmental data set

This example consists of a simulated environmental data set which was used in a previous work [29]. Analyzed data consists of four environmental monitoring campaigns (monthly, from May to August 2005) at 11 sampling sites in the Ebro river region. For each sample, the concentration of 15 pesticides was measured, giving four individual data matrices of 11 rows (sites) and 15 columns (pesticides). Different types of uncertainties (**E**) were considered based on homoscedastic and heteroscedastic (proportional) noise properties. For homoscedastic noise, all uncertainties associated with the values of a variable (pesticide concentrations) were considered to come from the same population with a zero mean and with a standard deviation equal to the 10% of the maximum intensity (concentration) value of the considered variable. In the case of proportional noise, uncertainties were varying with a standard deviation proportional (10%) for every measured value of the variables (pesticide concentrations). Finally, in both cases, these uncertainties were added to the simulated data and the variable **Daug** was obtained by matrix column augmentation of the four individual data matrices corresponding to every individual monitoring campaign ($\mathbf{Daug} = [\mathbf{D}_1;\mathbf{D}_2;\mathbf{D}_3;\mathbf{D}_4]$). Data uncertainties are given in a variable called **Eaug** of the same size with **Daug**. All these variables are in a MATLAB file called *enviro.mat*, together with the identifiers of the measured samples and variables.

5. Operating procedure

New features of the updated version of the MCR-ALS interface are shown next. For a more detailed visualization of the operation procedure, the readers are referred to Supplementary material where videos with step by step operation procedures are included.

5.1. Single matrix data analysis

One of the chromatographic data sets described in Section 4.1 will be used to show the determination of the number of components and the procedure for the selection of initial estimates and for the selection of ALS optimization constraints, for both row and column profiles.

The first screen of the program is launched by calling the *mcr_main* function (Fig. 1). The user has to select which is the data set that is going to be analyzed that, in this case, is **chrom_data**. The "Plot" button allows representing the data, both in row and column directions, for visual inspection. Next, two options are available for the determination of the number of components. The "Manual" button is used when prior knowledge about the correct number of components is available and the "SVD" button is used when this estimate is performed considering the number of largest singular values obtained by the Singular Value Decomposition algorithm.

A new aspect of this version of the MCR-ALS GUI 2.0 program is the possibility of introducing information about data uncertainties, if available. In case of having this information, a Maximum Likelihood Principal Component Analysis (MLPCA) initial subspace projection, is possible to diminish noise propagation effects on MCR solutions (more details will be given in Section 5.7).

In any ALS optimization algorithm an initial estimate of one of the two factor matrices, **C** or $\mathbf{S}^T$, is required. There are three different options: "Manual" if they are already available, "Pure" for determining initial estimates either of **C** or $\mathbf{S}^T$ by means of a purest variable detection method [5], or "EFA" by means of Evolving Factor Analysis [4], only suitable for the case of analyzing evolving processes. In the chromatographic example, four different species were considered (estimated by SVD) and initial estimates were obtained by means of Evolving Factor Analysis.

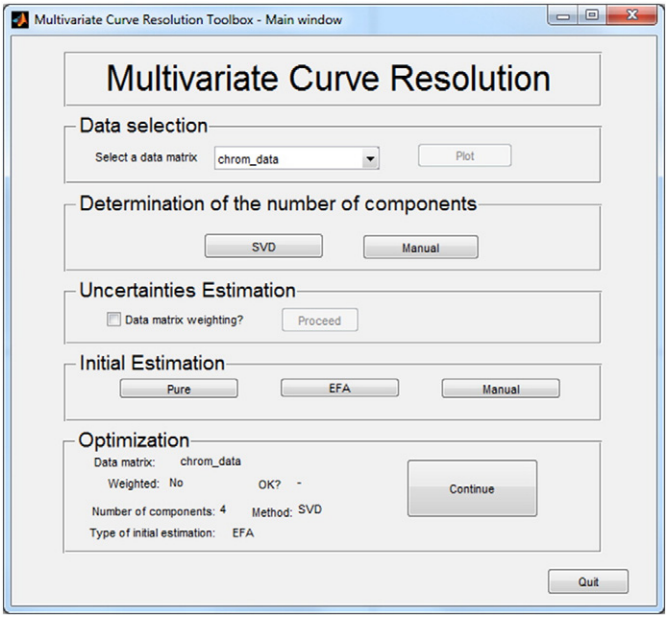

Fig. 1. MCR-ALS GUI main window ("*mcr_main*").

After clicking the "Continue" button, a summary screen in which the top plots represent the row and column profiles of the experimental data, the middle plots show the initial estimate and the **C** and $\mathbf{S}^T$ profiles obtained by a least-squares step and the bottom plots represent the score and loading plots obtained by PCA of the analyzed data matrix **D** with the previously selected number of components appears. Above these plots, the user can modify the number of matrices simultaneously analyzed by MCR for multiset analysis but, in this first example, only one single data matrix is analyzed.

The next step is the selection of constraints for ALS optimization. In this new version of the interface, there are two differentiated screens for the selection of constraints, one for the profiles linked to the row mode (i.e. concentration profiles, **C** matrix) and another for the profiles related to the column mode (i.e. spectral profiles, $\mathbf{S}^T$ matrix). In the first screen, constraints for the row mode profiles can be selected (Fig. 2a). Common natural constraints (such as non-negativity, unimodality, closure and equality) and more advanced constraints (such as correlation or kinetic hard-modeling) are available. In this example, constraints selected were non-negativity using the *fnnls* algorithm [30] and unimodality using the average option with a 10% of tolerance for all the species, and the equality constraint algorithm to introduce selectivity and local rank information about the concentration of the different components [27], especially if they are absent or with very low contributions. It forces concentration values to be equal or lower to those values provided by the user in an external variable (**csel_matrix**). In the second screen, constraints for column mode profiles (spectral direction) can be selected analogously for non-negativity, unimodality, closure or equality options (Fig. 2b). In this case, only non-negativity constraints were selected.

In the case of no closure (i.e. no mass balance in concentrations) constraints, the possibility of normalizing the resolved spectral profiles (i.e. normalizing them to have equal height, total sum norm or Euclidean norm) is offered prior to starting the ALS optimization. This is recommended to avoid scale instabilities during the evolution of the ALS optimization and it fixes the possible intensity ambiguities. In this example, normalization using the Euclidean norm was selected. Finally, the user can modify general optimization parameters (such as the number of iterations or the convergence criterion) and the name of different output variables.

After ALS optimization, the screen of results is presented (Fig. 3a). In addition to the information about the convergence, lack of fit and explained variance, this updated version offers the possibility to obtain and visualize more detailed information about the evolution of the ALS optimization. By clicking the "Information" button, different plots regarding the ALS evolution of the explained variance, lack of fit, logarithm of the sum of squared residuals and evolution of row/column (concentration/spectra) profiles are available (Fig. 3b). Indication of the extent of rotational ambiguities still associated with the finally obtained MCR-ALS solutions can be then evaluated selecting the interface connection to the MCR-Bands program, clicking the "MCR Bands" button (see example below).

5.2. Multiset data analysis

This example consists of the analysis of the augmented data matrix obtained in the simultaneous analysis of four different chromatographic runs with common coeluted components (variable **augmented_data** in the *multiset.mat* file).

MCR analysis starts with the determination of the number of components and initial estimates. In this example, the purest spectra are recommended with a noise level of 5%. Now, in the "Selection of the data set" window, the number of simultaneously analyzed data matrices is 4 (one matrix for each of the four chromatographic runs). Then, the program asks for the type of multiset data structure (see [26,31] for more details about multiset data types). The "Column-wise augmented data matrix" is selected in this case, with all matrices having the same number of rows. After clicking the "Continue" button, the selection of row mode constraints window appears, but now allowing for the possibility to deal with multiple and different constraints for every analyzed **C** submatrix. At the top of the screen, a panel regarding the multiset data structure is presented. It contains the total number of **C** submatrices included in the augmented data set, an option to apply the same constraints to all **C** submatrices, or the possibility to change the constraints according to the different C submatrices. Finally, at the right corner, the possibility to apply the constraint of correspondence among species by selecting which components are present in every considered **C** submatrix is offered (Fig. 4).

In this case, the same constraints will be applied to all **C** submatrices (checked option) and the **isp_matrix** variable needs to be chosen at the pop-up menu. **isp_matrix** contains the following values:

$$\mathbf{isp\_matrix} = \begin{bmatrix} 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 0 \\ 1 & 0 & 1 & 1 \\ 1 & 1 & 1 & 1 \end{bmatrix}.$$

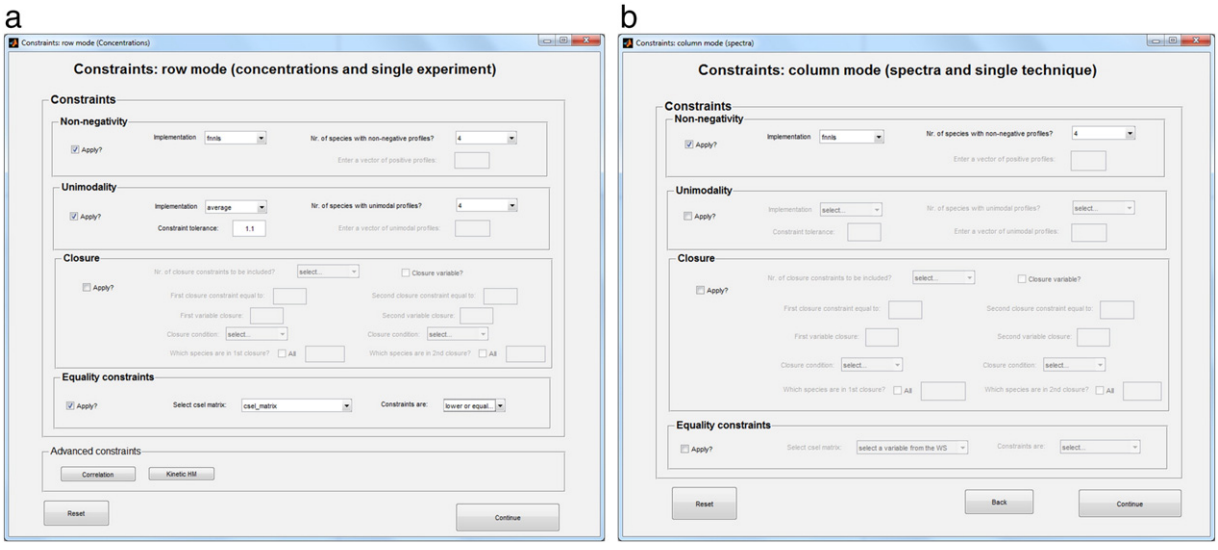

Fig. 2. Single matrix data analysis: selection of constraints windows for a) row and b) column modes.

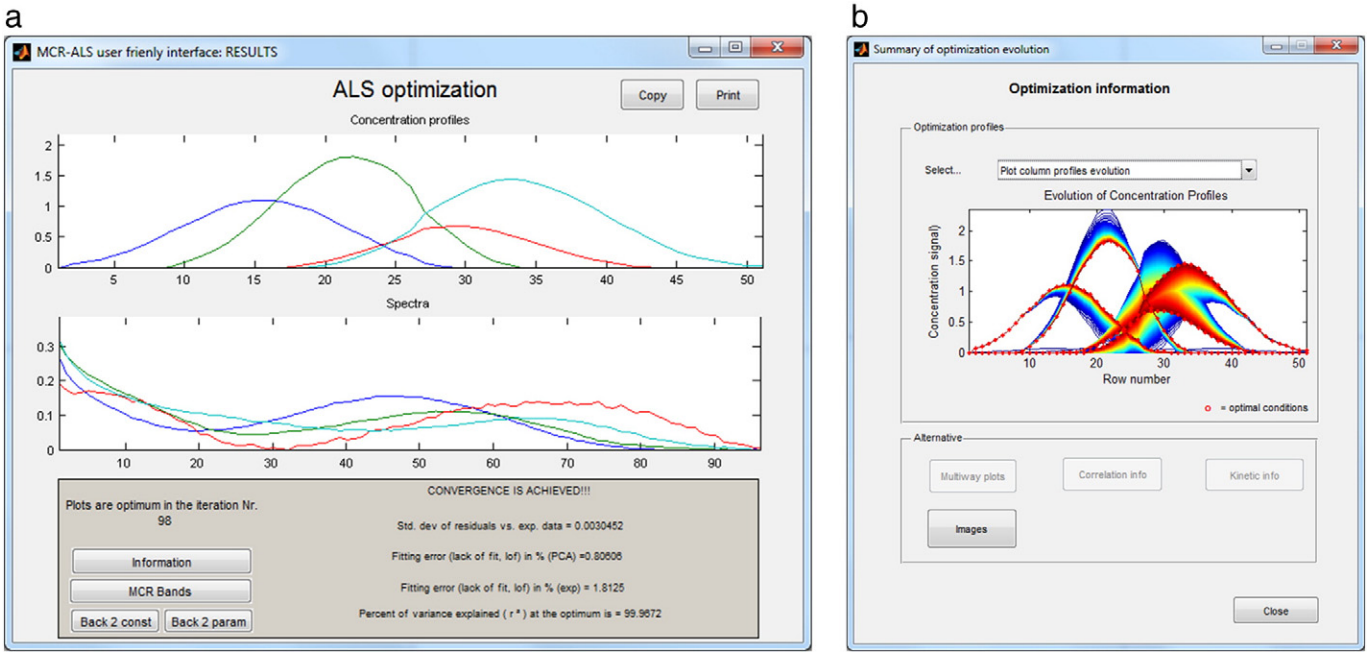

Fig. 3. MCR-ALS results window. a) General information and b) detailed information about the optimization and additional options.

In this matrix, each row corresponds to the **C** submatrix of an HPLC run and each column to one of the species detected by the purest variable detection method. As stated in the description of the data set, the first and fourth HPLC runs contain all the components considered in the analysis while the fourth component is absent from the second HPLC run and the second component from the third HPLC run. In addition, non-negativity constraints are applied. In the case of column matrices, as in the previous section, only non-negativity constraints are applied to spectra. After selecting spectral normalization (dividing by the Euclidean norm), ALS optimization is performed and the results

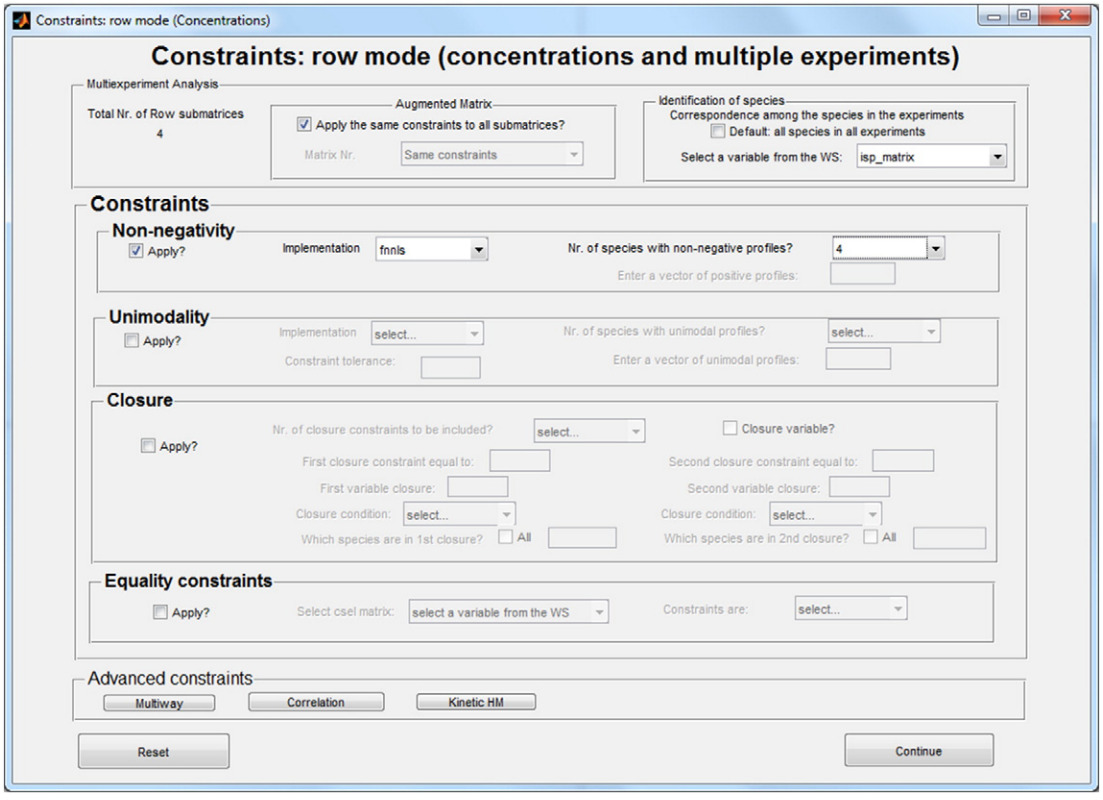

Fig. 4. Multiset data analysis: selection of constraints windows (row mode).

window shows the finally resolved elution and spectral profiles of the four coeluted components (for more details check Supplementary material).

5.3. Kinetic modeling

The third data set shows an example of hybrid hard-soft modeling of a kinetic process, where the application of a new kinetic hard-modeling constraint is included as one of the novelties of the present version of the graphical interface. Data can be found at the *kinetic.mat* file and the name of the variable is **kin_data**.

MCR-ALS analysis starts with the determination of the number of components and initial estimates. In this case, the number of selected components was three and the EFA method was chosen to obtain the initial estimates of the concentration profiles.

With regard to the row mode constraints, non-negativity, closure (total concentration of $1 \cdot 10^{-3}$) and kinetic constraints were applied. The latter will be explained in more detail. After clicking the "Kinetic HM" button a new screen appears (Fig. 5).

First, the number of kinetic reactions or processes should be given. In this example, a two consecutive reaction kinetic model A → B → C is proposed and, therefore, the number of models is set to 1. Then, the kinetic model system has to be written in the mechanism edit box. The proposed two-step consecutive reaction model is written in two lines: A > B and B > C. The program then requires giving the initial concentration values of the three reactive species (A, B and C). In this particular case, only the initial concentration of A is different from zero and it is set to a value equal to $1 \cdot 10^{-3}$. Also, it is necessary to indicate whether the considered species is colored (i.e. contributes to the measured signal or not). Initial guesses for reaction rates are also required and, as it can be expected, the closer these values are to the correct ones, the faster the kinetic optimization will be. Finally, it is necessary to select from the workspace a variable containing the time axis (necessary to calculate the numerical value of the reaction rate constants). A simulation of the concentration profiles using the proposed mechanism, initial concentrations and reaction rate guesses can be obtained by clicking the "Simulate" button.

A key step before applying this kinetic constraint is to ensure the correct correspondence between the MCR-ALS resolved species and the kinetic modeled species. For this reason, the user has to match MCR-ALS resolved species with kinetic species in the model one to one. An initial help to facilitate this identification is the comparison of the simulated plot of concentration profiles using the kinetic parameters with the plot of the concentration profiles obtained from the initial estimates (either from EFA or purest variable detection methods).

In the case of column mode constraints, only non-negativity constraints are selected.

Finally, ALS optimization with the kinetic constraints is carried out. In the "Information" button of the results window, there is the detailed information about the finally fitted kinetic model. When clicking the "Kinetic info" button, information about each fitted model is given. In this case, concentration profiles for the A → B and B → C model are plotted, and values of the related reaction rates and of the sum of the squares of the residuals for the hard-modeling fitting of the concentration profiles are given.

5.4. First-order calibration

The next example shows the application of the correlation constraint for the calibration of first order data. Data used in this example can be found in the *correlation_data.mat* file.

The same steps as in previous examples are followed. Initially, selection of the **correl_data** variable and determination of the number of components (three) and of initial estimates (in this case using the "Manual"

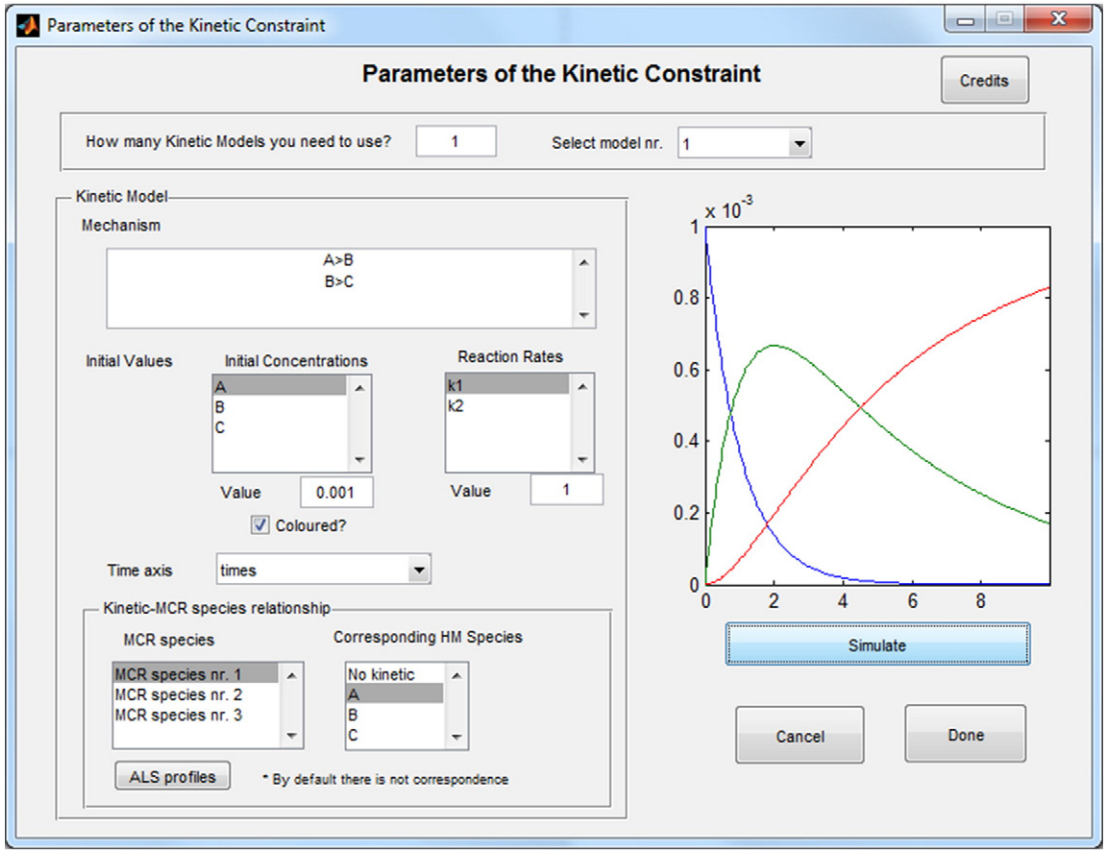

Fig. 5. Kinetic constraint options window.

option by selecting the **Sint** variable provided) are required. Then, in the row mode constraints, the correlation constraint is selected in addition to non-negativity constraints (the closure constraint is not recommended to avoid inconsistencies in the concentration scale with the correlation constraint). After clicking the "Correlation" button, a new window appears (Fig. 6a). First, the variable that contains the quantitative information for the calibration samples is selected. In this example, the **Cknown** variable is selected. As stated before, the **Cknown** variable contains quantitative information for the first species in the first twenty-eight samples (calibration samples). So, it can be considered that there is one analyte and two interferences in each sample. Next, the species to which the correlation constraint will be applied should be defined. A binary codification is used, where '1' is for calibrated species and '0' for non-calibrated species. Here, only the first species is constrained and, the codification variable is, therefore, equal to '1 0 0' in the edit box. Note that, in this case, it is mandatory to establish a correct correspondence between the sequence of species defined in the initial estimate and the sequence of species in the variable containing the quantitative information.

In the case of column mode constraints, only non-negativity constraints will be applied and none of the normalization methods of the spectra have to be selected (due to their incompatibility with the calibration constraint).

During the ALS optimization, the evolution of the calibration model fitting is shown and, at the end of the optimization, a window with extended information regarding the regression model is available (Fig. 6b). Here, for each constrained species, figures of merit of the regression model, such as RMSEC, and the predicted versus actual concentration value plot and calibration curve parameters (slope, offset and correlation coefficient) are shown.

Finally, if unknown samples are present, the prediction of their concentration values can be obtained from the resolved MCR-ALS profiles. In this case, samples 29 to 35 were not included in the calibration model and their concentrations were estimated with a relative error in their prediction equal to 2.9% with an error interval of 0.3% to 11.6% (compared to the reference values stored in the **Cval** variable).

5.5. Spectroscopic imaging

This example shows the application of the MCR-ALS toolbox to the analysis of spectroscopic imaging data. Data used in this example can be found in the *image.mat* file that contains two variables: a data cube with spectra at each pixel (**imageC**) and the unfolded data cube into a two-way matrix (**imageM**) necessary to perform the MCR-ALS analysis. After loading the file and launching the MCR toolbox, this last variable (**imageM**) is selected. Then, the number of components is selected (four components) and initial estimates are obtained by means of the detection of the purest spectra. The number of considered matrices is one and only non-negativity constraints are applied for row and column modes. Finally, spectral normalization by using the Euclidean norm is selected before the optimization.

From resolved column profiles, spectral properties of the four considered components are obtained. This allows for the possible identification of resolved components. However, it is more difficult to visualize directly the information contained in the resolved row mode profiles. For this reason, in this new version of the program interface, refolding of the resolved row profiles to show the 2D spatial distribution maps of each component is possible, provided that the number of pixels in x and y directions and the number of images are adequately defined.

As seen in Fig. 7, this new tool allows an easier and more direct way to interpret the obtained row and column mode profiles by showing simultaneously the distribution map of the considered component in the sample and its related Raman spectrum. In Fig. 7, the first resolved component was mostly present in the central part of the image. It is also worth to mention that distribution maps and relative intraimage quantitation information can be saved in the MATLAB workspace by clicking the "Save" button. Finally, all components can be also plotted simultaneously by clicking the "Multiplot" button.

5.6. Multilinear fluorescence spectra

The next example shows the application of multilinear constraints during the ALS optimization and the assessment of the rotational ambiguities associated with the resolved profiles using the MCR-Bands program. In this example, the **EEMMatrix** variable found in the *fluorData.mat* file is used.

As in the previous cases, the number of components is first estimated by means of the SVD algorithm and three species were selected. Next, the purest spectral initial estimates were selected. In this case, in the "Selection of the data set" screen, it is mandatory to set the number of matrices equal to 20 (as described above, the number of rows of **EEMMatrix** is the product of 20 samples by 301 channels of the excitation spectra and the number of columns is 321 channels of the emission

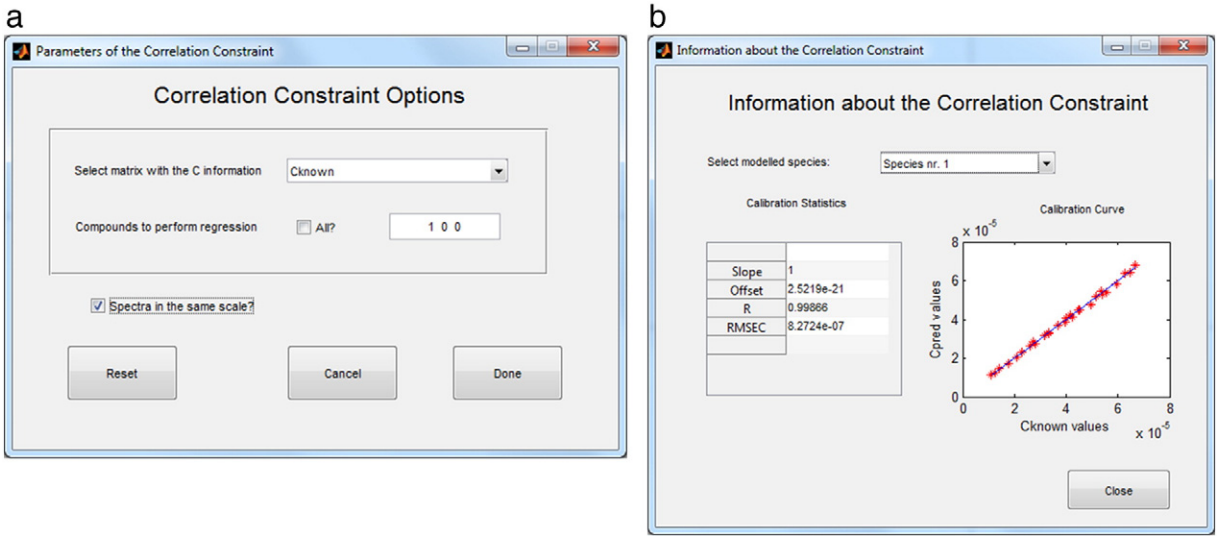

Fig. 6. Correlation constraint windows: a) options and b) results.

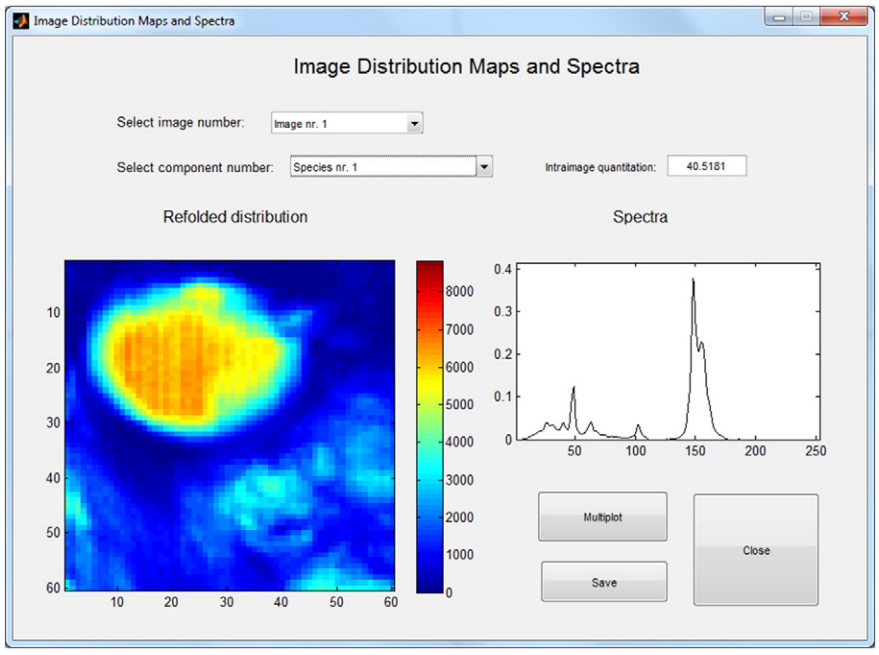

Fig. 7. Imaging example: distribution maps and spectra for each individual component.

spectra). In the definition of the three-way data set, the column-wise augmented data matrix has to be selected indicating that all matrices have the same number of rows.

Since multiple matrices are present in the row mode, multiset structure options can be selected. In this case, the same constraints to all the matrices and the same species were present in all the matrices. In the row mode, non-negativity and multi-way constraints are applied. After clicking the "Multiway" button, a new screen appears and the "multilinear, equal shape and synchronization (all species)" option is selected. The other parameters can be left to the default values and the trilinear model for all the species is tested. However, other options such as quadrilinear models or partial multilinear models (only for some species) can also be selected. Finally, spectral non-negativity constraints and spectral normalization (Euclidean norm) options are also selected.

Pure emission spectra of the different species are recovered in an augmented intermixed profile matrix, not easy to interpret. A new option has been included to refold these profiles in order to untangle them in two modes [32]. After clicking "Information" and "Multiway plots", a new screen showing a single resolved profile per each of the three modes for each individual component is launched. As can be seen in Fig. 8, profiles corresponding to excitation and emission spectra and to relative concentrations of every resolved component are given separately. In this case, the "Save in WS" button allows to save three

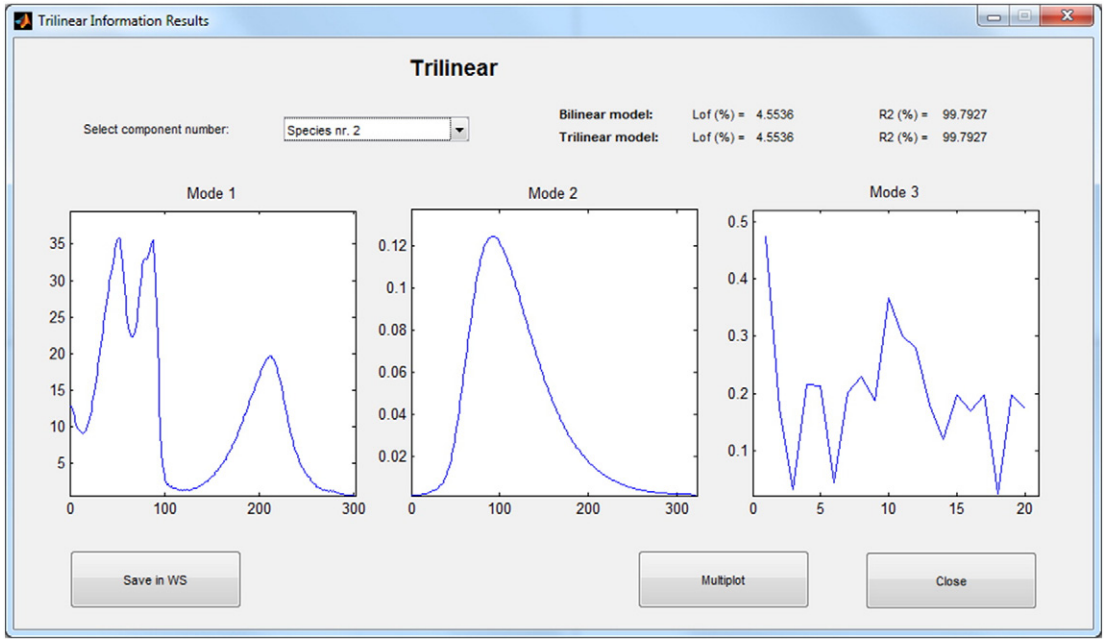

Fig. 8. Multilinear example: resolved profiles for each individual component per each one of the three modes.

mode profiles in the workspace and the "Multiplot" button allows plotting in the same window simultaneously these profiles for all resolved components.

This example finishes with the assessment of the indication of the rotational ambiguities associated with the obtained solutions. The "MCR Bands" button is clicked on the MCR-ALS results window in order to launch the MCR-Bands program. Concentration and spectral profiles resolved by MCR-ALS are loaded directly and the user only has to select the same constraints that have been previously used in the ALS optimization. In this case, spectral normalization, non-negativity for both concentration and spectra and trilinearity constraints are selected. Results of the MCR-Bands method show clearly that the extent of rotational ambiguities (measured by the difference between fmax and fmin values [33]) is negligible. The coincidence between maximum and minimum values of the optimized function can be checked graphically and numerically [33] (the maximum difference between fmax and fmin values is lower than 10^{-4}). When the trilinearity constraint is not applied during MCR-Bands calculations, maximum and minimum band profiles of the same component differ significantly and the extent of rotation ambiguity is much larger and ranges from 0.35 to 0.45. Further information about the MCR-Bands method and about its use can be found at previous works [22,23].

5.7. MCR-ALS including data uncertainties: MLPCA preliminary data projection

The last example shows the application of a MLPCA preliminary data projection and noise filtering, previous to MCR-ALS optimization. It has been recently shown [13] that the MCR-ALS analysis using MLPCA filtered data provides reliable results specially for the case of non-homoscedastic highly noisy data systems, as for instance environmental tables or microarray data sets. Propagation of noise to the resolved profiles ($\mathbf{C}$ and $\mathbf{S}^T$) can be avoided with this approach, and very reliable profile estimates are obtained. The requirement is, however, to have reliable estimates of data uncertainties, which are not easy to obtain in many applications.

In this case, the environmental data set is used as example (*enviro.mat*). After launching the interface, the **Daug** variable is selected. Then, the MLPCA preliminary step is selected before the ALS optimization, just after the determination of the number of components. In the main window, the "Data uncertainties" option is ticked in order to launch the options for the MLPCA application (Fig. 9).

The operating procedure selects the variable **Eaug** that contains data uncertainties (a matrix of the same size as the data matrix giving the standard deviation estimate of the uncertainty associated with each experimental data value). The dimensionality of the model is initially fixed by the number of components selected for the MCR-ALS optimization. Then, auxiliary parameters (number of iterations and convergence limit) related to the iterative process of the MLPCA algorithm can be modified. It is important to emphasize that depending on the size of the data matrix, MLPCA preliminary data projection can be a slow process. After this preliminary MLPCA step, the user is informed about the end of the process (convergence, divergence, maximum number of iterations reached) and the MLPCA projected data matrix can be then examined using the traditional MCR-ALS approach. Starting with the selection of the initial estimates (in this example, the purest variables were preferred), only non-negativity for all species in both modes (rows and columns) and spectral (normalization) constraints are selected.

6. General tips and troubleshooting

A short list with tips regarding common questions about the toolbox operation is presented:

A) Determination of the number of components
   - When the estimation of the number of components is not straightforward (e.g. there is a doubt whether to use three or four components), a reasonable option is to carry out the MCR-ALS analysis for different numbers of components. The optimum number of components is obtained by checking the model fit for each MCR-ALS analysis and the

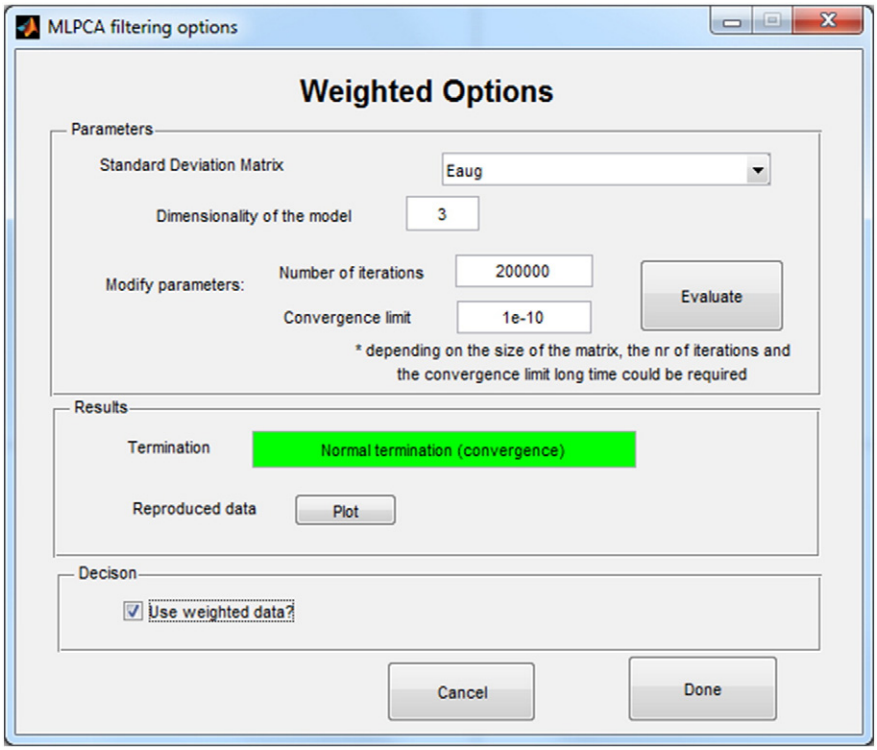

Fig. 9. MLPCA filtering options selection window.

interpretability of the obtained profiles in the different MCR models tested.

B) Initial estimates
   - In the case of using the purest variable detection method, two aspects should be considered. On the one hand, the amount of filtering noise is recommended to be above the noise level of the data. If this amount of noise is unknown, different values can be tested and the profiles of the initial guesses compared. When filtering noise is too low, selected profiles have too noisy features. Once the selected noise level is higher than the experimental one, selected profiles are not noisy and have the expected features (e.g. spectral, elution or reaction profiles). On the other hand, if the data contain negative values, the purest variable selection algorithm does not work properly as described by Winding and Stephenson in their original work [5] in which the authors stated that negative regions of the spectra (in their case, second-derivative spectra) can be discarded if the component shows more than one positive peak. In our case, it can be recommended to use it only for the positive part of the data signal or force all the data signal to be positive (i.e. a baseline correction could be applied in case negative values are present).
   - When dealing with multiple experiments, initial estimates should not be obtained by performing EFA on the column-wise augmented matrix. In this case, if an EFA initial estimate is desired, the initial guess should be obtained for each experiment individually (as many EFA analysis as experiments) and, then, concatenate the obtained individual initial guesses in a single column-wise augmented estimate respecting the right correspondence of species among experiments. This procedure is not implemented in the interface and should be done externally.

C) Application of constraints
   - It is important to apply constraints that are really fulfilled by the initial feasible solution. If constraints not obeyed by this initial solution are imposed, the optimization procedure will probably not work properly, and a significant change in the lack of fit value will happen giving non-feasible profiles.
   - In the case of multiset analysis (column-wise augmented data sets), the application of the correspondence among the species in the experiments can be recommended, since it can provide information on the presence/absence of species in different experiments which is extremely helpful for a proper resolution to decrease rotation ambiguities. However, this identification of the species should be done correctly and should match the same species sequence in the initial estimates. If not known, correspondence of species can be derived from the results of a previous MCR-ALS analysis of the multiset data assuming the presence of all species in all experiments.

D) MCR-Bands operation
   - Assessment of rotational ambiguities by using the MCR-Bands GUI should be carried out using exactly the same constraints to those used during the MCR-ALS optimization. Do not apply different constraints to those fulfilled by the initial feasible solution.
   - In case that the non-linear optimization does not progress from the initial values or does not converge, "Rotation Matrix" in the "Optimization parameters" menu can be changed from the default identity matrix to a different one, to move the optimization from the initial solution (for instance, starting with a random one).

To sum up, the first results usually obtained from initial MCR analysis can be improved by studying the resolved profiles and by reconsidering the number of components initially proposed and/or introducing/removing some of the constraints used in the ALS optimization. For final selection of the optimal solution, the proper explanation of the experimental data (data fitting aspects) and the recovery of feasible profiles (chemically meaningful shapes) from the knowledge of the investigated system should be considered.

7. Conclusions

An updated version of the MCR-ALS graphical user friendly interface is presented including some of the most recent developments of the MCR-ALS algorithm in a simple and intuitive manner. Data sets can be analyzed easily by this MCR-ALS GUI including the selection of the number of components, initial estimates and ALS constraints. On the other hand, experienced users can apply advanced constraints, such as correlation or kinetic hard-modeling constraints, and, at the end of the optimization, assess the effects of the rotational ambiguities on the obtained solutions using the MCR-Bands program. The option for MLPCA preliminary subspace projection has been included to consider cases where data uncertainties are known and to avoid error propagation in MCR-ALS results. Finally, several output options have been added to facilitate the interpretation of the results and evaluate the performance of the optimization.

8. Validation

Alejandro García-Reiriz[1], PhD

1. Departamento de Química Analítica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Instituto de Química Rosario (IQUIR-CONICET), Suipacha 531, Rosario S2002LRK, Argentina.

In this work the authors present the version 2.0 of the graphical interface to apply MCR-ALS.

MCR-ALS is a very general method that can be applied to a wide variety of chemometric studies. Because each problem involves different requirements to MCR-ALS, the new version is adapted to each type of study making easier to apply the GUI in each particular case. With this new GUI, it is avoided that data have to be processed in auxiliary routines because it has more options, making it simpler and direct in obtaining the results. Several aspects about the algorithm were improved. Now the representation of results with the trilinearity constraint or concentration prediction results in multivariate calibration is easier to be obtained because these topics have been included. Also it included MCR-Bands to evaluate the rotation ambiguities of results and MLPCA to noise filtering of the data.

The tutorial videos presented are very good examples to learn how to use the interface and they show how it has been adapted to each specific needs.

Dr. Lionel Blanchet[1,2], PhD

1. Radboud University Nijmegen, Institute for Molecules and Materials (IMM), Department of Analytical Chemistry, Nijmegen, The Netherlands. 2. Radboud University Medical Centre, Nijmegen Centre for Molecular Life Sciences, Department of Biochemistry, Nijmegen, The Netherlands.

Jaumot et al. present an updated version of their popular graphic user interface of MCR-ALS. A number of new functionalities are now available. They reflect the progresses observed in curve resolution over the last decade. The classical MCR tools (SVD, MCR-ALS, EFA, SIMPLISMA) are obviously still present and are now integrated into a seamless procedure. The addition of new tools is here more interesting.

First, prior to the analysis extra information on the data structure can be used to weight appropriately the variables. Second, new and more advanced constraints are available within MCR-ALS itself e.g., simple kinetic models or correlation constraint. These new features allow the user to implement straightforwardly more sophisticated models. Third, beyond the MCR-ALS resolution, the analysis can now be followed by the evaluation of ambiguities in the MCR results. This allows researcher to explore the consequence of the rotation ambiguities often mentioned in the "MCR" community but also often forgotten

during the interpretation of the results. Note that this additional step requires the optimization toolbox from MATLAB.

The new MCR-ALS GUI represents a significant improvement as compared to the previous versions. Experienced users will be able to navigate easily through both the usual and new features. Beginners are clearly directed through the most useful steps. Yet they might get confused by the number of available options in the most advanced settings which require more theoretical understanding.

The interface remains general enough to be useful in a wide range of applications. Depending on the first choices, more specific tools progressively appear allowing to adapt the analysis to the nature of the data. Specific visualization tools are available for multi-way data enabling the analysis of hyperspectral imaging or multi-dimensional chromatography.

Conflict of interest

We certify that there is no conflict of interest with any financial organization regarding the material presented in the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.chemolab.2014.10.003.

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