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Exploring the potential of combining chemometric approaches to model non-linear multi-way data with quantitative purposes – A case study

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Abstract

Second-order based calibration methods have been widely investigated capitalizing on the inherent benefits of the data structure and the decomposition models, demonstrating that second-order advantage is a property that conspires to a high likelihood success in the resolution of systems of varying complexity. This work aims to demonstrate the applicability of a combined chemometric strategy to solve non-linear multivariate calibration systems in the presence of non-multilinear multi-way data. The determination of histamine by differential pulse voltammetry at different pH is presented as case study. The experimental system has the outstanding difficulty arisen from the large displacement along the potential axis by the pH, which was successfully overcome by implementation of the presented combined strategy. For data modeling, MCR-ALS, U-PLS/RBL and U-PCA/RBL-RBF were used. MCR-ALS allowed unraveling the non-linear behavior between the signal and the concentration, and extracting the underlying profiles of the constituent. Quantitative analysis was performed through the three models, and a comparative evaluation of the predictive performance was done. The best results were achieved with U-PCA/RBL-RBF (mean recovery= 101%) whereas, MCR-ALS yield the lowest mean recovery for all samples (70%)

Keywords: non-trilinear type 3 data; pH-voltammetry; non-linear regression model; MCR-ALS; U-PLS/RBL; ANNs

1. Introduction

Multivariate calibration techniques have been attracting the attention of researchers in several investigation fields. The interest in the use of multivariate analysis relies on the fact that the inclusion of multiple signals can significantly improve the applicability of quantitative analyses [1, 2].

The central purpose of multivariate calibration is to establish empirical models relating unselective multiple instrumental signals to known analyte concentrations, which are then utilized to predict the analyte concentration of the test sample [3]. The multivariate calibration methodologies are categorized into first-, second- or higher-order calibration in association to the instrumental modes of the acquired signal [4, 5]. One particularity of first-order calibration methods is the requirement of a large number of calibration samples that must contain similar composition than the target sample, i.e., to contain the same potential interferents, for the success of the resolution and the prediction [3]. Nonetheless, second- and higher-order calibration models outweigh this attribute through a property that enables to selectively identify the components of a mixture even in the presence of non-modeled constituents, which is universally known as “second-order advantage” [5, 6].

The evolution of the modern analytical instrumentation has facilitated the acquisition of multidimensional signals allowing to enlarge the chemical information that can be obtained from the system under study. Some of the most common analytical instruments can yield first-order data for a single sample (e.g., infrared spectrum [7], UV spectrum [8], DSC signals [9]), which are acquired as a unidimensional vector data array. Second-order data could be obtained with a unique instrument, but it can be generated by instrumental hyphenation or experimental setups, allowing arranging the signals in bidimensional data arrays, i.e., data matrix. For instance,

excitation-emission fluorescence matrices [10], chromatography coupled to spectral detection [11] and kinetic with spectral monitoring [12] are cases of second-order data acquisition.

In the literature, it is possible to find several techniques for multivariate quantitative analysis. Notwithstanding this great diversity of methods offers exceptional versatility in the development of quantitative models, it usually leaves the analyst uncertain as to which is the most appropriate for a given set of data. This situation is not a trivial matter since each data set comprises particular underlying factors, e.g., noise level, signal overlapping, among others, that cannot be generalized. One of the underlying factors of the data that must be considered before modeling is the data linearity. In this regard, it becomes necessary to make a distinction between the types of linearity that can be observed in the multivariate calibration field.

First, it should be evaluated if the instrumental modes in which the data was collected are mutually independent. This condition guarantees the concept of *multi-linearity* of the multiway data. When lack of multi-linearity is observed, it is relevant to identify its origin to evaluate the different strategies that can be implemented for the resolution. The lack of multi-linearity has been well described elsewhere by Olivieri and Escandar, who introduced a classification for the different types of non-linearity in three- and four-way data structures [4]. On the other hand, in analytical chemistry, many quantitative procedures apply linear calibration models to describe the relationship between the instrumental measurement (dependent variable) and the property of interest (independent variable) [13]. However, there are systems in which the linear law is not obeyed, e.g., violation of the Beer-Lambert law, and non-linear functions ought to be applied to fit the data. Hence, the evaluation of the lack of linearity and multi-linearity is an unavoidable start in any multivariate quantitative procedure.

Among calibration models, partial least square (PLS), principal component analysis (PCA) or classical least square (CLS) are the most common algorithms [13] for first-order calibration methods, while unfolded-PLS and multi-way-PLS (U-PLS and N-PLS) are two of the used algorithms in second-order calibration procedures [14]. All these algorithms perform linear calibration models, i.e., linear regression modeling, and only data that conform to the principle of multi-linearity can be subjected to decomposition [13, 15]. Besides, parallel factor analysis (PARAFAC) [16] and multivariate curve resolution coupled to alternating least-squares (MCR-ALS) [17] are algorithms based on alternating least-square optimization that allows decomposing higher-order data and gathering *loadings*, which comprise the individual profiles of the constituents, and *scores*, that are the contribution of the analytes in each sample [18]. The obtained scores can be then utilized to build the proper regression model in predictive studies, even in systems where the analytical signals do not vary linearly with the analyte concentration. However, when the non-linear relationship between the dependent and the independent variable is present, artificial neural networks (ANNs) are the most common methods used in building the empirical non-linear calibration model [19]. ANNs are non-parametric techniques, so they do not assume any specific model form providing them with especial flexibility and ability to model diverse kind of data [20].

This work aims to demonstrate the applicability of a combined chemometric strategy to solve non-linear multivariate calibration systems in the presence of lack of multi-linearity in multi-way data. In this research, the quantitation of histamine in the presence of histidine by differential pulse voltammetry at different pH is presented as case study. These data represent an outstanding chemometrics challenge because of the large potential-displacements of the electroanalytical signal arisen from the pH variation. Moreover, for the best of our knowledge,

no reports regarding second-order DPV-pH data coupled to chemometrics with quantitative aims have been published yet.

2. Experimental section

2.1. Chemical, reagents and samples

Histamine (HIM) and histidine (HIS) were purchased from Sigma-Aldrich (Stenheim, Germany) and Fluka (Buchs, Switzerland), respectively. Boric acid (p.a. H_3BO_3) was obtained from Merk (Darmstadt, Germany). Glacial acetic acid (HAc), *o*-phosphoric acid (H_3PO_4), sodium hydroxide (NaOH) and dimethylformamide (DMF), all of analytical grade, were acquired from Panreac (Barcelona, Spain). Ultra-pure water was obtained with a Milli-Q purification system from Millipore (Bedford, USA).

Analytes stock solutions of 500 mg L^{-1} were prepared by dissolution of the appropriate amount of the powder presentation in ultrapure water and stored under refrigeration in the dark. Britton-Robinson Buffer (BRB) was prepared by mixing the proper amount of H_3BO_3, HAc and H_3PO_4, in order to obtain a final concentration of 40 mmol L^{-1} of each substance. To perform the pH-dependent experiments, the pH of the BRB was adjusted with NaOH, as appropriate, prior to the sample preparation.

2.2. Calibration and validation samples

A calibration set of HIM pure standard sample was daily prepared in triplicate by transferring the proper aliquot of the analyte stock solutions and 2.0 mL of BRB of the corresponding pH to a 10.00 mL volumetric flask and completing to the mark with ultra-pure water. The final HIM concentrations were ranging between 0.0 and 10.0 mg mL^{-1}.

A sample validation set was built considering HIM concentrations different than those used for the calibration samples. In these samples, HIS was incorporated as non-modeled interferent in 3 different concentrations as detailed in Table 1. The validation samples were prepared as previously described for the calibration samples.

2.3. Instrumentation and experimental procedure

Differential pulse voltammetry (DPV) measurements were conducted using a Metrohm 663 VA and an AUTOLAB PGSTAT 10 potentistat/galvanostat (ECOChemie. Utrecht, The Netherlands) with General-Purpose Electrochemical software (GPES) version 4.9.006 (ECOChemie. Utrecht, The Netherlands) for the data acquisition and instrument control.

The DPV experiments were carried out in a 10 mL cell using a conventional three-electrode system configuration including a 3.0 mm diameter glassy carbon electrode (GCE) as working electrode, an Ag/AgCl (saturated KCl) reference electrode and a 0.3 mm platinum wire auxiliary electrode, all of them commercially acquired (ECOChemie. Utrecht, The Netherlands).

At the beginning of the working day, the surface of the GCE was mechanically cleaned using a cotton pad soaked in DMF for 2 minutes and in ultrapure water for 30 seconds. Then, an electrochemical cleaning was made by applying three reduction cycles in the range of +1.5 – +0.9 V, at 15 mV s^{-1}, with an amplitude of 0.05 V, to the supporting electrolyte solution. The supporting electrolyte solution consisted in a BRB solution adjusted at the appropriate experimental pH. After electrode conditioning, DPV measurements were conducted by scanning the potential range of +0.9 V to +1.5 V at a scanning rate of 15 mV s^{-1}, with an amplitude of 0.05 V. Between measurements, the surface of the electrode was regenerated through mechanical cleaning. All experiments were carried out at room temperature.

For the pH measurements, a Crison Micro pH 501m (Barcelona, Spain) equipped with a combined glass/saturated calomel electrode, was used.

2.4. Software

Data processing and analysis were performed in MATLAB 2015b [21]. MCR-ALS GUI 2.0 [22] codes for MATLAB were freely downloaded from www.mcr-als.info. U-PLS/RBL and U-PCA-RBL-RBF were implemented in MVC2 [23] and MVC1 [24], respectively, for which the MATLAB codes are available at http://www.iquir-conicet.gov.ar/eng/div5.php?area=12. *i*-coshift [25-27] tool for MATLAB was acquired from http://www.models.life.ku.dk/icoshift.

For baseline correction, a moving average procedure (peak width of 0.01), which is available in the GEPS software, was implemented (Figure S1).

3. Results and discussion

3.1. General considerations

3.1.1. Chemometrics in electroanalytical chemistry

Curiously, the number of publications reporting the use of chemometrics in electrochemistry is small in comparison with the application of other analytical methodologies, such as spectroscopy or chromatography. This observation has been recently stated by Esteban *et al.* in Ref [28] whose proposed the hypothesis that the dearth of research in this topic might be caused by the close relationship between mathematics and electrochemistry and, also, the lack of linearity between the signal and the concentration of the electroactive species [29, 30]. Nevertheless, there is an ongoing interest in exploit the potentialities of electrochemical

instrumentation by applying chemometrics and promote its use to solve electroanalytical problems.

First-order calibration methods have been extensively used in electroanalytical chemistry by the implementation of the classical calibration models as multi-linear regression (MLR), PLS or PCR, among others [30]. Moreover, higher-order calibration methods have been recently explored and presented in several publications reporting the application of MCR-ALS, PLS-based methods and ANNs techniques for the predictive analysis of compounds in samples of variate nature [31, 32]. Among the electroanalytical techniques, differential pulse voltammetry (DPV) and stripping voltammetry (SP) are the most used for the acquisition of second-order data with quantitative aims [29, 31, 33, 34]. However, some features inherent to the electrochemical systems are cumbersome and made difficult the direct implementation of a chemometric model. One of the most common issues present in electrochemistry is the signal displacement by empirical phenomena that leads to a lack of linearity of the signal-analyte concentration relationship and a loss of multi-linearity in the multi-way data. Hence, recent works are focused on the development of new strategies that enable either the modeling of non-linear data [34] or the correction of shifted data [29, 35, 36]. Nevertheless, there is a noticeable dearth of research in this field when large signal-shifts occur, for example, as it is the case of the displacement along the potential axis of some irreversible signals as a function of the pH [29].

3.2. Data properties

3.2.1. Multi-linearity

When second-order data are obtained for a set of samples, the data matrices can be organized into a three-way structure, which can whether conform to the *low-rank trilinearity*

principle (henceforth referred to as trilinearity) or not. In this case, it is appropriate to be referred to the classification tree for a three-way data of a set of samples proposed by Olivieri and Escandar elsewhere [4]. Briefly, four types of data are distinguished according to the trilinearity concept: 1) *trilinear*; 2) *non-trilinear type 1*; 3) *non-trilinear type 2*; and *non-trilinear type 3* (for more details, the reader must be referred to Ref [4]). The latter corresponds to the case when individual second-order data do not conform to the bilinerity principle. It should be highlighted that the most of the experimental second-order calibration investigations reported in the literature have performed the acquisition and modeling of trilinear three-way data set or non-trilinear type 1 three-way data. Furthermore, a small number of publications report calibration methods built with non-bilinear data modeling with quantitative purposes, i.e., non-trilinear type 3 data [37, 38].

The experimental data presented in this report consist of a set of second-order data comprising the DPV signals of HIM at six different pH (DPV-pH). Figure 1.A depicts the DPV-pH second-order data of HIM at a given concentration, while Fig. 1.B shows the DPV signals acquired for different concentrations of HIM at the same pH (pH 6). In Fig. 1.A, it is clear to observe that the DPV-pH matrix presents severe deviations of bilinearity as result of the displacement of the oxidation peak potential with the pH: *the HIM peak shifts negatively with rising the pH value,* in agreement with previous reports [39]. Additionally, a significant shift between DPV signals arisen from the different concentrations at the same pH is observable (Fig. 1.B).

**** Figure 1 ****

These observations lead to the conclusion that the multi-way data set, i.e., DPV-pH-concentration, is a severe case of non-trilinear type 3 data, since neither bilinearity in the DPV-pH nor bilinearity in the DPV-concentration arrays are fulfilled. It is worth to highlight that this is the first time that this type of non-trilinear data is used with quantitative purposes.

Under this complex scenario, several alternatives were implemented to carry out the chemometric modeling. After an in-depth evaluation of the possible strategies, the following procedure was achieved. Aiming to recover de bilinearity in the DPV-concentration data array, a shift correction procedure based in correlation shifting method (i-*coshift*) [25-27] was implemented since no shape distortions were observable. Figure 2 shows the DPV signals acquired at three different pH level for several concentrations before and after shifting correction.

**** Figure 2 ****

In this way, a bilinear DPV-concentration array at each pH was obtained. Nonetheless, the DPV peak shifts arisen from the pH change were not corrected since the large displacements between signals would hinder the right implementation of a correction strategy and, more importantly, would suppress the selectivity of the second-mode. This phenomenon is a troublesome situation for which solutions were comprehensively analyzed and are described in this work.

3.2.2. Regression model

To implement the most appropriate regression model, a first evaluation of the trend line describing the relationship between the dependent (DPV signal) and the independent variable (concentration of the analyte) at every pH was performed. Figure 3 shows the aligned DPV signals for the calibration set (1.0-10.0 mg L^{-1}) acquired at pH 4, 6 and 9.

**** Figure 3 ****

A clear difference in the regression trend at the different pH levels is noticeable with the naked eye. However, to accurately establish a function that explains the regression model for the entire system, it is necessary to perform a thorough evaluation by decomposing the data through specific chemometric techniques. Hence, experimental data were subjected to MCR-ALS to explore the empirical behavior of the constituents.

3.3. Data modeling

3.3.1. MCR-ALS

MCR-ALS is a wide-spread soft-modelling method that performs bilinear decomposition of a data matrix into two sets of profiles, which comprise relevant physicochemical information about the constituents of the system under study [17]. The application of this method has been successfully demonstrated in numerous situations proving its potential to reach significant results. It has been implemented for many purposes, either quantitative analysis [11, 40-42] or descriptive studies allowing unraveling the behavior of chemical or biological processes where species are totally or partly unknown [43-45]. In this matter, the fact that MCR-ALS can extract the individual contribution of the constituents when no prior information of the system is available is rather appealing, especially, for investigations of highly complex systems.

The bilinear decomposition of MCR-ALS is performed under the application of constraints that are implemented during the iterative ALS phase. It is well-documented that if no constraints are implemented, uncountable possible solutions that equivalently represent the system might be obtained. The most common constraints are non-negativity, unimodality and correspondence between species, which relies on chemical principles [46]. However, it has been proved that the application of a full set of constraints helps to diminish the range of feasible solutions, albeit not completely. This phenomenon is known as *rotational ambiguity* [47, 48].

With the spread of multivariate data analysis escorted by the evolution of analytical instrumentation, diverse data structures have arisen, that coerced chemometricians to explore new alternatives for the data modeling. Along those lines, MCR-ALS has evolved into a very versatile strategy for the modeling of a wide range of data structures. Even though MCR-ALS is a pure bilinear decomposition model, it has been extended to higher-order multivariate analysis, e.g., third-order data [49-51]. Moreover, it is well-known that first-order calibration models can perform a reliable prediction only in the case that test samples contain the same composition that the calibration samples; however, it has been demonstrated that this drawback can be overcome with the aid of MCR-ALS. Ahmadi et al. have proved the ability of soft-modelling methods in analyzing first-order data sets containing unmodeled components, in which the rotational ambiguity associated to the resolution is minimized by applying specific constraints during iteration [52]. In addition, Esteban's group has demonstrated that the implementation of constraints that model the signal feature shall enhance the performance of the MCR-ALS modeling [53-56].

In the present case, pH dimension is the bilinear breaking mode, whereas the concentration mode fulfils the concept of low-rank bilinearity. Thus, to perform a bilinear

decomposition, the path of Ahmadi work was followed and a *first-order* MCR-ALS strategy was implemented by unfolding the DPV-pH data to arrays of lower dimension, i.e., an unfolded one-way vector. To achieve the decomposition, a bilinear row-wise concatenated/column-wise augmented matrix was built. First, a DPV-pH vector was obtained by concatenating the aligned DPV signals of the different pH for each evaluated sample. Then, the concatenated vector corresponding to each calibration and validation samples were column-wise appended. Figure S2 (supplementary material) graphically depicts the data arrangement used for MCR-ALS decomposition.

To start the modeling, initial concentration estimates were obtained by a SIMPLISMA-like procedure [57] and the number of components was evaluated through singular value decomposition (SVD). The imposed constraints during optimization were non-negativity of concentration and spectral modes, correspondence among the species and normalization in concentration. Voltammogram and concentration profiles achieved from the MCR-ALS decomposition for four validation samples containing 4.0 mg mL^{-1} of HIM and calibration data set are shown in Fig. 4.

**** Figure 4 ****

As can be seen in Fig. 4, two components were necessary to describe the behavior of HIM and one additional component was needed for the samples containing HIS as interferent. These results are in accordance with the afore-mentioned observations demonstrating that the regression model follows both linear and non-linear behavior against concentration in dependence to the pH. By evaluation of the unfolded voltammogram profiles, it can be noticed

that the non-linear regression trend prevails at lower pHs, whereas linearity arises with rising the pH values. For the predictive analysis, a pseudo-univariate calibration model was built by using the scores obtained for the factor with linear trend. These scores were plotted against the nominal concentration of the analyte and a least-square regression model was implemented to fit the data. Scores against nominal concentrations are depicted in Fig. 5 and predictive results are summarized in Table 1.

**** Figure 5 ****

As can be appreciated, at a higher concentration of the interferent, the contribution of the analyte significantly diminishes (*# Sample* 7, 8, 11 and 12 in Table 1). This effect is evidenced by performing a prediction analysis with the scores obtained for the factor with linear regression trend (the scores of the second factor were not considered in this study because of its unusual behavior against concentration). This effect can be the result of two different phenomena. One of them can be related to the possible interaction between the analyte and the interferent, in which the interferent precludes the analyte detection. Besides, it should be considered that in first-order MCR-ALS decomposition, rotational ambiguity is heavily present and gets relevant at higher concentration of the unmodeled component due to the strong signal overlapping [52].

Based on these observations, it can be concluded that linear multi-way calibration models cannot be applied and other methodologies that enable the prediction of the analyte in the presence of non-linear regression trend need to be explored.

3.3.2. U-PLS/RBL

The unfolded multi-way extension of the renowned PLS method is the U-PLS methodology that performs decomposition to higher dimension arrays. U-PLS it is a multi-way extension of PLS methodology and operates after unfolding the multi-way data arrays into vectors [58]. Those algorithms are applicable on systems showing small deviations of trilinearity, although the second-order advantage is not achieved. The U-PLS methodology consists of performing the classical PLS model calibration with the unfolded calibration data (not including the test samples) using a suitable number of latent variables. If no potential interferents are expected in the test samples, the amplitude of residuals in the prediction step is in the order of the instrumental noise level. Then, the concentration ($\hat{y}_u$) prediction of the sample is obtained through $\hat{y}_u = \mathbf{t}_u^T\mathbf{v}$, where $\mathbf{t}_u$ is the score vector obtained for the sample data matrix and $\mathbf{v}$ are the regression coefficients acquired from the U-PLS model. However, when interferents are present, the residuals are extraordinarily large in comparison to the instrumental noise and, consequently, this formulation is not suitable for the prediction of the samples. Hence, to overcome this situation, the test sample is subjected to the residual bilinearization (RBL) procedure that enables to separate the signal that is explained by calibration from the contribution of the potential interferents, which means, achieve the second-order advantage [58-60]. To put it succinctly, the residual variance of a test sample is estimated considering the matrix $\mathbf{E}$ which comprise the residuals of the U-PLS model. In the RBL process, the $\mathbf{E}$ matrix is subsequently subjected to SVD decomposition for which results contain information about the interferents. This information that is then added to rebuild the original data matrix. Last, during the residual minimization step, the loadings obtained in the calibration stage remains constant while the scores are adjusted to minimize the residual variance. At the end for the RBL process, when the scores of the test sample are adjusted, the concentration of the analyte can be properly estimated

[61, 62]. The combination of U-PLS and RBL has already been described and demonstrated in the relevant literature [58, 60].

In the present case, as it is shown in Fig. 5, the regression model shows a noticeable global non-linear signal behavior. Hence, U-PLS/RBL was implemented because it is one of the most flexible linear second-order multivariate calibration techniques and can cope with mild non-linearities. The calibration step was carried out using the well-known leave-one-out methodology. It is worth noticing that for linear regression system, only one or two PLS factors are necessary to explain the model; however, in the present experimental system, four PLS factors were needed to model the complexity of the data. This fact supports the presence of non-linearity that was observed with the MCR-ALS analysis.

The best prediction results were obtained when two RBL components were included in the resolution, which explains the presence of the interferent in the test samples. Table 1 comprises the prediction results obtained with the U-PLS/RBL model for the validation samples. These values are better - albeit rather low ($\bar{R}$% 82 %) - than those obtained from MCR-ALS. It is noticeable that the lower recoveries were obtained for those samples containing the highest amount of the unmodeled component, in accordance with the MCR-ALS results. This conclusion strength the fact that a possible interaction between analyte and interferent is present during detection.

3.3.3. Artificial neural networks

Artificial neural network (ANN) constitutes a family of multivariate nonparametric regression techniques, which, after a convenient training procedure, are able of learning the correlation of a set of predictor variables with the desired response. The ANN family can be

subdivided into three groups of techniques: 1) multi-layer perceptron networks (MLP), 2) radial basis functions (RBF), and 3) support vector machines (SVM) [62]. The first two are the most frequently reported in the literature to solve multivariate calibration problems.

Briefly, ANNs contain operating units called neurons, which are arrayed in three layers: 1) input, 2) hidden, and 3) output layers. Input and output neurons correspond to the measured signals and the properties predicted by the model, respectively. The hidden nodes represent the computing core. During training or calibration process, each hidden and output node receives weighted contributions, that are then projected on a transfer function to generate a non-linear output. The procedure of learning consists of adjusting the relationship between signals and concentrations by modifying the weights related to the inter-neural connections. Thus, the final output is close to the nominal concentration value for the analyte [20].

In second-order data, the number of original variables comprised in the instrumental signal is usually large and contain redundant information. Then, the information can be compacted into a small number of latent variables. For instance, the principal components (PCs) or scores obtained by computing the explained variance of the unfolded training data can be used as the input layer. When unexpected components are in the test samples, its scores shall not be adequate for the analyte prediction using the trained ANN [63]. Hence, it is necessary to implement a strategy capable to indicate the sample as an outlier, to separate the contribution of the unexpected component from the analyte signal and, then, to build the calibration model to perform the analyte prediction. It has been reported that unfolded PC analysis coupled to RBL (U-PCA/RBL) and subsequent ANN modeling is an excellent alternative to cope with this situation [62, 63]. In this approach, the adjusted score vector $\mathbf{t}_u$ obtained at the end of the RBL process is introduced into the ANN network for the subsequent concentration prediction.

In this work, the number of PCs used as input neurons was four, and two RBL factors were necessary for the modeling with U-PCA-RBL and subsequent prediction. RBF was chosen to perform the calibration model and the implemented architecture was (4-10-1) for input-hidden-output layers, respectively. The prediction results are shown in Table 1. As can be appreciated, the performance of U-PCA/RBL-RBF is superior in comparison to the linear regression methods, yielding excellent recoveries values with a $\bar{R}$% of 101 % (s=8).

Figure 6.A depicts the relationship between the nominal and predicted concentration for the three chemometric models and the corresponding least-square fitting lines. Figure 6.B shows the elliptical joint confidence region for the slope and the intercept of the linear regression between the actual and predicted concentration, with a 95% confidence level, for each model. Moreover, Fig 6.A and 6.B indicate the theoretical point and line, respectively, for intercept=0 and slope=1 [64]. Noticing that the ellipse built for U-PCA/RBL-RBF contains the theoretical expected value for the intercept and slope and, moreover, the fitting line of nominal vs predicted relationship overlaps the theoretical line. These results reinforce the fact that with an in-depth evaluation of the system behavior leading the use of the proper chemometric tool is possible to leverage the predictive properties of an analytical methodology

**** Figure 6 ****

**** Table 1 ****

4. Conclusions

Second-order based calibration methods have been widely investigated capitalizing on the inherent benefits of the data structure and the decomposition models, demonstrating that second-order advantage is a property that conspires to a high likelihood success in the resolution of systems of varying complexity.

In this work, a qualitative and quantitative analysis was performed by implementing parametric and non-parametric methodologies for the determination of histamine in the presence of histidine by differential pulse voltammetry at several pHs as case study.

First, MCR-ALS was performed to evaluate the empirical behavior of the constituents. It was proved that first-order data, built from unfolded second-order data, can be solved with the aid of MCR-ALS, allowing to separate the individual contribution of each sample constituent. Based on the acquired results, it was concluded that at least two regression models were necessary to perform the calibration of the analyte, one of which presents a pronounced non-linear behavior. Moreover, it was possible to unravel the presence of a potential interaction between the analyte and the interferent, phenomenon that was reinforced with the results achieved from U-PLS/RBL.

On the other hand, U-PCA/RBL combined with RBF modeling significantly outweigh the performance of the parametric models demonstrating the capability to handle non-multilinear data in non-linear regression systems.

The results allowed to demonstrate the importance of performing a comprehensive analysis to accurately select the method to implement for the resolution and thus obtain reliable results. The most remarkable accomplishment achieved in this work was the successful implementation of chemometric techniques to bear the foresee difficulty arisen from the large displacement along the potential axis by the pH, which, to the best of our knowledge and belief,

has not been reported yet. The modeling of non-multilinear data has been a matter of paramount interest for chemometricians and represents an outstanding challenge in the field of quantitative electroanalytical chemistry. The inception of this research line encourages the development of new chemometric methodologies that lead to a coercive expansion of its range of application.

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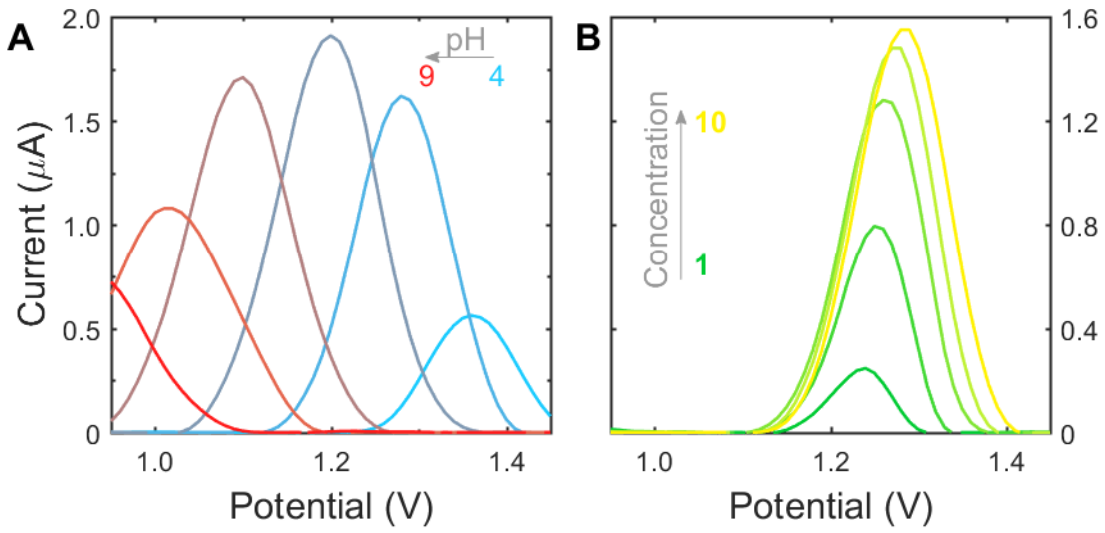

Figure 1. (**A**) Differential pulse voltammograms obtained at GEC in BRB solution containing of 5.0 mg L^{-1} HIM at the pH range of 4-9 (from cyan to red lines) and the (**B**) Differential pulse voltammograms obtained at GEC registered for concentrations of 1.0-10.0 mg L^{-1} HIM at pH 6.

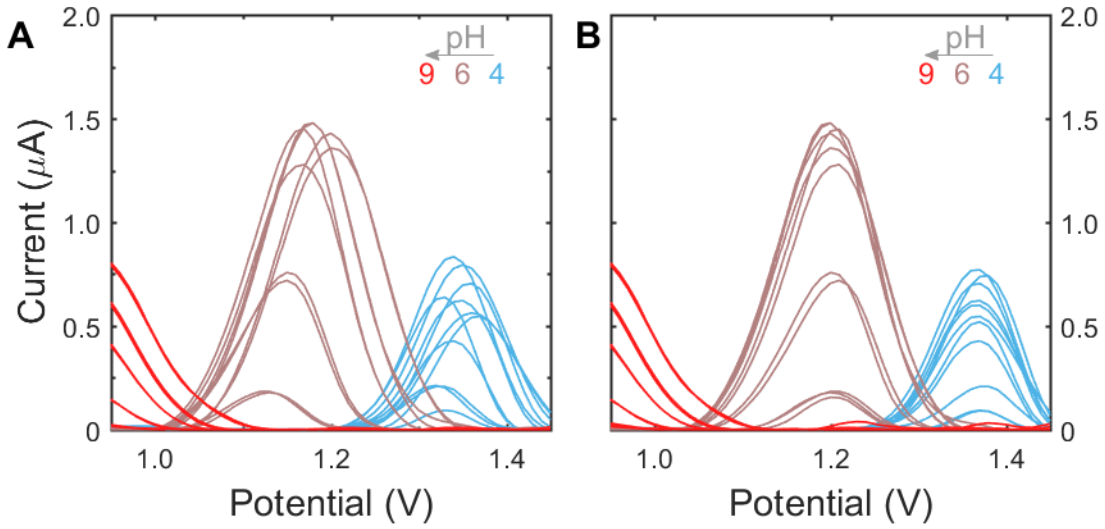

Figure 2. (**A**) Original and (**B**) aligned DPV signals acquired for several concentrations of HIM at pH 4 (cyan) 6 (purple) and 9 (red).

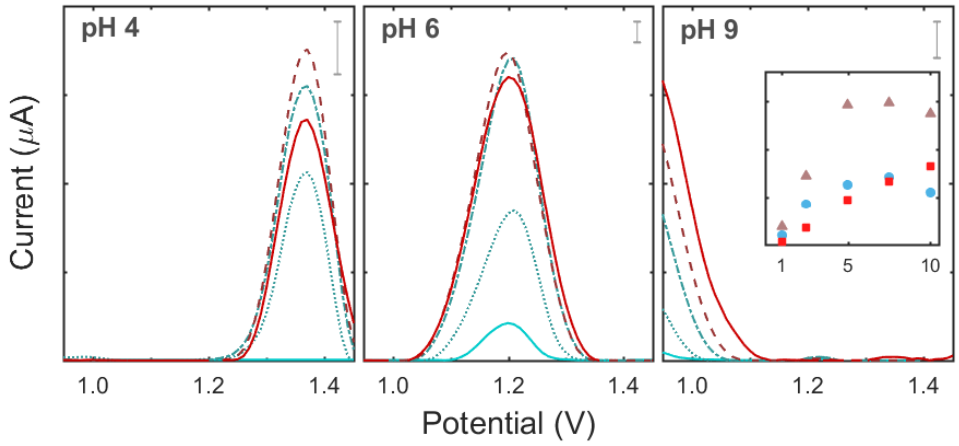

Figure 3. Aligned DPV signals of 1.0 mg L^{-1} (solid cyan line) and 10.0 mg L^{-1} (solid red line) of HIM at pH 4, 6 and 9. DPV signals of 2.5 mg L^{-1} (dotted line), 5.0 mg L^{-1} (dash-dotted line) and 7.5 mg L^{-1} (dashed line) mg L^{-1} of HIM are shown. Grey bars indicate the current of 0.1 µA. Inset: maximum current intensity against concentration at pH 4 (cyan) 6 (purple) and 9 (red).

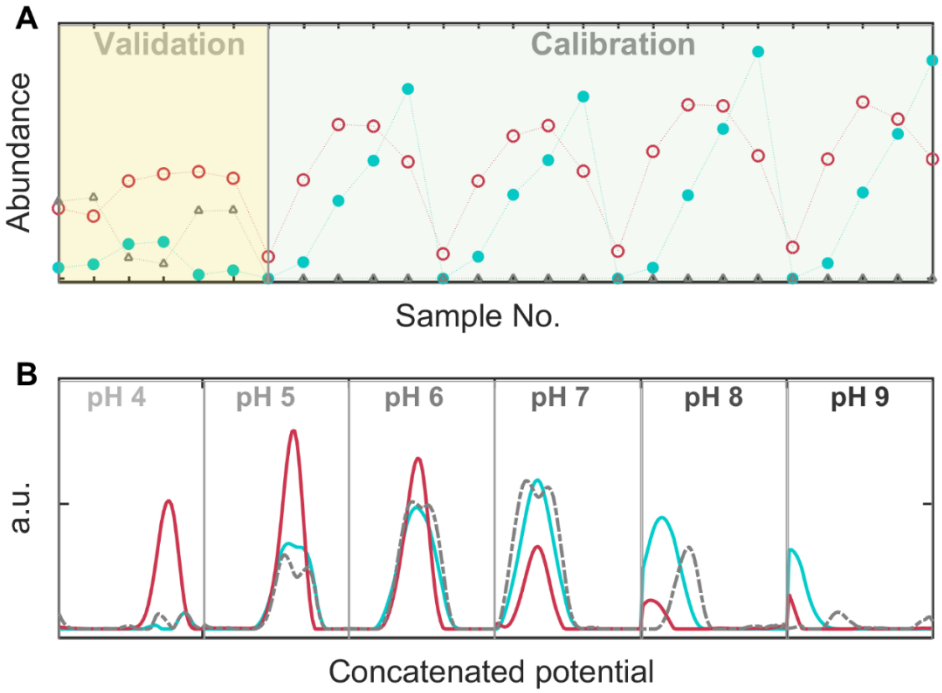

Figure 4. (**A**) Concentration and (**B**) unfolded voltammogram profiles acquired after MCR-ALS decomposition of DVP-pH signals. The first six samples correspond to validation samples containing 4.0 mg L^{-1} HIM (empty red and full cyan circles) and 25.0, 5.0 and 15.0 mg L^{-1} HIS (grey triangles) in duplicate, respectively.

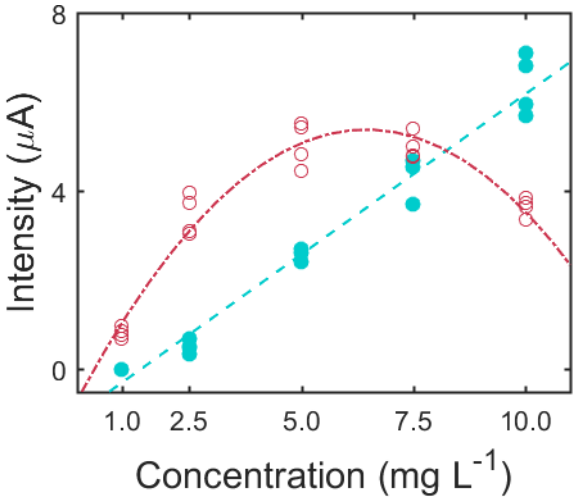

Figure 5. MCR-ALS concentration scores obtained from the calibration signals of HIM against nominal concentration. Scores of first (full cyan dots) and second (empty red dots) MCR-ALS factors show the linear and nonlinear regression trends, respectively. Dashed cyan line and dash-dotted red line are the fitted lines through linear and quadratic function, respectively.

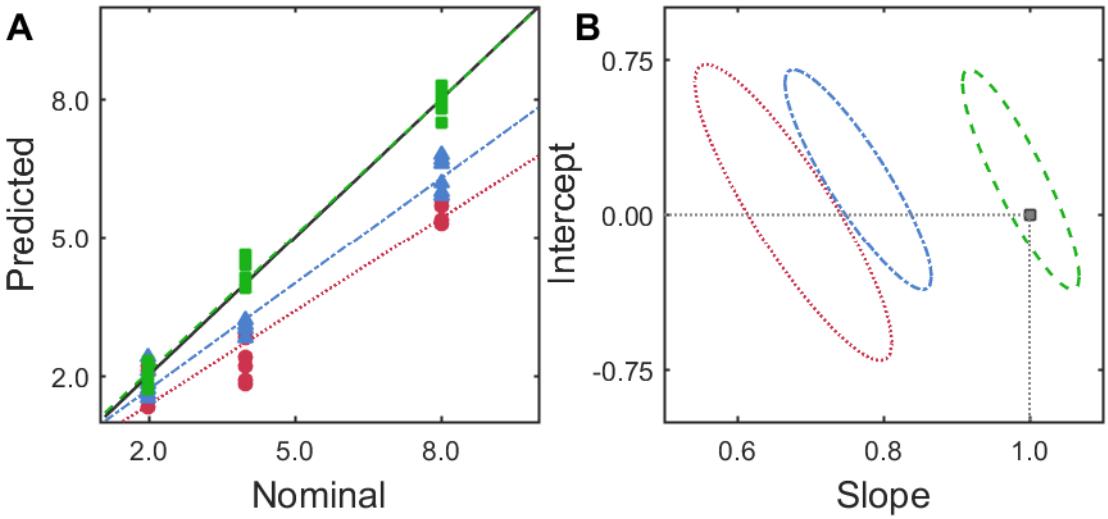

Figure 6. (**A**) Nominal against predicted concentrations of HIM (in mg mL^{-1}) and the corresponding least-square fitting lines. (**B**) Elliptical joint of confidence region (95% confidence level) for the slope and the intercept of the linear regression between the nominal and the predicted concentrations of HIM. MCR-ALS (dotted red line), U-PLS/RBL (dash-dotted blue line) and U-PCA/RBL-RBF (dashed green line). Results for MCR-ALS are in red, U-PLS/RBL in blue and U-PCA/RBL-RBF are in green. The theoretical regression line (**A**) and the ideal point(**B**) (slope=1, intercept=0) are shown in black.

Table 1. Recovery results obtained from validation samples using parametric and nonparametric methodologies.

# Sample	HIM (mg L^{-1}) [a]				HIS [b] (mg L^{-1})
	Nominal	Predicted			
		MCR/ALS	UPLS/RBL	U-PCA/RBL - RBF	
1	2.0	2.1 (105)	2.4 (120)	2.2 (110)	25.0
2	2.0	2.2 (110)	2.1 (105)	1.8 (90)	25.0
3	2.0	1.3 (65)	1.7 (85)	1.9 (95)	5.0
4	2.0	1.5 (75)	1.8 (90)	2.1 (105)	5.0
5	2.0	1.3 (65)	1.6 (75)	2.3 (115)	15.0
6	2.0	1.3 (65)	1.5 (74)	1.7 (85)	15.0
7	4.0	2.2 (55)	3.0 (75)	4.4 (110)	25.0
8	4.0	2.4 (60)	3.1 (78)	4.6 (115)	25.0
9	4.0	2.9 (73)	3.2 (80)	3.9 (98)	5.0
10	4.0	2.8 (70)	3.1 (78)	3.9 (98)	5.0
11	4.0	1.8 (45)	2.8 (70)	4.1 (103)	15.0
12	4.0	1.9 (48)	2.8 (70)	4.1 (103)	15.0
13	8.0	5.7 (71)	5.9 (70)	7.5 (94)	25.0
14	8.0	5.8 (73)	6.0 (75)	8.1 (101)	25.0
15	8.0	5.3 (66)	6.2 (78)	7.9 (99)	5.0
16	8.0	5.4 (68)	6.8 (85)	8.3 (104)	5.0
17	8.0	5.4 (68)	6.7 (84)	7.8 (98)	15.0
18	8.0	5.7 (71)	6.6 (83)	8.2 (103)	15.0
$\bar{R}$%[c]		**70 (*s*=16)**	**82 (*s*=12)**	**101 (*s*=8)**	

[a] Recoveries (%) are between parenthesis
[b] HIS as an interferent
[c] Mean recovery in %

Table 1

Table 1. Recovery results obtained from validation samples using parametric and nonparametric methodologies.

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		MCR/ALS	UPLS/RBL	U-PCA/RBL - RBF	
1	2.0	2.1 (105)	2.4 (120)	2.2 (110)	25.0
2	2.0	2.2 (110)	2.1 (105)	1.8 (90)	25.0
3	2.0	1.3 (65)	1.7 (85)	1.9 (95)	5.0
4	2.0	1.5 (75)	1.8 (90)	2.1 (105)	5.0
5	2.0	1.3 (65)	1.6 (75)	2.3 (115)	15.0
6	2.0	1.3 (65)	1.5 (74)	1.7 (85)	15.0
7	4.0	2.2 (55)	3.0 (75)	4.4 (110)	25.0
8	4.0	2.4 (60)	3.1 (78)	4.6 (115)	25.0
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10	4.0	2.8 (70)	3.1 (78)	3.9 (98)	5.0
11	4.0	1.8 (45)	2.8 (70)	4.1 (103)	15.0
12	4.0	1.9 (48)	2.8 (70)	4.1 (103)	15.0
13	8.0	5.7 (71)	5.9 (70)	7.5 (94)	25.0
14	8.0	5.8 (73)	6.0 (75)	8.1 (101)	25.0
15	8.0	5.3 (66)	6.2 (78)	7.9 (99)	5.0
16	8.0	5.4 (68)	6.8 (85)	8.3 (104)	5.0
17	8.0	5.4 (68)	6.7 (84)	7.8 (98)	15.0
18	8.0	5.7 (71)	6.6 (83)	8.2 (103)	15.0
$\bar{R}\%$ [c]		**70 (*s*=16)**	**82 (*s*=12)**	**101 (*s*=8)**	

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Figure 1

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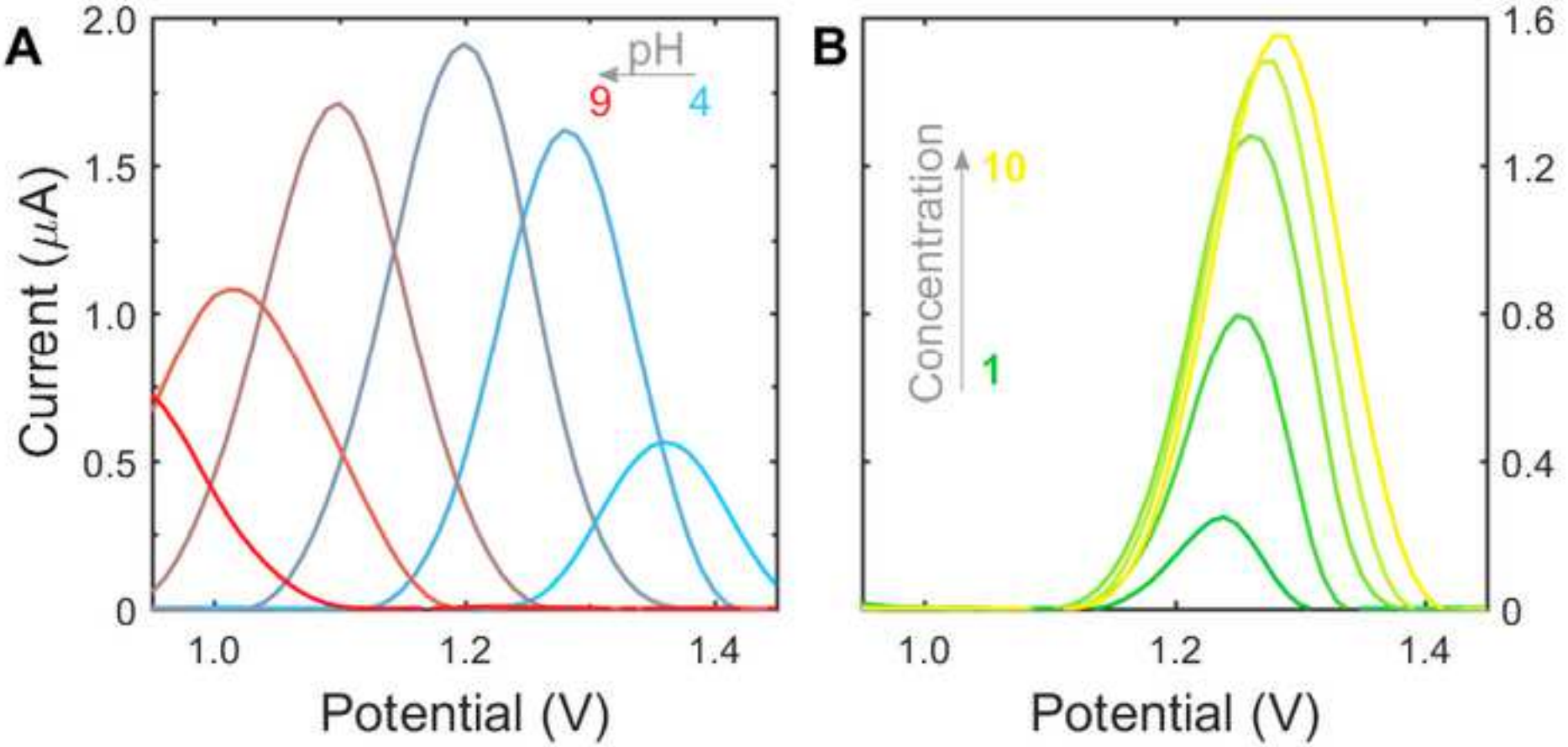

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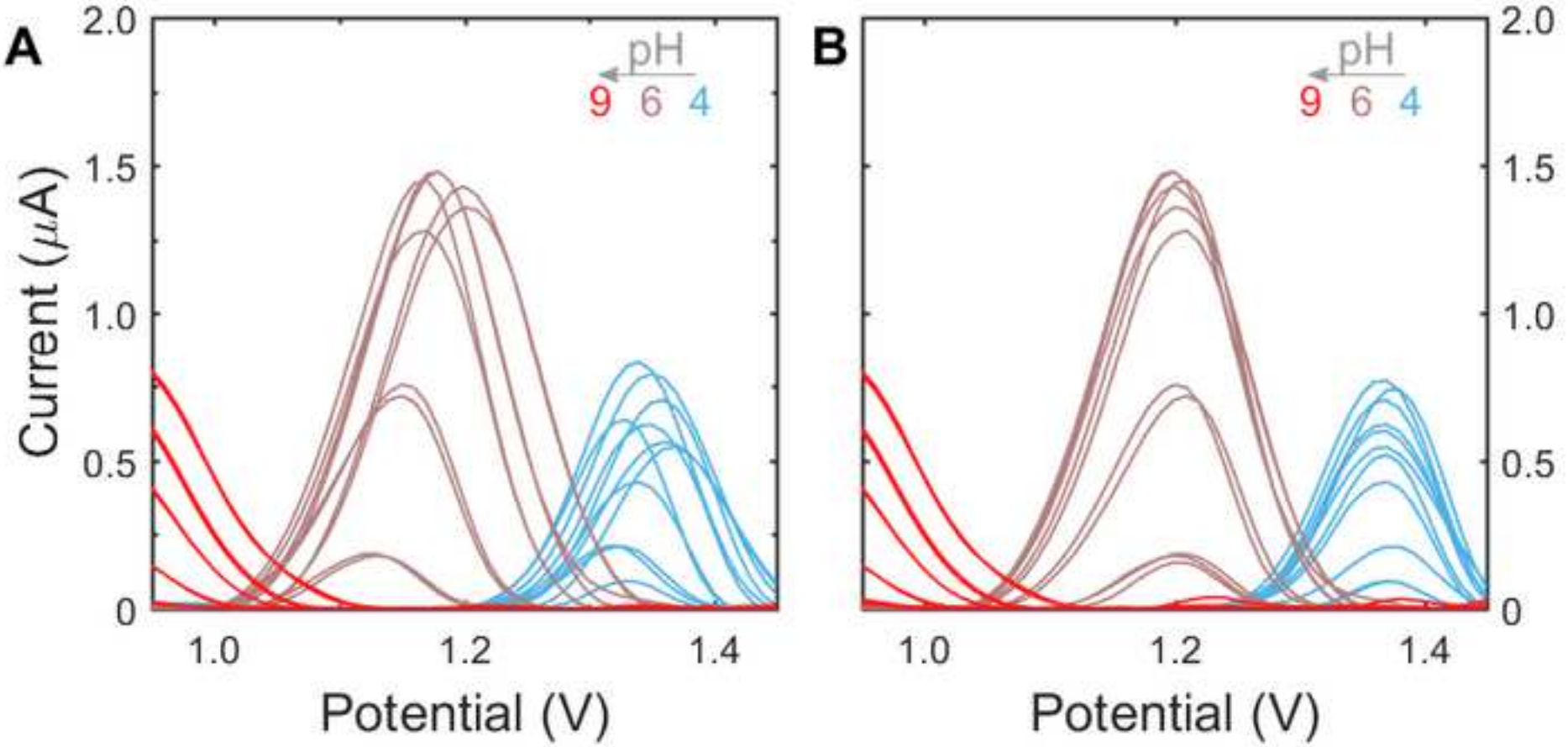

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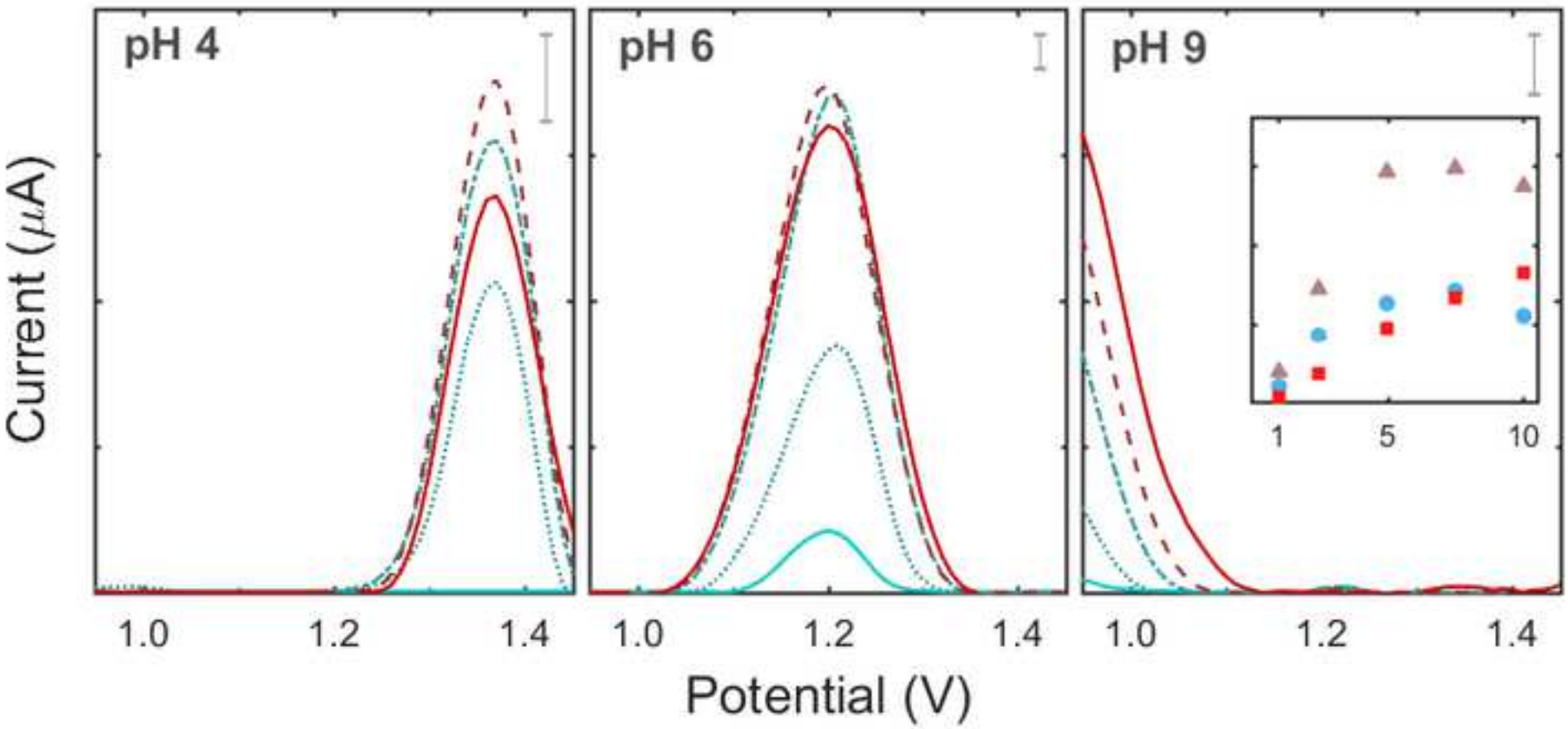

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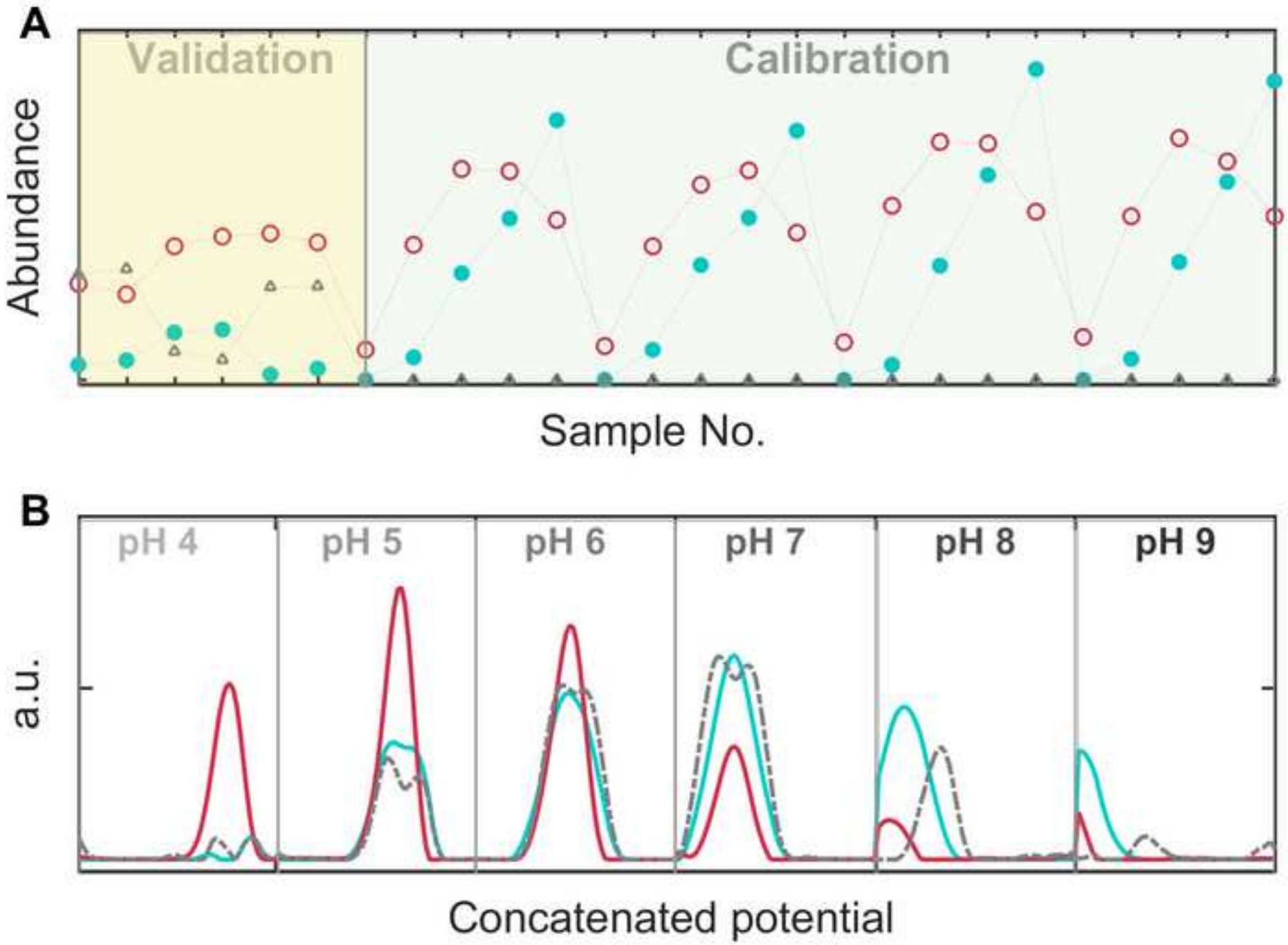

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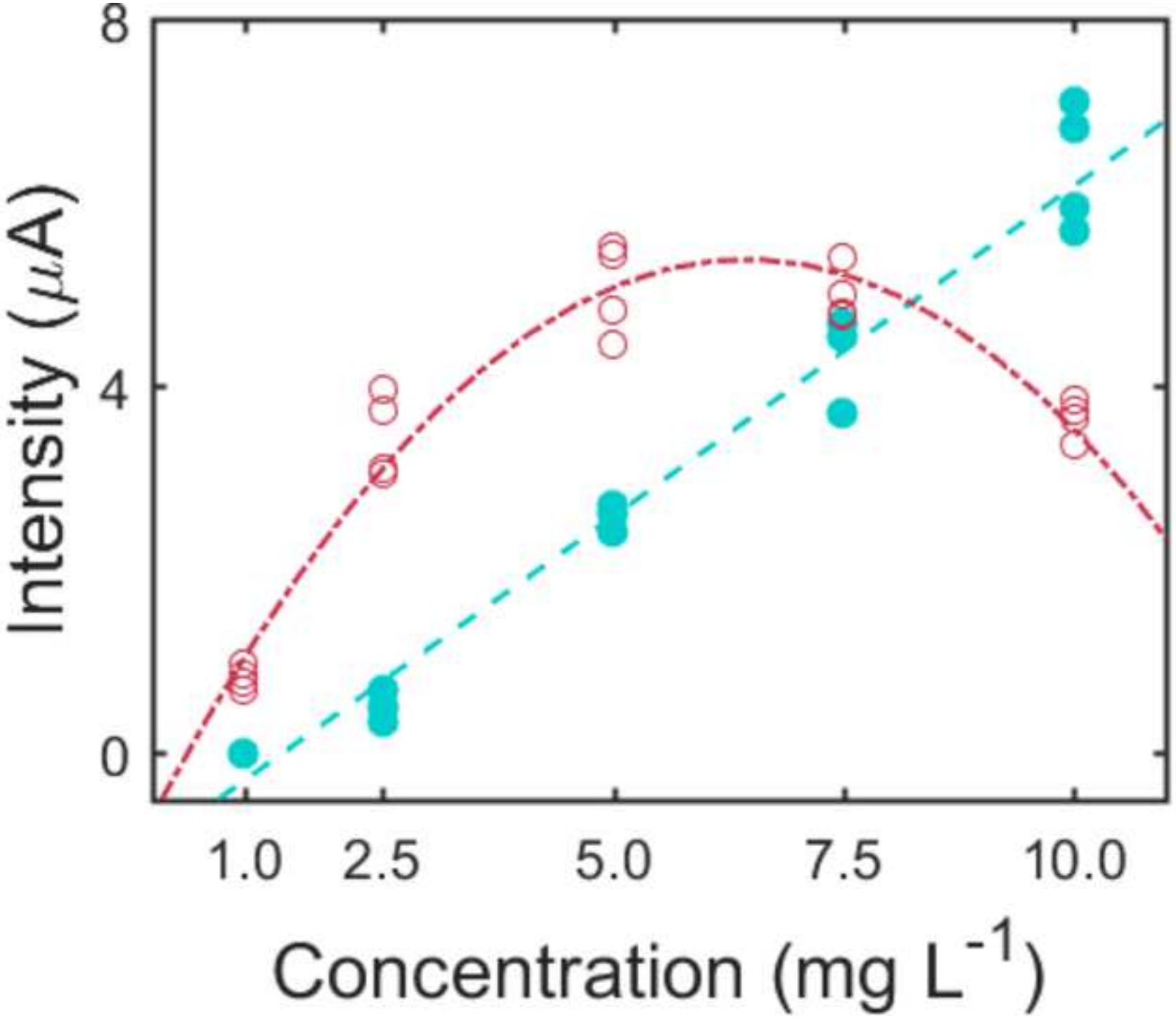

Figure 6

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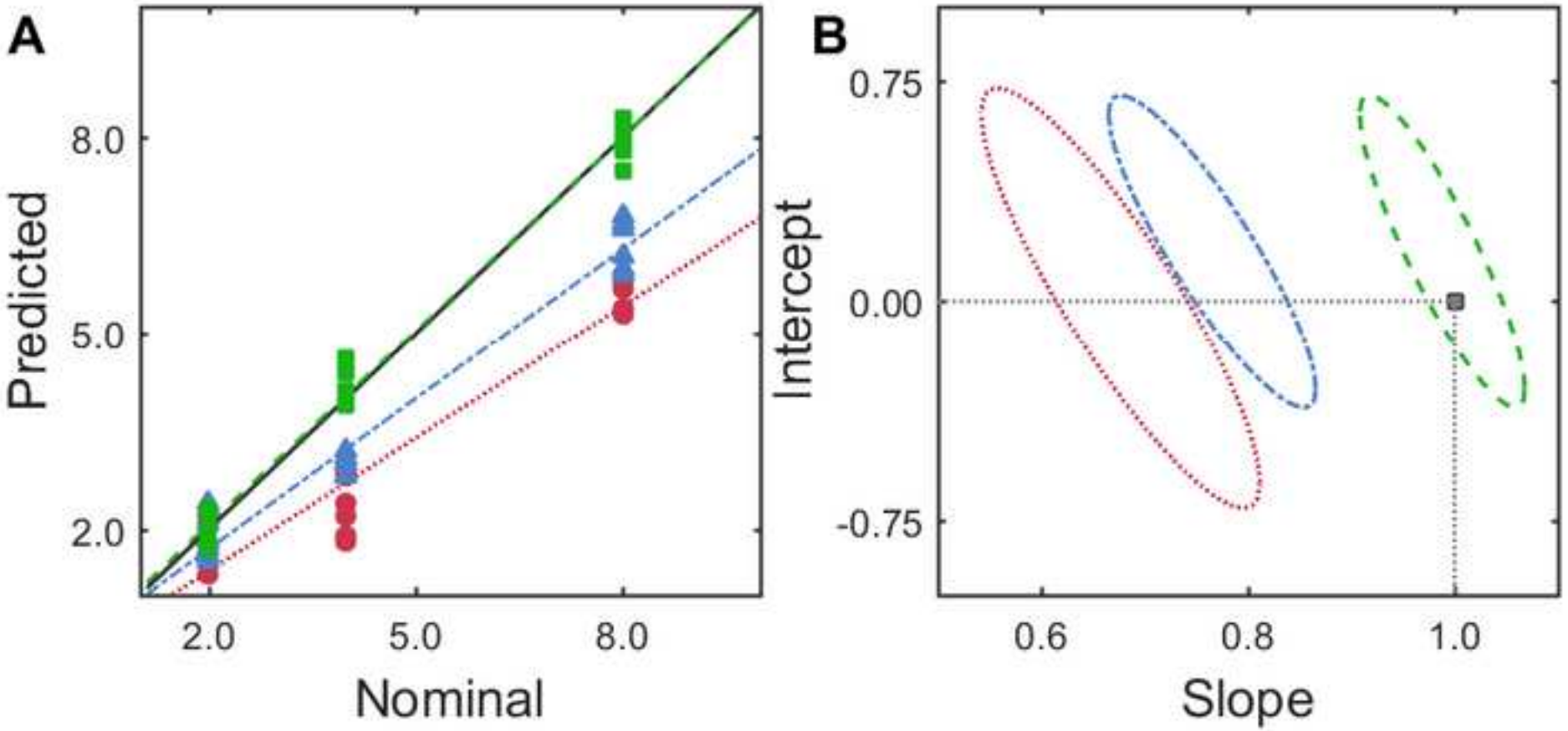

Exploring the potential of combining chemometric approaches to model non-linear multi-way data – A case study

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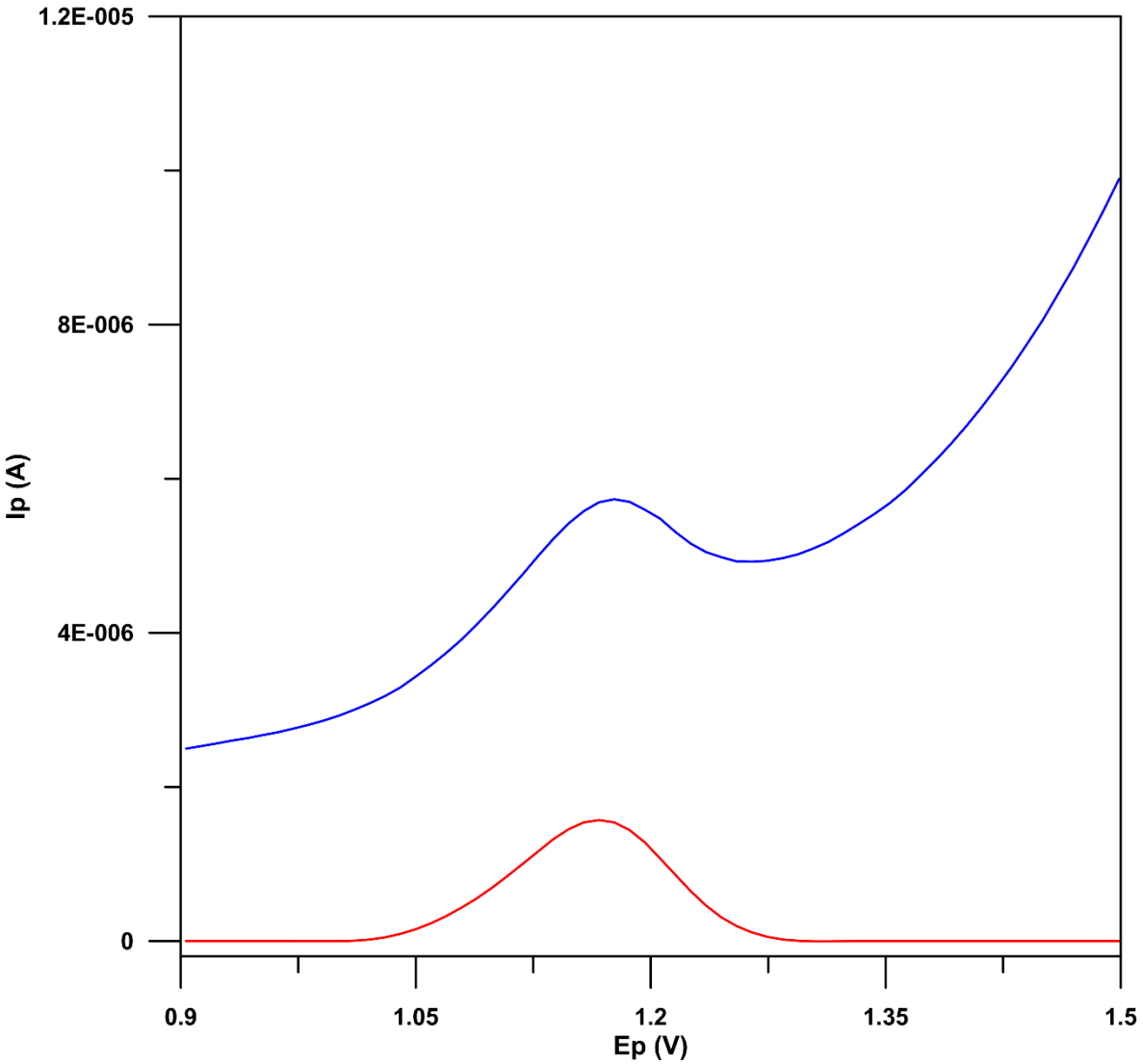

Figure S-1. Example of the baseline correction employing GPES. Voltammogram without any treatment in blue, and after baseline correction in red.

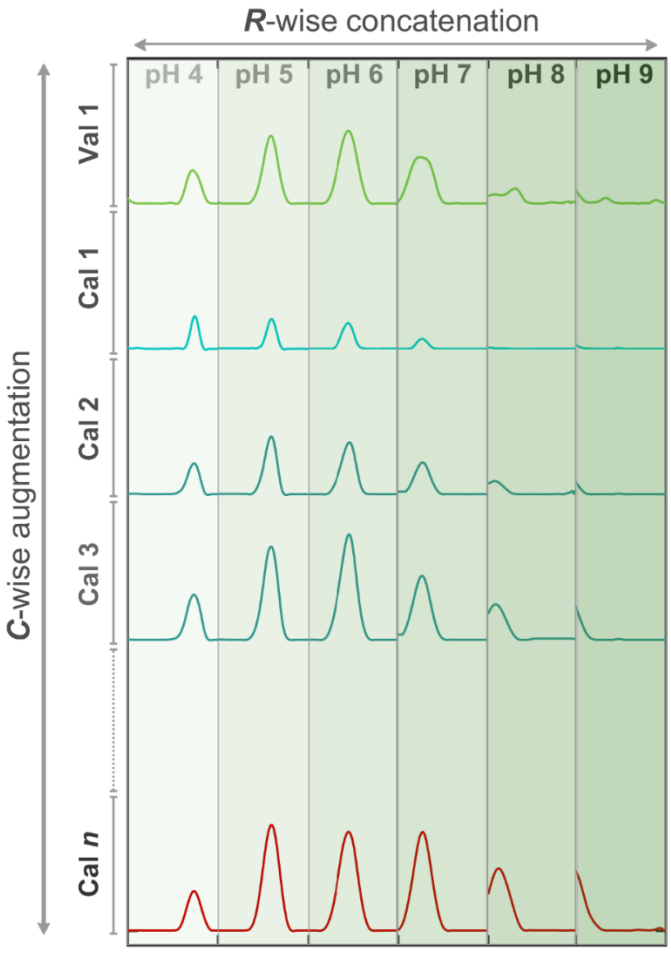

Figure S-2. Data arrangement for MCR-ALS resolution. Cal 1-*n* are de DPV-pH signals corresponding to the calibration samples and Val 1 is the corresponding signal of validation samples. (SUPLEMENTARIA?)