A new approach to multivariate standard addition based on multivariate curve resolution by alternating least squares: Application to voltammetric data.

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ABSTRACT

A multivariate version of the classical univariate standard addition method is proposed for the analysis of samples generating overlapping signals in the presence of notorious matrix effects. Unlike previous versions based on multivariate calibration by partial least squares (PLS), the proposed strategy takes advantage of a self-modelling methodology: multivariate curve resolution by alternating least squares (MCR-ALS) enhanced with signal shape constraints based on parametric functions. In this way, there is no need for the full multivariate response of a blank solution and, in multianalyte determinations, the standard additions can be made with a solution containing all the analytes, which constitutes a clear advance as compared to PLS approach. The proposed method has been successfully tested in the voltammetric determination of hydroquinone and catechol in solutions of increasing complexity and appears to be a promising tool in the field of electroanalysis.

Keywords: multivariate standard addition; multivariate curve resolution by alternating least squares (MCR-ALS); shape constraint; voltammetry; matrix effects.

INTRODUCTION

The typical calibration strategy in univariate instrumental analysis is the external standard method, which builds a calibration model (a straight line) with standard solutions to predict by interpolation the concentration of the analyte in unknown samples from their instrumental responses [1,2]. Nevertheless, this strategy fails in the presence of matrix effects, *i.e.* in samples with many components affecting the relationship between concentration and response. A solution for this may be to include the main components of the samples in the standard solutions (matrix matching), but this is not always possible. Then, an alternative approach is the standard addition method. It is based on the construction of a calibration model (again a straight line) with the responses of the sample before and after successive additions of a standard solution of the analyte. Then, the concentration of the analyte in the sample is predicted by extrapolation to a zero response value[1,2].

The standard addition method is particularly recommended for voltammetric measurements, which are especially sensitive to matrix effects[3]. Although voltammetric data are intrinsically multivariate, the heights or the areas of the peaks are usually taken instead of the full voltammograms to carry out univariate standard addition[3]. Moreover, if different analytes are present in the sample producing well-resolved signals, additions of a solution containing all analytes can be made and separated standard addition plots can be obtained for every analyte.

Unfortunately, voltammetric signals are frequently overlapping each other, which makes necessary to analyse the full voltammogram to guess the contribution of each analyte to the overall response. This can be done by means of different chemometric methods[4–7]. Among these, partial least squares (PLS) calibration is especially efficient and versatile. However, it is based on the methodology of external standard calibration, since standard solutions are prepared with different proportions of the analytes according to a convenient experimental design and are measured to build a calibration model that is further applied to the prediction of concentrations from the voltammograms of unknown samples. Nevertheless, PLS models only work properly if the relationship between concentration and response is linear and if all interfering substances integrating the matrix of the samples are equally present in the standard solutions. Small deviations from linearity can be compensated by a higher number of latent variables but severe non-linearity demands more sophisticated chemometric methods[5,7]. In the case of matrix effects, matrix matching is a good alternative, but it is not easy to synthetically reproduce the contents of interfering substances in the standards. Therefore, it would be very helpful that a multivariate version of the classical standard

addition method was available for these situations where the only way to ensure a reproducible matrix is by carrying out all measurements in the original sample with controlled additions of the standards.

As early as 1979, Kowalski and coworkers had already proposed a multivariate extension of the univariate standard addition method[8–10]. It was called generalised standard addition method (GSAM) and was successfully applied to data obtained by UV-vis spectrophotometry[11], inductively coupled plasma spectroscopy[12,13] and anodic stripping voltammetry[14]. GSAM method applies classical least-squares calibration (CLS) to a 'signal increase' matrix obtained by subtracting the spectra of the sample from the matrix containing all the spectra measured after successive additions of standards. Some years later, a PLS version of GSAM was also developed[15]. Although GSAM methodology was frequently used in the 1980's, its popularity decayed along the 1990's and the focus of multivariate standard addition moved to the study of second order data, mostly in spectroscopic measurements[16–19].

In 2012 Melucci and Locatelli[20] proposed an alternative strategy which, unlike GSAM, applies PLS to the original voltammograms obtained along the successive additions of standards, without subtraction of the voltammogram of the sample. Then, a calibration model is built to predict the relationship between voltammograms and added concentrations. Finally, the model is applied to a blank voltammogram (*i.e.*, measured in a solution containing the same matrix as before, but without analyte) to predict by extrapolation the concentration of the analyte in the sample. This PLS extrapolation approach was successfully tested in solutions containing Pb(II)-ions as the only electroactive species. Recently, our group extended this approach to multicomponent analysis[21]. However, such extended method still requires the extrapolation to the voltammogram of a blank, which can be easily acquired when working with spiked samples (the blank is just the non-spiked sample) but can be very difficult to obtain in real situations. In the case of stripping voltammetry, the blank can be reasonably estimated by carrying out the stripping scan without previous accumulation[22]. Another drawback of PLS methodology is that the additions of the different analytes have to be done separately to prevent that PLS confuses them in a single latent variable (if all analytes are added inside the same solution they act as a single analyte generating several overlapping peaks). This is not a great inconvenience but complicates the analysis since it involves many standard solutions and requires a longer time to carry out all the individual additions.

The goal of the present work is to overcome such problems by designing a new strategy of multivariate standard addition, which does not need neither blank signals nor alternate

additions of individual standards. For this purpose, PLS will be replaced by multivariate curve resolution by alternating least squares (MCR-ALS), a method developed by Tauler *et al.* for spectroscopic data[23–26] but with great potential in electroanalysis[7,27–29]. On the one hand, MCR-ALS allows one to obtain the concentration profiles of all the analytes, which transforms the multivariate problem (a full voltammogram for every addition) into a set of univariate problems (the concentration of every analyte after each addition) that can be easily solved using the classical univariate standard addition method. On the other hand, the shape constraint of MCR-ALS takes advantage of the well-defined shape of the analytes (usually a peak) to distinguish them even when added with the same solution. For comparison purposes, we have used in this work the same analytes, hydroquinone and catechol, previously considered by our group in calibration studies by PLS[21,30].

THEORY

Figure 1 summarizes the scheme of MCR-ALS adapted to standard addition. Matrix **R** contains the voltammograms measured in the sample before (first row) and after successive additions of a solution containing known concentrations of all analytes (second and successive rows). MCR-ALS consists on the decomposition of **R** into a product of two matrices: **C**, which contains the concentration of the analytes (in columns), and $\mathbf{S}^T$ (*i.e.*, transposed **S** matrix) that includes the pure responses of all analytes (in rows):

$$\mathbf{R} = \mathbf{C} \cdot \mathbf{S}^T \quad (1)$$

If the only contributions to the overall response were these of the analytes, a classical least squares calibration (CLS) could be done. Then, a matrix containing a prediction of the concentration, $\mathbf{C}_{pred}$, could be obtained in a single operation by dividing **R** by a $\mathbf{S}^T$ matrix constructed with experimental measurements of every analyte alone:

$$\mathbf{C}_{pred} = \mathbf{R} \cdot (\mathbf{S}^T)^+ \quad (2)$$

where '+' indicates pseudoinverse matrix. Then, for every analyte, the plot of $\mathbf{C}_{pred}$ as a function of the added concentration would generate a linear plot that could be extrapolated in the same way as in classical standard addition method to determine the original concentration of the analyte in the sample.

Nevertheless, when the complexity of the samples demands the standard addition method, there are far more contributions to the response than these of the analytes and this greatly

decreases the accuracy of CLS[4–6]. In contrast, MCR-ALS does not work only with known species, but with components that also take into account the contribution of unknown species and electrochemical processes. In this case, **C** and $\mathbf{S}^T$ matrices are obtained from Eqn. (1) through an iterative process, called alternating least squares (ALS):

$$\mathbf{C} = \mathbf{R} \cdot (\mathbf{S}^T)^+ \quad (3a)$$

$$\mathbf{S}^T = (\mathbf{C})^+ \cdot \mathbf{R} \quad (3b)$$

Starting from a rough estimation of the pure spectra $\mathbf{S}^T$, the cycle of Eqns. 3a and 3b is repeated several times until the reproduced matrix (the product $\mathbf{C}\cdot\mathbf{S}^T$) gets as close as possible to the experimental matrix **R**. The similarity between the reproduced matrix and **R** matrix is evaluated by means of the percentage of lack of fit (lof):

$$lof = \sqrt{\frac{\sum_{ij}(x_{ij} - \hat{x}_{ij})^2}{\sum_{ij}(x_{ij})^2}} \text{ x } 100 \quad (4)$$

where x_{ij} and $\hat{x}_{ij}$ are the elements of the experimental and reproduced matrix, respectively. At this point, MCR is not too different from principal component analysis (PCA), which decomposes the **R** matrix as a product of a scores matrix **T** and a loadings matrix $\mathbf{P}^T$ based on a certain number of principal components. However, in PCA both scores and loadings are just mathematical entities without physical meaning, whereas in MCR-ALS different constraints (*e.g.*, non-negativity, selectivity, closure ...) are applied during the iterative process to confer physical meaning to **C** and $\mathbf{S}^T$ matrices as the concentration profiles and the pure signals, respectively[28].

Among such restrictions, the shape constraint is especially useful for the standard addition method. It consists on fitting the individual signals of the components (usually peak-shaped) to parametric functions such as the simple and symmetric Gaussian peak and the more complex and asymmetric double-Gaussian peak, asymmetric logistic peak, log power peak and exponentially modified Gaussian peak[7,31–33]. The Gaussian peak can also be used to provide estimations of the pure signals ($\mathbf{S}^T$) to start MCR-ALS iterations by means of the home-made program *peakmaker*[32]. These initial estimations and the application of the signal shape constraint along the iterative process ensure that MCR-ALS can distinguish the different analytes that are simultaneously added in the same proportion from a single standard solution.

When MCR-ALS is applied with the shape constraint, it generates a pair of optimal **C** and $\mathbf{S}^T$ matrices. For quantitative purposes, the most important is **C** matrix, which contains the

concentration profiles of every analyte (*i.e*., the evolution of its concentration along the whole addition process). Then, the linear plot of every column of **C** as a function of the added concentration of the corresponding analyte can be submitted to the same extrapolation process as in the classical standard addition method. Thus, MCR-ALS transforms the multivariate problem into a set of univariate problems easily manageable with the classical method.

Finally, it must be pointed out that the fitting of parametric peaks requires a flat background close to zero, which can be achieved by blank subtraction or, if the blank is not available, with an effective method of baseline correction. Alternatively, an intricate background can be included as an additional component in MCR-ALS modelled with an appropriate parametric function (*e.g.* an exponential increase) or free from any shape restriction.

EXPERIMENTAL

Reagents

Hydroquinone (HQ), catechol (CC), NaH_2PO_4 and NaH_2PO_4 were provided by Sigma-Aldrich (St. Louis, MO, USA). In all cases, analytical grade reagents were used and solutions were prepared with ultrapure water (Milli-Q plus 185 system, MilliporeSigma, Burlington, MA, USA).

Solutions of dihydroxybenzene isomers were prepared daily and stored in the dark at 4° C to prevent oxidation.

Instrumentation

Differential pulse voltammetric (DPV) measurements were carried out in a VA Stand 663 (Metrohm, Herisau, Switzerland) connected to a computer-controlled potentiostat – μAutolab Type III) with GPES version 4.9 data acquisition software (EcoChemie, Utrecht, The Netherlands).

Pt wire and Ag/AgCl/KCl (3 mol L^{-1}) were purchased from Metrohm and used as counter and reference electrodes respectively. Graphene modified screen-printed electrodes (SPGPHE) with a disk diameter of 4 mm (ref. 110GPH, DS SPCE) were acquired from Metrohm Dropsens (Oviedo, Spain) and used as working electrodes, coupled to the Autolab System by means of a flexible cable (ref. CAC, DropSens).

All voltammetric measurements were carried out under a purified nitrogen atmosphere (Linde N50) in a glass cell at room temperature (20 °C).

pH measurements were performed using a Crison micro pH 2000 (Hach Lange Spain, L'Hospitalet de Llobregat, Spain).

Voltammetric measurements

Differential pulse voltammograms (DPV) were recorded in 0.1 mol L^{-1} phosphate buffer at pH 7 from −0.2 V to 0.9 V applying a step potential of 5 mV, a pulse amplitude of 50 mV and a pulse time of 50 ms.

For multivariate standard addition curves either 0.1 mol L^{-1} phosphate buffer at pH 7 (synthetic samples) or tap water (natural samples) were spiked by appropriate concentrations of HQ and CC. Then, the sample was deaerated with pure nitrogen for 10 min prior to voltammetric measurement. Finally, five simultaneous additions of HQ and CC were carried out and the respective curves were recorded.

Voltammetric measurements

Data treatment was done inside Matlab® environment[34] by means of a modified version of the 'official' MCR-ALS program[35]. Baseline corrections were carried out according to automatic weighted least squares (AWLS), available in the package PLS_Toolbox by Eigenvector[36].

RESULTS AND DISCUSSION

Preliminary studies with simulated data

Initial studies to test the validity of the proposed multivariate standard addition method assisted by MCR-ALS were performed according to the scheme shown in Figure 1 with simulated data, in which two analytes with different overlapping degrees and concentration ratios were considered. Taking into account that the shape constraint is particularly important in this method, simulated data were analysed by MCR-ALS to select the most effective parametric function to be used in the shape constraint. As Figure 2a shows, asymmetric functions produced very good fittings but evidenced a clear trend to underestimate the contribution of small peaks overlapped to big ones (a significant part of the lower peak is understood as a 'queue' increasing the asymmetry of the higher peak) resulting in biased predicted concentrations. In contrast, the fitting of the fully symmetric Gaussian peak is not so

perfect, but it is much more robust in the presence of small peaks, which are better preserved from being 'absorbed' by bigger overlapping peaks (Figure 2b). This is why Gaussian peaks where selected for the shape constrains applied in the present work. The equation for such peaks is:

$$I = a \exp [- b (E - c)^2] \quad (5)$$

where I is the current, E is the potential and a, b, c are adjustable parameters related to the height, width and position of the peak, respectively.

The application of this symmetric Gaussian peak constraint in simulated data provides good adjustments, with a lack of fit between the original and reproduced data lower than 0.05 % in all considered cases, and with predicted concentrations identical to the expected ones.

Simultaneous determination of hydroquinone and catechol in synthetic samples

The applicability of the proposed multivariate standard addition method assisted by MCR-ALS was tested for the simultaneous voltammetric determination of HQ and CC, which are two isomers of dihydroxybenzene that usually coexist in the environment. The voltammetric determination of these two isomers is often problematic due to their close oxidation potential, which results in complete merged peaks when glassy carbon electrodes (GCE) are used. As it is reported in the literature[30,37], the use of carbon nanomaterial modified electrodes can partially overcome this situation, providing some discrimination between the oxidation potential of both peaks and resulting in partially overlapped peaks that allow the satisfactory determination of both analytes by the application of chemometric methods.

Therefore, in this work, SPGPHE was used for the simultaneous voltammetric determination of HQ and CC in 0.1 mol L^{-1} phosphate buffer at pH 7. Three different samples containing different HQ:CC ratios (1:1, 2:1 and 1:2) were considered as representative situations for the application of the presented multivariate standard addition method. As it can be seen in Figure 3a-c, the voltammetric signal obtained for each sample (thick black line) shows different prevalence of each peak depending on the considered HQ:CC ratio. Further simultaneous additions of HQ and CC were performed in each sample and voltammetric signals were recorded, observing a linear increase of the signals without changes in the overlapping degree between both peaks. For each sample, MCR-ALS was applied to the obtained signals considering both non-negativity and symmetric Gaussian peak shape constraints in order to obtain the predicted pure signals and associated concentrations of each isomer. Figure 3a-c

shows, for 1:1, 2:1 and 1:2 HQ:CC concentration ratios respectively, the good fitting achieved in all cases between the experimental data (black signals) and the reproduced data (blue signals) calculated from the pure signals and predicted concentrations. These good fittings can be also demonstrated by the low lack of fit provided by the three independent adjustments: 4.35, 3.76 and 3.87 % for 1:1, 2:1 and 1:2 HQ:CC concentration ratios respectively. The plotting of the predicted concentrations *vs.* the added concentrations (insets in Figure 3) followed by their extrapolation to zero allows the determination of HQ and CC concentrations in each sample. The obtained results for the three considered situations, which are summarized in Table 1, show a good accuracy of the predicted concentrations, as inferred by the low relative standard errors, demonstrating the suitability of the presented MCR-ALS strategy for the multivariate standard addition of HQ and CC. In comparison to previous multivariate standard addition method assisted by PLS[21], lower relative errors were achieved, with the additional advantage of a faster analysis provided by the simultaneous additions of analytes instead of the alternate additions required for PLS strategies.

Application to the analysis of tap water samples

At the view of the above results, the applicability of the proposed MCR-ALS multivariate standard addition method in samples with more complex matrices was assessed through the simultaneous voltammetric determination of HQ and CC in a spiked tap water sample using a SPGPHE. It must be pointed out that no pretreatment was applied to the sample, thus it contained notorious concentrations of ions like calcium, magnesium, carbonate and chloride (derived from the hardness of water and the processes in the water treatment plant) as well as traces of organic matter that can interact with the analytes considered. Although the complexity of the sample is not too high, noticeable matrix effects are expected taking into account for instance the differences observed in the behaviour of the river water samples studied in ref. 21. Voltammetric measurements under the above-mentioned conditions were performed including both the spiked tap water sample and five successive and simultaneous additions of HQ and CC. A triplicate of this experiment was carried out and representative experimental voltammograms obtained are shown in Figure 4 (black signals). As it can be observed, HQ and CC also present partially overlapped peaks that difficult their determination through the most common univariate standard addition method. The modelling by means of MCR-ALS provided reproduced signals (in blue) that successfully match the experimental ones, with a mean lack of fit of 5.72 % for the three considered replicates of the tap water sample.

The predicted concentrations for both HQ and CC are reported in Table 2 and, as it can be observed, good precisions and accuracies inferred by the low relative standard deviations (RSD %) and relative errors (%) respectively were achieved.

These good results confirm the applicability of the proposed multivariate standard addition method assisted by MCR-ALS, not only for samples with simple matrices but also for more complicated samples with matrix effect. Furthermore, this presented method can overcome the major limitations of other multivariate standard addition methods based on the use of PLS.

CONCLUSIONS

In this work a new multivariate standard addition method assisted by multivariate curve resolution by alternating least squares was developed for the simultaneous determination of analytes presenting overlapped peaks in complex matrices. This method presents some important advantages with respect to previously reported PLS strategies for the multivariate standard addition since it does not need neither blank signals, which are not always possible to obtain, nor alternate additions of individual standards, which unnecessarily increase the experimental time. In this case, both analytes can be added simultaneously thanks to the enhancement provided by the application of a peak shape constrain, being the symmetric Gaussian peak the parametric function that provides better predicted concentration. The application of MCR-ALS gives rise to predicted pure signals and concentration profiles after the application of non-negativity and the above mentioned signal shape constraint. The extrapolation to zero predicted concentration allows the determination of both considered analytes in the test samples. The applicability of this method has been successfully proved at three levels: (i) simulated data; (ii) simultaneous voltammetric determination of HQ and CC in synthetic samples at three different concentration ratios; and (iii) simultaneous voltammetric determination of HQ and CC in a spiked tap water sample. In all cases very good fittings and predictions were achieved, inferred by very low lack of fits (lower than 0.05 % and 6 % in simulated and experimental data respectively) and good relative errors lower than 9 %. Furthermore, this method also presented good precision in the analysis of the spiked tap water sample, with RSD lower than 9 % for both HQ and CC. Taking into account the promising features of the proposed methodology, further investigations should confirm its suitability for samples with matrices more complex than that of tap water, such as wastewaters, food products or beverages. Also, the effects of non-linearity should be investigated and, if

necessary, non-linear algorithms like these of artificial neural networks (ANN) or support vector machine (SVM) should be adapted to the strategy of multivariate standard addition.

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Table 1. Expected and predicted concentrations for the simultaneous determination of HQ and CC in 0.1 mol L^{-1} phosphate buffer pH 7 at different HQ:CC ratios by multivariate standard addition calibration method using a SPGPHE.

	Expected concentration (μmol L^{-1})	Predicted concentration (μmol L^{-1})	Relative error (%)
Sample 1			
HQ	4.50	4.59	2.0
CC	4.49	4.36	2.9
Sample 2			
HQ	9.14	9.92	8.5
CC	4.57	4.92	7.7
Sample 3			
HQ	4.57	4.33	5.3
CC	9.13	9.41	3.0

Table 2. Expected and predicted concentrations for the simultaneous determination of HQ and CC in a spiked tap water sample by multivariate standard addition calibration method using a SPGPHE.

	Expected concentration ($\mu mol\ L^{-1}$)	Predicted concentration ($\mu mol\ L^{-1}$)	Relative error (%)	RSD (%)
HQ	4.58	4.90	7.1	8.7
CC	9.15	9.10	0.6	8.6

n=3 for RSD(%)

Figure captions

Figure 1. Schematic representation of the proposed multivariate standard addition method based on MCR-ALS.

Figure 2. Comparison of the pure signals (red and blue lines) typically obtained in the MCR-ALS analysis of a voltammogram measured for a mixture of two analytes (black line) by using whether a) two asymmetric functions or b) two symmetric Gaussian functions. Currents are given in arbitrary units.

Figure 3. Experimental (black) and calculated (blue) voltammograms with corresponding calibration curves (inset) for the simultaneous determination of HQ and CC in 0.1 mol L^{-1} phosphate buffer at pH 7 using a SPGPHE in a solution containing 4.5 μmol L^{-1} HQ and CC (a), 9.1 μmol L^{-1} HQ and 4.6 μmol L^{-1} of CC (b) and 4.6 μmol L^{-1} of HQ and 9.1 μmol L^{-1} of HQ (c).

Figure 4. Experimental (black) and calculated (blue) voltammograms with corresponding calibration curves (inset) for the simultaneous determination of HQ and CC in a spiked tap water sample using a SPGPHE.

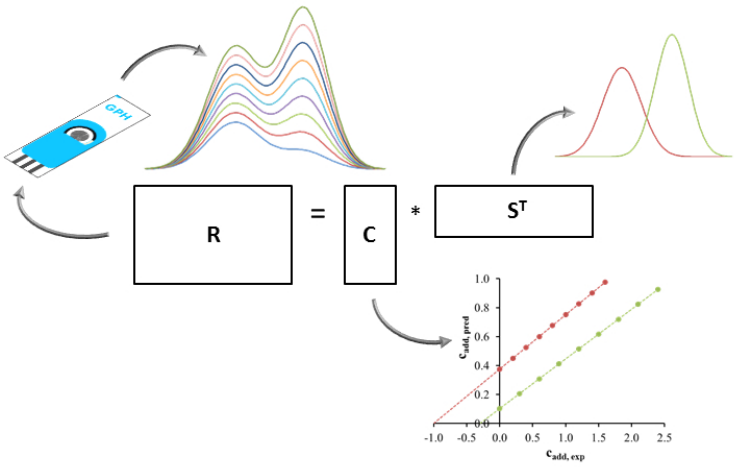

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Figure 1

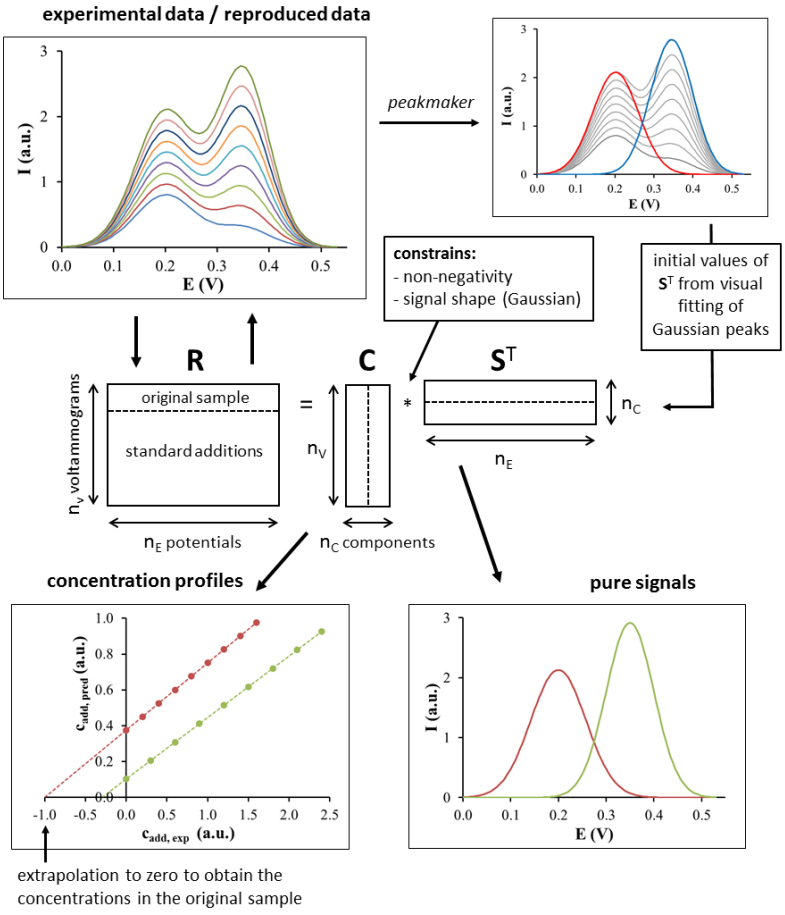

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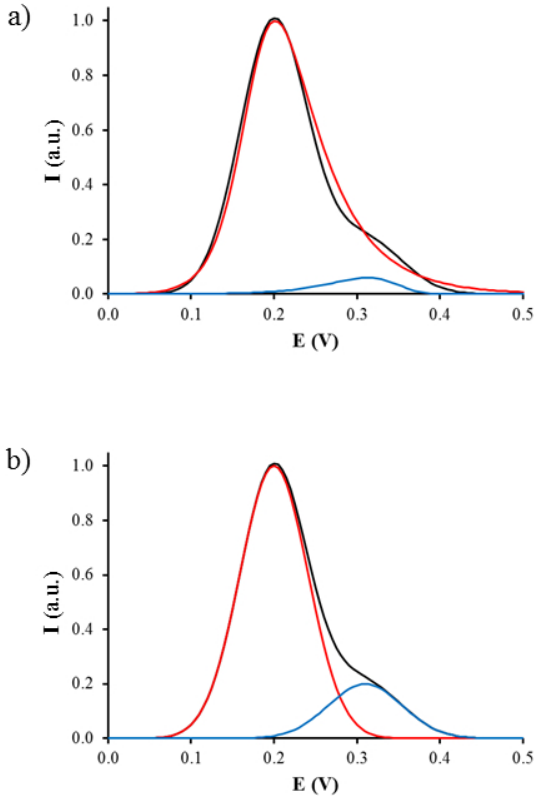
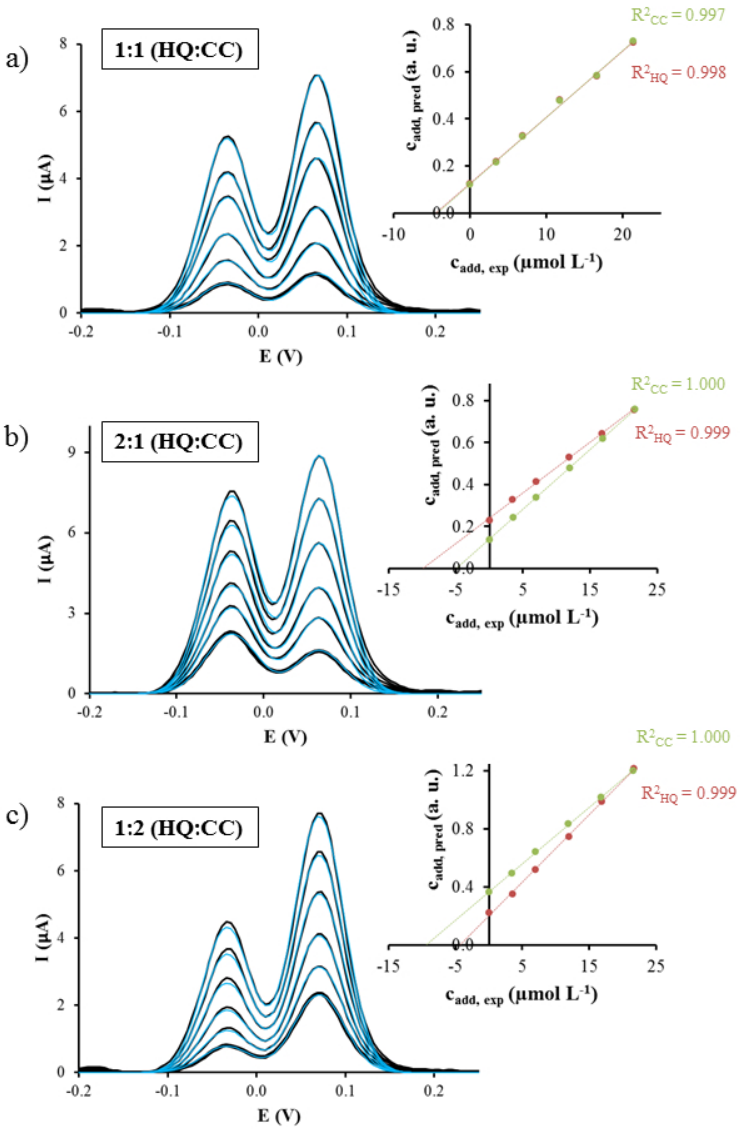
Figure 2



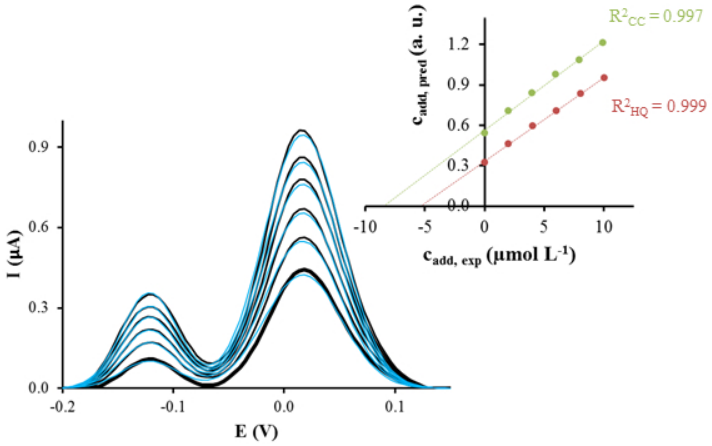
190x254mm (96 x 96 DPI)

Figure 3



190x254mm (96 x 96 DPI)

Figure 4



190x254mm (96 x 96 DPI)