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Spectrophotometric simultaneous determination of nitroaniline isomers by orthogonal signal correction–partial least squares

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Abstract

The simultaneous determination of nitroaniline isomer mixtures by using spectrophotometric methods is a difficult problem in analytical chemistry, due to spectral interferences. By multivariate calibration methods, such as partial least squares (PLS), it is possible to obtain a model adjusted to the concentration values of the mixtures used in the calibration range. Orthogonal signal correction (OSC) is a preprocessing technique used for removes the information unrelated to the target variables based on constrained principal component analysis. OSC is a suitable preprocessing method for partial least squares calibration of mixtures without loss of prediction capacity using spectrophotometric method. In this study, the calibration model is based on absorption spectra in the 200–500 nm range for 21 different mixtures of nitroaniline isomers. Calibration matrices were containing 1–21, 1–15 and 1–18 $\mu g\,ml^{-1}$ of *m*-nitroaniline, *o*-nitroaniline and *p*-nitroaniline, respectively. The RMSEP for *m*-nitroaniline, *o*-nitroaniline and *p*-nitroaniline with OSC and without OSC were 0.6567, 0.2692, and 0.3134, and 1.3818, 1.2181, and 0.3953, respectively. This procedure allows the simultaneous determination of nitroaniline isomers in real matrix samples and good reliability of the determination was proved.

Keywords: Nitroaniline; Determination; Partial least squares; Orthogonal signal correction

1. Introduction

Aniline and its substituted derivatives (*meta*, *ortho* and *para*-nitroanilines) are widely produced for a variety of industrial and commercial purposes, including dyestuff (as intermediates) and pesticide manufacturing. Since these compounds have significant water solubility, they are often present in wastewater discharges from such manufacturing facilities. The toxic nature of anilines dictates that their discharge concentrations be controlled, hence requiring the use of a reliable analytical method for determining anilines in such aqueous effluents and surface waters as rivers in the vicinity of the factories.

A variety of analytical methods have been reported for the determination of selected anilines. Such analytical techniques have been included: gas chromatography [1,2], high performance liquid chromatography [3], flow injection analysis [4], liquid chromatography [5], voltammetry [6–9] and spectrophotometry [10–13]. In general only the GC and HPLC approaches have been sufficiently sensitive and selective to detect anilines in complex environmental media. But a simple and rapid spectrophotometric method is demand for determination of these dangerous compounds in surface waters.

One of the main drawbacks of the application of spectrophotometric methods in the simultaneous determination of aniline derivatives is the high degree of spectral overlapping of these constituents. Nowadays combination of chemometrics methods with the computer-controlled instruments to monitor the molecular absorption spectra creates a powerful method in multicomponent analysis avoiding preliminary separation step [14].

The application of quantitative chemometrics methods, particularly partial least squares (PLS) to multivariate

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chemical data is becoming more widespread owing to the availability of digitized spectroscopic data and commercial software for laboratory computers. Each method needs a calibration step, where the relationship between the spectra and the component concentration is deduced from a set of reference samples, followed by a prediction step in which the results of the calibration are used to determine the component concentrations from the sample spectrum. The basic concept of PLS regression was originally developed by Wold [15,16] and application of PLS in spectrometry have been discussed by several workers [17–20]. In addition, several multicomponent determinations based on the application of these methods to spectrophotometric data have been reported [21–27].

Orthogonal signal correction (OSC) was introduced by Wold et al. [28] to remove systematic variation from the response matrix X that is unrelated, or orthogonal, to the property matrix Y. Therefore, one can be certain that important information regarding the analyte is retained. Since then, several groups [29–35] have published various OSC algorithms in an attempt to reduce model complexity by removing orthogonal components from the signal.

This paper describes an analytical methodology for simultaneous determination of nitroaniline isomers using spectrophotometric method and a multivariate calibration technique (partial least squares) with preprocessing by orthogonal signal correction. The aim of this work is to propose orthogonal signal correction–partial least squares (OSC–PLS) method to resolve ternary mixtures of nitroaniline isomers in river, tap and waste waters without prior separation. To our knowledge this is the first spectrophotometric report on the direct determination of nitroanilines, without any primary chemical reaction, like diazotization, or separating steps.

Generally for the evaluation of the predictive ability of a multivariate calibration model, the root mean square error of prediction (RMSEP) and relative standard error of prediction (RSEP) can be used [36]:

$$\mathrm{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n}(y_{\mathrm{pred}} - y_{\mathrm{obs}})^2}{n}}$$

$$\mathrm{RSEP}\,(\%) = 100 \times \sqrt{\frac{\sum_{i=1}^{n}(y_{\mathrm{pred}} - y_{\mathrm{obs}})^2}{\sum (y_{\mathrm{obs}})^2}}$$

where y_{pred} is the predicted concentration in the sample, y_{obs} the observed value of the concentration in the sample and n the number of samples in the validation set.

2. Experimental

2.1. Reagents and standard solutions

All the chemicals used were of analytical reagent grade, sub-boiling, distilled water was used throughout. Stock solutions of nitroaniline isomers were purchased from Fluka. Standards of working solution were made by appropriate dilution daily as required. A universal buffer solution (pH 7.0) was prepared by Ref. [37].

2.2. Instrumentation and software

A Hewlett-Packard 8453 diode array spectrophotometer controlled by a Hewlett-Packard computer and equipped with a 1 cm pathlength quartz cell was used for UV–vis spectra acquisition. Data acquisition between 200 and 500 nm were performed with UV–vis ChemStation program (Agilent Technologies), running under Windows XP. A Metrohm 692 pH-meter furnished with a combined glass-saturated calomel electrode was calibrated with at least two buffer solutions at pH 3.00 and 9.00.

The data were treated in an AMD 2000 XP (256 Mb RAM) microcomputer using MATLAB software, version 6.5 (The MathWorks). OSC and PLS calculus were carried out in the 'PLS Toolbox', version 2.0 (Eigenvectors Company).

2.3. Procedure

2.3.1. Standard calibration set

A mixture design was used to maximize statistically the information content in the spectra [38–40]. A training set of 21 samples was taken (Table 1). The concentrations of *m*-nitroaniline, *o*-nitroaniline and *p*-nitroaniline were varied between 1.0–21.0, 1.0–15.0 and 1.0–18.0 $\mu g\,ml^{-1}$, respectively. The mixed standard solutions were placed in a 10 ml volumetric flask and completed to the final volume with deionized water (final pH 7.0). The absorption spectra were recorded between 200 and 500 nm against a blank of universal

Table 1
Concentration data of the different mixtures used in the calibration set for the determination of nitroaniline isomers

Mixture	Meta	Ortho	Para
M1	1	1	18
M2	5	1	14.6
M3	9	1	11.2
M4	13	1	7.8
M5	17	1	4.4
M6	21	1	1
M7	17	3.8	1
M8	13	6.6	1
M9	9	9.4	1
M10	5	12.2	1
M11	1	15	1
M12	1	12.2	4.4
M13	1	9.4	7.8
M14	1	6.6	11.2
M15	1	3.8	14.6
M16	5	3.8	11.2
M17	9	3.8	7.8
M18	13	3.8	4.4
M19	5	6.6	7.8
M20	9	6.6	4.4
M21	5	9.4	4.4

Unit: $\mu g\,ml^{-1}$.

buffer. The spectral region between 200 and 500 nm, which implies working with 301 experimental points per spectra (as the spectra are digitized each 1.0 nm), was selected for analysis, because this is the zone with the maximum spectral information from the mixture components of interest. All absorption data are preprocessed by standard meancentring and scaling.

2.3.2. Prediction set and analysis of real samples

For prediction set, seven mixtures prepared, that were not included in the previous set were employed as an independent test (Table 2). The real samples in this study were collected in surface waters from Tag-e-Bostan (Fontal water), from Gar-e-Soo (waste water). The range concentrations were added to be 1.0–21.0, 1.0–15.0 and 1.0–18.0 μg ml^{-1} for *m*-nitroaniline, *o*-nitroaniline and *p*-nitroaniline, respectively.

3. Results and discussion

3.1. Selection of the optimum chemical conditions

Fig. 1 shows the absorption spectra in aqueous solution of individual nitroaniline isomers at pH 7.0. With the aim of investigation the possibility of determining nitroaniline isomers in mixtures, the optimum working conditions were studied under the conditions previously established for each nitroaniline isomers. A universal buffer solution of pH 7.0 was selected. In order to select the optimum pH value at which the minimum overlap occurs, influences of the pH of the medium on the absorption spectra of nitroaniline isomers were studied over the pH range 4.0–10.0.

Individual calibration curves were constructed with several points (Fig. 2), as absorbance versus nitroaniline isomers concentration in the range 1.0–21.0, 1.0–15.0 and 1.0–18.0 μg ml^{-1} for *m*-nitroaniline, *o*-nitroaniline and *p*-nitroaniline, respectively. The wavelengths used to generated calibration curves were 251, 225 and 381 nm for *m*-nitroaniline, *o*-nitroaniline and *p*-nitroaniline, respectively.

Table 2
Added and found results of synthetic mixtures of nitroaniline isomers

Added			Found			Recovery (%)		
Meta	Ortho	Para	Meta	Ortho	Para	Meta	Ortho	Para
By PLS method								
4.0	1.5	15.0	4.66	1.12	14.75	116.5	74.7	98.3
16.0	1.2	4.0	16.02	1.27	4.91	100.1	105.8	122.8
12.0	6.8	1.2	12.23	6.78	1.43	101.9	99.7	119.2
10.5	3.0	7.2	10.43	3.27	7.22	104.3	109.0	100.3
9.0	6.0	4.5	8.75	6.52	4.71	97.2	108.7	104.7
7.0	5.0	8.5	10.56	1.87	8.82	150.9	37.4	103.8
2.3	11.0	4.0	2.67	11.31	4.06	116.1	102.8	101.5
By OSC–PLS method								
4.0	1.5	15.0	4.17	1.58	14.60	104.3	105.3	97.3
16.0	1.2	4.0	17.18	1.12	4.10	107.4	93.3	102.5
12.0	6.8	1.2	12.75	6.64	1.16	106.3	97.6	96.7
10.5	3.0	7.2	11.12	3.18	6.75	111.2	106.0	93.8
9.0	6.0	4.5	9.77	6.18	4.25	108.6	103.0	94.4
7.0	5.0	8.5	6.76	5.21	8.00	96.6	104.2	94.1
2.3	11.0	4.0	2.30	11.6	4.03	100.0	105.5	100.8

Unit: μg ml^{-1}.

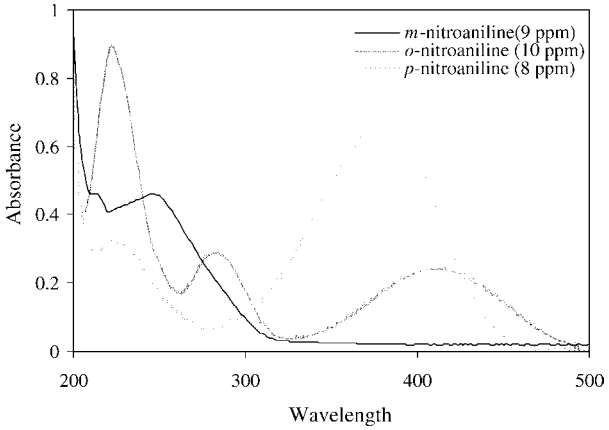

Fig. 1. Typical spectrum of the individual nitroaniline isomers at pH 7.0.

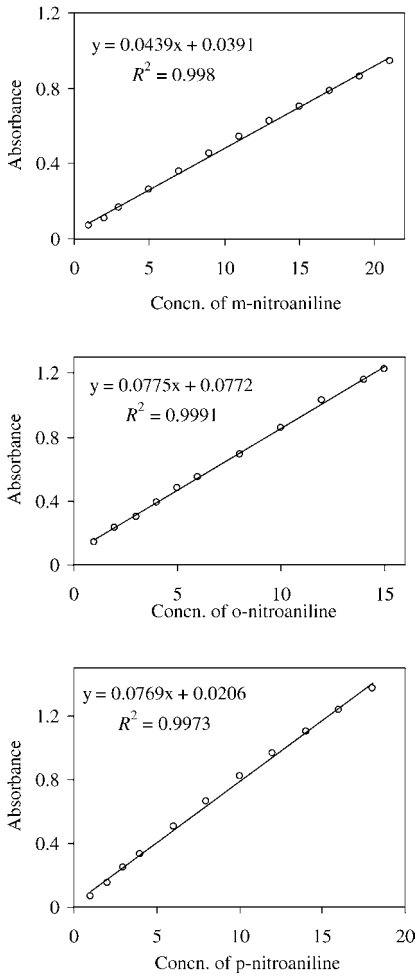

Fig. 2. Analytical curves for univariate determination of nitroaniline isomers.

Linear regression results, line equations and R^2 are shown in Fig. 2.

3.2. Calibration and validation

According to Section 2.3.1, the calibration matrix was designed. In Table 1, the compositions of the ternary mixtures used in the calibration matrices are summarized. For prediction set, seven mixtures were prepared according to Section 2.3.2 (see Table 2). To ensure that the prediction and real samples are in the subspace of training set, the score plot of first principal component versus second was sketched and all the samples are spanned with the training set scores.

3.3. Preprocessing by orthogonal signal correction

For calibration set three OSC components were used for filtering. Evaluation of the prediction errors for the validation set reveals that the OSC treated data give substantially lower RMSEP values than original data. Also, the OSC-filtered data give much simpler calibration models with fewer components than the ones based on original data. The results imply that the OSC method indeed removes information from UV–vis data that is not necessary for fitting of the *Y*-variables. In some cases the OSC method also removes non-linear relationships between *X* and *Y*. The score plots for the PLS and OSC–PLS are shown in Fig. 3. As score plots reveal the geometrical placement of the solutions in principal components space. The experimental noise can destroy this relation but by removing the noise using OSC filtering, the OSC–PLS score plots depicted in a more clear way the location of the solutions in the scores map which are the same as triangular experimental design was used in preparation of calibration and prediction solution sets.

3.4. Selection of the optimum number of factors

The optimum number of factors (latent variables) to be included in the calibration model was determined by computing the prediction error sum of squares (PRESS) for cross-validated models using a high number of factors (half the number of total standard + 1), which is defined as follows:

$$\text{PRESS} = \sum (y_i - \hat{y}_i)^2$$

where y_i is the reference concentration for the ith sample and $\hat{y}_i$ represents the estimated concentration. The cross-validation method employed was to eliminate only one sample at a time and then PLS calibrate the remaining standard spectra. By using this calibration the concentration of the sample, left out was predicted. This process was repeated until each standard had been left out once.

One reasonable choice for the optimum number of factors would be that number which yielded the minimum PRESS. Since there are a finite number of samples in the training set, in many cases the minimum PRESS value causes overfitting

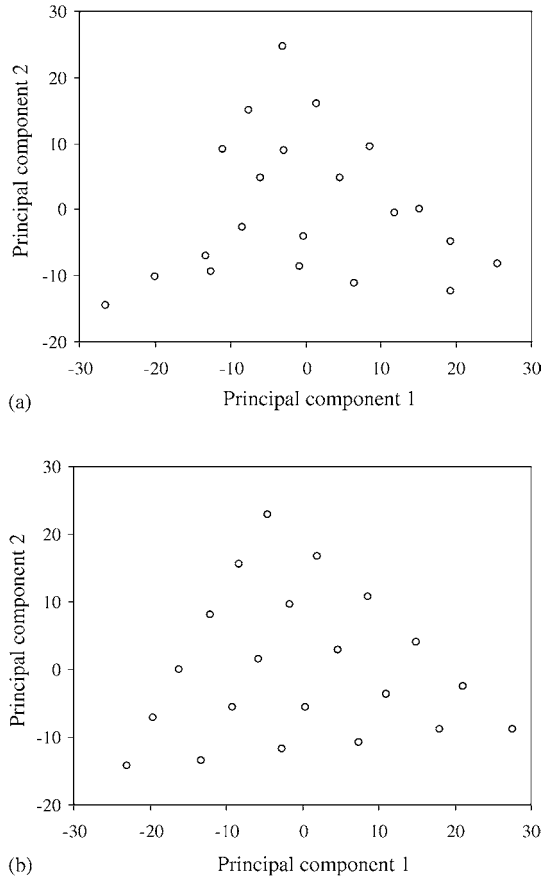

Fig. 3. Plots of first principal component against second principal component for nitroaniline isomers determination: (a) by PLS model, and (b) by OSC–PLS model.

for unknown samples that were not included in the model. A solution to this problem has been suggested by Haaland et al. [38] in which the PRESS values for all previous factors are compared to the PRESS value at the minimum. The *F*-statistical test can be used to determine the significance of PRESS values greater than the minimum.

The maximum number of factors used to calculate the optimum PRESS was selected as 11 and the optimum number of factors obtained by the application of PLS and OSC–PLS models are summarized in Table 3. In all instances, the number of factors for the first PRESS values whose *F*-ratio probability drops below 0.75 was selected as the optimum. In

Table 3
Statistical parameters of the optimized matrix using the OSC–PLS and PLS

Nitroaniline isomer	NPC[a]	PRESS	RMSEP	RSEP (%)
m-Nitroaniline[b]	4	0.0677	0.6567	6.7551
o-Nitroaniline[b]	4	0.0083	0.2692	4.5887
p-Nitroaniline[b]	4	0.0628	0.3134	4.1314
m-Nitroaniline[c]	4	0.8630	1.3818	14.2146
o-Nitroaniline[c]	4	0.7034	1.2181	20.7625
p-Nitroaniline[c]	4	0.6277	0.3953	5.2116

[a] Number of principal component.
[b] Using OSC–PLS.
[c] Using PLS.

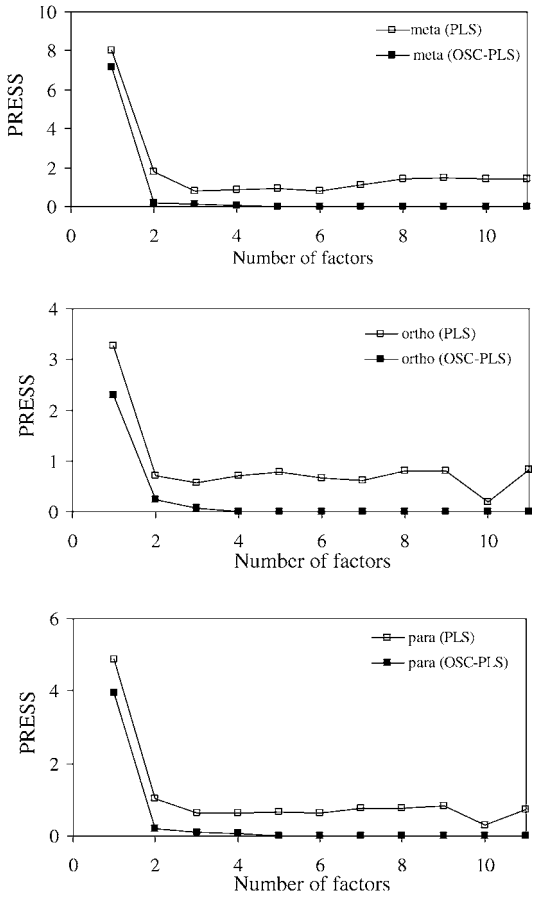

Fig. 4. Plots of PRESS vs. number of factors by PLS and OSC–PLS.

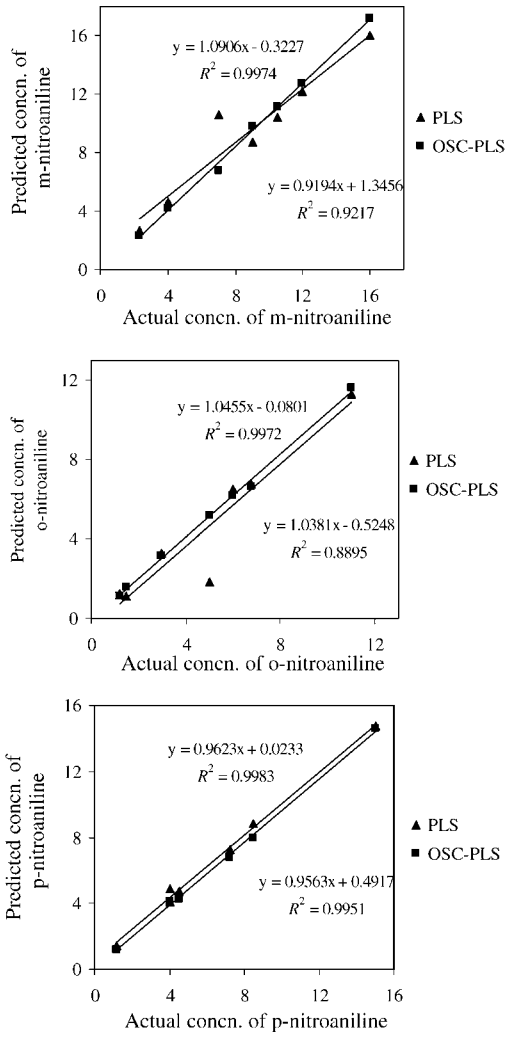

Fig. 5. Plots of predicted concentration vs actual concentration for nitroaniline isomers by PLS and OSC–PLS.

Fig. 4, the PRESS obtained by optimizing the calibration matrix of the absorbance data with PLS and OSC–PLS models is shown.

3.5. Determination of nitroaniline isomers in synthetic mixtures

The predictive ability of method was determined using seven three-component nitroaniline isomers mixtures (their compositions are given in Table 2). The results obtained by applying PLS and OSC–PLS algorithm to seven synthetic samples are listed in Table 2. Table 2 also shows the recovery for prediction series of nitroaniline isomers mixtures. As can be seen, the recovery was also quite acceptable. The root mean square error of prediction and relative standard error of prediction results are summarized in Table 3. The plots of the predicted concentration versus actual values are shown in Fig. 5 for nitroaniline isomers (line equations and R^2 values are also shown).

Table 4
OSC–PLS results applied on the real matrix samples

Type of water	Added			Found		
	Meta	Ortho	Para	Meta	Ortho	Para
Tap water	5.0	10.0	4.0	5.27 (2.42)[a]	9.55 (1.64)	3.99 (2.86)
Tap water	10.5	6.0	4.5	10.43 (1.42)	5.57 (2.13)	4.43 (2.42)
Tag-e-Bostan (frontal)	12.0	5.0	4.0	11.83 (1.21)	4.79 (2.06)	4.19 (2.75)
Gar-e-Soo (waste)	6.5	11.0	1.1	6.33 (2.06)	11.13 (1.08)	1.77 (3.36)

Unit: $\mu g\,ml^{-1}$.
[a] Value in parentheses are relative S.D. for $n = 3$.

3.6. Determination of nitroaniline isomers in real samples

In order to test the applicability and matrix interferences of the proposed method to the analysis of real samples, the method was applied in a variety of situations. For this purpose, diverse spiked samples and reference materials were analyzed. Table 4 shows the results obtained for real matrix samples. Therefore, the OSC–PLS model is able to predict the concentrations of each nitroaniline isomers in the real matrix sample.

4. Conclusion

The nitroaniline isomers mixture is an extremely difficult complex system due to the high spectral overlapping observed between the absorption spectra for their components. For overcoming the drawback of spectral interferences PLS multivariate calibration approaches are applied. In addition, the present study shows that the OSC can be a good method to remove systematic variation from the response matrix X that is unrelated, or orthogonal, to the property matrix Y. Therefore, one can be certain that important information regarding the analyte is retained. The good agreement clearly demonstrates the utility of this procedure for the simultaneous determination of nitroaniline isomers, without tedious pretreatment with complex samples in synthetic and natural water samples.

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