*Article*

# Flow-Injection Solid Phase Partial Least-Squares Spectrophotometric Simultaneous Determination of Iron, Nickel and Zinc

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Um método de calibração multivariada PLS-2 foi desenvolvido para a determinação simultânea de ferro, níquel e zinco em misturas ternárias utilizando a espectrofotometria em fase sólida acoplada à análise em fluxo. Fe(II), Ni(II) e Zn(II) formam complexos coloridos com 1-(2-tiazolilazo)-2-naftol (TAN), imobilizado em sílica funcionalizada C18 em pH 6,4. O procedimento proposto foi baseado nas diferentes razões de reação/retenção dos íons estudados sobre o suporte sólido. Os dados espectrofotométricos dos analitos, fixados no suporte sólido, foram obtidos entre 400 e 800 nm como função do tempo e um algoritmo utilizando o método dos mínimos quadrados parciais (PLS-2) foi usado para prever a concentração dessas espécies em amostras sintéticas. O lote de calibração empregado foi constituído por 8 misturas ternárias e um branco. Misturas contendo 0,040 a 0,20 mg L$^{-1}$, de cada espécie, foram resolvidas satisfatoriamente, usando 3 fatores para cada analito com dados espectrofotométricos obtidos em uma faixa de comprimento de onda entre 560 e 650 nm.

A PLS-2 multivariate calibration method has been developed for the simultaneous determination of iron, nickel and zinc in ternary mixtures by solid phase spectrophotometry associated with flow injection analysis. Fe(II), Ni(II) and Zn(II) form color complexes with 1-(2-thiazolylazo)-2-naphthol (TAN), immobilized on a C18 bonded silica support, at pH 6.4. The proposed procedure is based on the different reaction/retention ratios of the studied ions on the solid support. Bilinear spectrophotometric data of the analytes, fixed in the solid support, were recorded in the 400-800 nm wavelength range as a function of time and a partial least squares (PLS-2) algorithm was used to predict results of synthetic samples. The calibration set employed was integrated by 8 ternary mixture standards and a blank solution. Mixtures containing 0.040 to 0.20 mg L$^{-1}$, of each species, were successfully resolved, using 3 factors for each analyte and a restricted number of absorbance data obtained in the wavelength range from 560 to 650 nm.

**Keywords:** solid phase spectrophotometry, FIA, multicomponent determination, partial least-square analysis

## Introduction

Solid phase spectrophotometry (SPS) provides effective methods to combine metal ion preconcentration and their spectrophotometric determination, by integrating the formation of colored complexes and their fixation on the surface of a solid support placed inside the measurement cell.[1,2] The coupling between flow injection analysis (FIA) and SPS (FI-SPS) makes it unnecessary to reach equilibrium conditions, thus providing faster and more selective methods than those found in batch conditions. Additionally, it offers a simple and easy way of automation and provides low detection limits, small amounts of samples and reagents[3] being employed.

Since flow injection analysis was introduced, the simultaneous determination of several analytes in a same sample has been a challenge in order to make FIA competitive with other analytical techniques. In this way, the use of SPS and FIA has been applied in several studies for the determination of binary mixtures of metal ions,[4-7] amines[8] and mixtures of dyes.[9-11]

Approaches employed for multicomponent determination in solution can be also applied in FI-SPS.

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Thus, several processes, without a previous separation or masking steps, can be employed based on multiwavelength detection with mathematical data processing.

There are various mathematical algorithms and chemometric methods associated with the advanced analytical instrumentation that allow on-line multi-component analysis. These methods are based on calibration procedures, such as principal component regression (PCR)[12] and partial least-squares (PLS) which, in fact, have been employed in our research for the simultaneous determination of four carbamate pesticides[13] and five phenolic compounds.[14]

In the present work, 1-(2-thiazolylazo)-2-naphthol (TAN), immobilized on a C18 bonded silica support, was employed for the PLS spectrophotometric determination of ternary mixtures of Fe, Ni and Zn in water based in previous studies[3,15] which showed that Fe(II), Ni(II) and Zn(II) were retained on TAN-C18 forming complexes in the 5.5 to 8.0 pH range.

## Experimental

### Reagents and solutions

All solutions were prepared with analytical grade reagents and high purity de-mineralized water. Iron, nickel and zinc standard solutions (0.040 - 0.20 mg L$^{-1}$) were prepared by dilution of 1000 mg L$^{-1}$ stock ones. The buffer employed was 1.0 mol L$^{-1}$ hexamine with pH adjusted to 6.4 with HCl. TAN solution was prepared by dissolving 1.0 mg of 1-(2-thiazolylazo)-2-naphthol Merck (Darmstadt, Germany) in 1 mL of ethanol and completing the volume to 100 mL with a 1 % (w/v) Triton X-100 Panreac (Barcelona, Spain) solution. An HCl solution (0.5 mol L$^{-1}$) was prepared by proper dilution of concentrated acid. Hydroxylammonium chloride 1 % (w/v) was used to guarantee the total iron reduction to iron (II). TAN reagent was immobilized on-line in C18 bonded silica (60 - 100 $\mu$m) obtained from Waters Sep-Pak cartridges and placed inside the flow cell. De-mineralized water was used as the carrier stream.

The chromogenic reagent was immobilized on 35 mg of the solid support contained in the flow cell by pumping the TAN solution through the cell at 1.5 mL min$^{-1}$ during 5 min. Afterwards, the flow cell was washed with HCl and water to remove the excess of TAN and then it was inserted in the optical path of the spectrophotometer.

Standard solutions and synthetic mixtures of Fe(II), Ni(II) and Zn(II) were prepared by adding appropriate aliquots of the metal reference solutions to 50-mL volumetric flasks containing 5 mL of hexamine buffer and 5 mL of hydroxylammonium. The blank solution was prepared in de-mineralized water, containing buffer and the reducer.

### Apparatus and software

The set-up consisted of a Hewlett-Packard (Avondale, PA, USA) Model HP8452A diode array-spectrophotometer, equipped with HP89530A MS-DOS UV-visible software, and a home-made flow cell of 1.0 mm optical path and 78 mm$^2$ cross section.[3] A peristaltic pump Gilson Miniplus 2 (Villiers Le Bel, France) equipped with flexible poly-vinyl chloride tube was employed for carrier transport and a sliding bar commutator[16] was used for standard and sample injection. Loops and connections were made from PTFE tubes with 0.8 mm id.

Absorption spectra were recorded and processed with the help of the spectrophotometer software. Data were transferred to an ASCI format and processed using a PC/AT 486 personal computer equipped with a PLS software from NICOLET (Madison, USA).

### Flow diagram and procedure

The loop L, of the manifold depicted in Figure 1, was filled with HCl and simultaneously the sample solution was flowed through the analytical path at 2.0 mL mim$^{-1}$. Fe(II), Ni(II) and Zn(II), present in the samples, reacted with the chromogenic reagent immobilized on the C18 beads and these ions were retained on the solid support forming the respectively complexes. Simultaneously, spectrophoto-
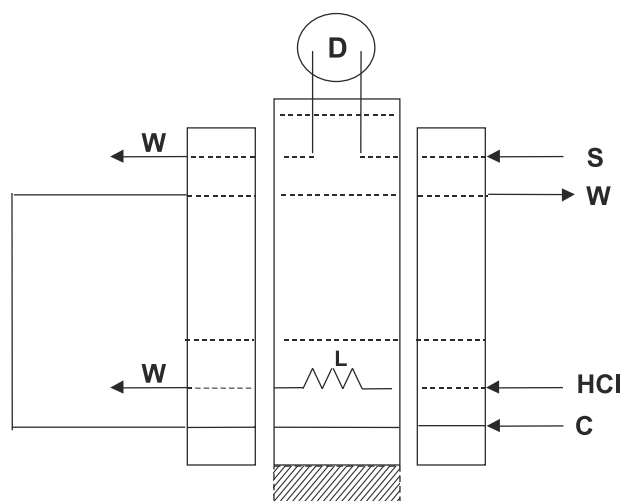


**Figure 1.** Set-up employed for FI-SPS determination of Fe(II), Ni(II) and Zn(II). S - sample (buffered at pH 6.4); C - carrier stream (water); L - HCl loop (625 mL); D - spectrophotometer equipped with a home-made flow cell; W - waste. 0.5 mol L$^{-1}$ HCl was employd as eluent.

metric measurements were carried out in the 400 - 800 nm wavelength range with a resolution of 2 nm using a scan acquisition time of 9 s in consecutive cycles of 10 s. After 100 s acquiring time of spectra, a 625 $\mu$L HCl volume was inserted in the carrier by sliding the commutator central bar, and transported towards the flow cell in order to provide the elution of the ions without removing the TAN chromogenic reagent. Data found for a calibration set (see Table 1) of 8 ternary mixtures of the ions studied, considered at two concentration levels, and a blank containing hexamine buffer and hydroxylammonium, were employed to obtain the PLS-2 parameters which were used in the prediction of the concentration of unknown samples.

**Table 1.** Composition of the calibration matrix employed for the PLS spectrophotometric determination of iron, nickel and zinc using FI-SPS.

| | Amount added/mg L$^{-1}$ | | |
| --- | --- | --- | --- |
| Standard | Fe | Ni | Zn |
| 1 | 0.040 | 0.040 | 0.040 |
| 2 | 0.040 | 0.040 | 0.20 |
| 3 | 0.040 | 0.20 | 0.040 |
| 4 | 0.20 | 0.040 | 0.040 |
| 5 | 0.040 | 0.20 | 0.20 |
| 6 | 0.20 | 0.20 | 0.040 |
| 7 | 0.20 | 0.040 | 0.20 |
| 8 | 0.20 | 0.20 | 0.20 |
| Blank | 0 | 0 | 0 |

## Results and Discussion

*Reaction between the metal ions and TAN-C18*

TAN, immobilized on a C18 support, was very stable in neutral and acidic solutions, allowing the use of the modified solid phase for more than 100 measurements without affecting the retention of analytes. Using 0.5 mol L$^{-1}$ HCl as eluent of the ions, the removal of the TAN reagent was not perceptible during the time of analysis.

Iron(II), nickel(II) and zinc(II) complexes with TAN, adsorbed on C18, present a red color and showed absorption maximum at 580 nm. However, as can be see in Figure 2, the spectra of all the complexes studied are highly overlapped, thus prohibiting the differentiation among Fe(II), Ni(II) and Zn(II) in samples containing mixtures of these elements. The effect of pH on the reaction/retention of Fe(II), Ni(II) and Zn(II) on C18-TAN was first studied within the 4.5 to 8.5 range. No significant variation in the retention efficiency was observed in the 6.4 to 7.8 pH range for Fe(II), 5.5 to 7.3 pH range for Ni(II), and in the 5.8 to 6.7 pH range for Zn(II). Thus, in further experiments an hexamine buffer solution at pH 6.4 was added to the samples.

Figure 3 shows the evolution, as a function of time, of the absorbance of each colored species at its maximum absorbance wavelength and, as it can be seen, the retention/reaction processes take place with different relative sensitivities and with the rate of the process being similar for Fe(II) and Ni(II) but different for Zn. In principle, this fact can be used to do the simultaneous determination of Fe(II), Ni(II) and Zn(II) in samples containing these ions, in order to take advantage of the differences of behavior of the three elements considered (see Figure 3).

*PLS-1 treatment of spectrophotometric data*

Mixtures of the three ions considered, at two concentration levels, were employed as the calibration set (see Table 1). 8 standards plus a blank solution were
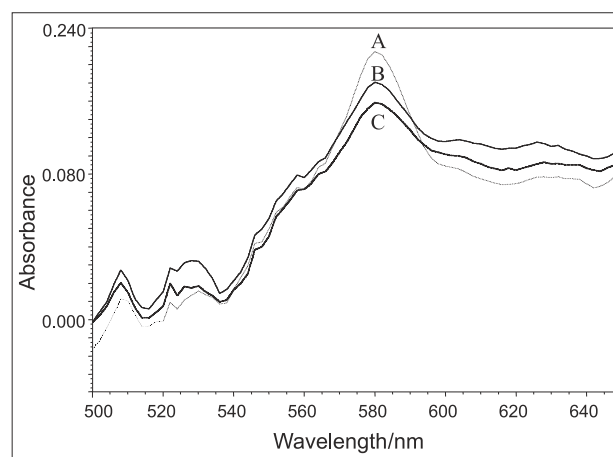


**Figure 2.** Absorbance spectra of complexes formed between TAN-C18 and studied ions at pH 6.4. (A) Zn(II), (B) Fe(II) and (C) Ni(II).
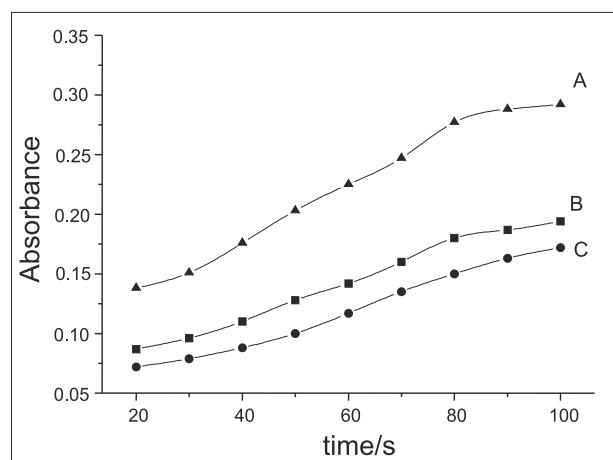


**Figure 3.** Absorbance signals at 580 nm obtained for the complexes formed with TAN-C18 as a function of time. (A) Zn(II), (B) Fe(II) and (C) Ni(II).

introduced into the cell and absorbance data were taken from 400 to 800 nm under wavelength intervals of 2 nm. Thus 201 points, which were measured every 10 s for a period of 100 s, were obtained for each solution. Spectral data found for each measurement time were mean centered and variance scaled and then treated by PLS-1. The evaluation of the model by cross-validation (see Table 2) showed difficulties to resolve correctly these ternary mixtures due to the overlap of the different ion spectra (see Figure 2). At the best conditions, corresponding to measurements carried-out after 30 s, PLS-1 provided excellent values for Fe(II) but mean errors higher than 10 % for Ni(II) and Zn(II). So, PLS-2 treatment was evaluated, by considering data found at different times, as an alternative to determine simultaneously Fe(II), Ni(II) and Zn(II) with immobilized TAN on C18 beads in spite of the strong overlapping between spectra of the complexes of the elements considered.

**Table 2.** Relative mean standard errors of prediction, as a function of the measurement time, found by cross validation of the calibration data employed for PLS-1 treatment of mixtures of Fe, Ni and Zn.

|  | Relative MEP (%) | | | |
| Time/s | Fe | Ni | Zn | Nr. of factors[*] |
| --- | --- | --- | --- | --- |
| 20 | 42.8 | 5.3 | 41.3 | 1, 2, 1 |
| 30 | 0.80 | 12.6 | 10.4 | 4, 2, 2 |
| 40 | 60.6 | 3.4 | 19.8 | 1, 3, 2 |
| 50 | 32.0 | 12.8 | 4.5 | 2, 4, 4 |
| 60 | 4.5 | 16.3 | 19.4 | 3, 3, 2 |
| 70 | 15.4 | 112.4 | 22.3 | 2, 1, 2 |
| 80 | 14.5 | 91.9 | 22.0 | 2, 1, 2 |
| 90 | 13.5 | 104.3 | 39.1 | 2, 1, 2 |
| 100 | 11.4 | 105.4 | 24.8 | 2, 1, 2 |

[*]Nr of factors corresponds to the best number of factors for data evaluation, by PLS-1, of mixtures of the three ions studied.

*PLS-2 treatment of spectrophotometric data*

After 20 s, injected sample solutions reached the flow cell providing significant retention of the analytes. Spectral data found, between this time and 100s at 10s intervals, were grouped (see Figure 4) obtaining new files containing 9 spectra in a sequential order. When spectra were collected from 400 to 800 nm wavelength range, 201 x 9 absorbance data points were obtained. These data were mean centered and variance scaled, being then treated by PLS.

Using the whole wavelength range, relative mean errors of prediction of 6.2 % for Fe(II), 29.8 % were found for Ni(II) and 2.2 % for Zn(II) for 3, 2 and 3 considered factors respectively. These results indicate some advantages offered by the simultaneous consideration of spectral data found at different measurement times, as compared with PLS-1 data treatment which provided mean error higher than 10 % for Ni(II) and Zn(II). Additional tests were carried

out to improve the obtained results using PLS-2 treatment of spectrophotometric data.

In order to select the best spectral range for PLS data evaluation, several restricted intervals were considered, by reducing from 201 to 46 absorbance points. Table 3 shows that the lowest mean errors obtained for the calibration set are those found between 560 and 650 nm, due probably to the high background of the solid support under 560 nm and above 650 nm.

The percentage of residual variance explained in the cross-validation process was evaluated for the concentration matrix and it was found that the use of 4 factors provided explained levels higher than 93% for the three elements considered. On the other hand, it was found (see Figure 5a) that the mean standard error of prediction (MEP) for the cross-validation process reached a minimum value for 4 factors in all cases. However Figure 5b shows that 3 factors
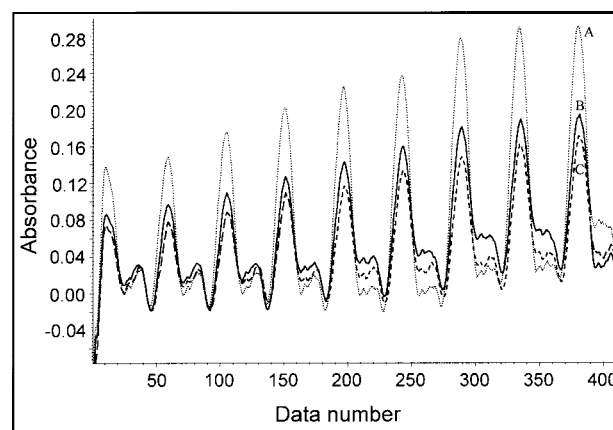


**Figure 4.** Absorption recording of data found for (A) Zn(II), (B) Fe(II) and (C) Ni(II) at concentration levels of 0.10 mg L$^{-1}$ measured 9 times in the range 560-650 nm. Each 46 points set corresponds to successive measurement times collect at intervals of 10s.

**Table 3.** Effect of the spectral range on the relative MEP obtained for the calibration set using PLS-2. Data were obtained by accumulating 9 scans in cycles of 10 s.

| | | Relative MEP | | | |
| Wavelength range/nm | No. of points | Fe | Ni | Zn | No. of factors* |
| --- | --- | --- | --- | --- | --- |
| 400-800 | 201 | 6.2 | 29.8 | 2.2 | 3, 2, 3 |
| 400-650 | 126 | 6.1 | 49.0 | 5.1 | 3, 2, 3 |
| 560-800 | 121 | 2.7 | 13.8 | 8.2 | 3, 2, 2 |
| 500-700 | 101 | 8.7 | 9.0 | 19.3 | 3, 3, 2 |
| 560-650 | 46 | 4.9 | 0.42 | 0.37 | 3, 3, 3 |

*The number of factors was chosen for each determination to obtain the minimum mean error of prediction (MEP) for the validation set. In each instance the three values given refer to iron, nickel and zinc respectively.

**Table 4.** Results obtained for the simultaneous determination of iron, nickel and zinc in synthetic mixtures by FI-SPS using PLS-2.

| Mixture | Amount added/ mg L$^{-1}$ | | | Amount found/ mg L$^{-1}$ (3 factors) | | | Amount found/ mg L$^{-1}$ (4 factors) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Fe | Ni | Zn | Fe | Ni | Zn | Fe | Ni | Zn |
| Sample 1 | 0.10 | 0.10 | 0.050 | 0.090 | 0.094 | 0.045 | 0.091 | 0.092 | 0.046 |
| Sample 2 | 0.080 | 0.040 | 0.040 | 0.086 | 0.036 | 0.038 | 0.086 | 0.038 | 0.037 |
| Sample 3 | 0.10 | 0.12 | 0.060 | 0.093 | 0.11 | 0.065 | 0.095 | 0.11 | 0.067 |
| Sample 4 | 0.050 | 0.080 | 0.080 | 0.054 | 0.080 | 0.069 | 0.056 | 0.078 | 0.070 |
| Sample 5 | 0.12 | 0.12 | 0.060 | 0.11 | 0.11 | 0.062 | 0.11 | 0.11 | 0.062 |
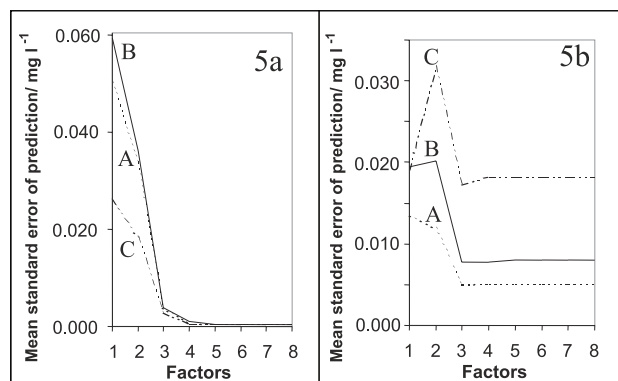


**Figure 5.** Mean standard error of prediction, expressed in mg L$^{-1}$, for each ion considered as a function of the number of factors: (A) Zn(II), (B) Fe(II), (C) Ni(II). 5a: cross-validation of the calibration set. 5b: prediction of the validation-set.

provided the lowest MEP´s for all the three ions considered in the case of concentration prediction for samples included in the validation set. So, from these facts, it was concluded that the use of 3 factors for Fe(II), Ni(II) and Zn(II) is enough to obtain accurate results in the analysis of water samples containing mixtures of these elements.

*Determination of Fe(II), Ni(II) and Zn(II) in water samples*

In order to test the performance of the proposed method, the aforementioned model was applied to predict the concentrations of the three ions in five synthetic mixtures, not employed in the calibration process. Results shown in Table 4 evidenced the ability of this method to obtain accurate results. By applying the t$_{95\%}$ paired test, it can be said that there is no significant difference between the obtained values using the proposed procedure and the amount added. On the other hand, as indicated before, the use of 3 factors provided the best results.

## Conclusions

Fe(II), Ni(II) and Zn(II) are retained on TAN immobilized in C18 bonded silica, forming complexes allowing *in situ* analyte separation and concentration of these ions from a water matrix.

The use of a multivariate calibration method, in the reduced range of 560-650 nm, in association with solid phase spectrophotometry and flow injection analysis, was appropriate for the determination of iron, nickel and zinc using a PLS-2 algorithm.

The method developed takes advantage of sensitivity and selectivity enhancements provided by the use of solid phase spectrophotometry and also of the benefits of multivariate calibration methods, *i.e.* the rapid determination of the analytes in a mixture without previous masking, preconcentration or separation steps.

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