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# Continuous flow titration system for the generation of multivariate spectrophotometric data in the study of acid–base equilibria ☆

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**Abstract**

In the present work a continuous flow system to carry out spectrophotometric titrations is developed. The titrant solution is generated on-line from mixing two different stock buffer solutions. The composition of the titrant agent is sequentially varied along the titration by changing the ratio of flow rates of both buffer channels, and therefore the pH can be modified. One spectrum is recorded at each pH value when the absorbance achieves the steady state. The spectral data have been treated by means of a recently developed self-modelling multivariate curve resolution method (SPFAC procedure). This method has been applied to the study of the acid–base equilibria of 1,2-naphthoquinone-4-sulfonate (NQS). Four titrations with concentrations of NQS ranging from $1.6 \times 10^{-4}$ to $7.3 \times 10^{-4}$ M have been performed, and, in every case, 24 spectra at different pH have been registered. Three species are detected in the range of pH studied (6.9–12.3). Their distribution plot with pH and their unit spectrum have been obtained.

*Keywords:* Flow system; Titrimetry; Chemometrics; Multivariate curve resolution; SPFAC procedure; 1,2-Naphthoquinone-4-sulfonate

## 1. Introduction

Several methods have been described in the literature to carry out titrations by using continuous flow methodologies [1–5]. These methods take profit of the advantages of flow systems such as the speed, reproducibility, high degree of automation and low expense of apparatus and reagents. Continuous flow titration manifolds are mostly based on the mixing of analyte and titrant solutions in a reaction coil followed by on-line detection. The analyte usually flows at a constant flow rate while that corresponding to the titrant solution is modified. In this way, parameters of the medium such as pH can be modified by generating a gradient along the titration when this titrant flow rate is varied. Several acid–base systems have been studied, although redox or complexation equilibria are also available. The recently reported flow injection titrations [6–9] are based on the injection of variable volumes of titrant into the system in order to obtain the corresponding titration curve. In both continuous flow and flow injection titration methods the detection of the process is usually per-

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formed by spectrophotometric or potentiometric techniques.

In this work, a continuous flow system for spectrophotometric titrations is developed. The titrant solution is generated on-line in the flow system from two different stock buffer solutions. During the titration, the ratio of flow rates of these buffers is sequentially modified. Therefore the concentration of sodium hydroxide of the titrant solution is varied at each step, and then the pH of the final solution. At each pH value, the corresponding spectrum is registered when the absorbance achieves the steady state. In this way, the set of spectra contains the information referent to the spectrophotometric changes along the titration. The treatment of these data by means of chemometric methods permits to extract chemical information about the analyte.

The method has been applied to the study of acid–base behaviour of 1,2-naphthoquinone-4-sulphonate (NQS). NQS is a reagent increasingly used for analytical determinations of amines and amino acids by using UV–visible spectroscopy [10–14]. One drawback of this substance is its poor stability in basic media where it progressively decomposes, and, as a consequence, its difficulty of being titrated by classical procedures. The continuous flow methodology proposed allows to titrate unstable reagents, overcoming their degradation with time by means of continuous renewing of the reagent solution, as it occurs in this work with NQS. An important remark of the present methodology with respect to other methods previously mentioned, is that the total flow rate as well as the analyte flow rate are maintained constant during the titration. This fact simplifies the mathematic data treatment since it does not require any correction due to the variation of the ratio between them.

The constituents and compositions of solutions used to buffer the chemical system permit stable, reproducible and controlled modifications of pH, which confers robustness to the procedure. One experimental limitation of classical titration methodologies consists of the difficulty to reproduce the same pH values for different titration runs and to get pH synchronization. This problem could be solved with a previous mathematical synchronization based on interpolation methods. However, interpolation errors must be considered in this case. This problem is solved using the proposed continuous flow system when solutions are sequentially titrated at the same pH values. On this basis, the spectrophotometric data generated will be synchronised, helping further mathematical calculations.

In recent years, several multivariate curve resolution methods have been developed [15–19]. In this work, the treatment of continuous flow titration data with a self modelling multivariate procedure (SPFAC) [20–22] has allowed the resolution of NQS acid–base equilibria, i.e., the estimation of the number of species present, their unit spectra and their distribution plot with pH. This methodology could be applied to the study of other reaction based systems such as redox, metal ion complexes, derivatization and others.

## 2. Experimental

### 2.1. Reagents

All chemicals were of analytical-reagent grade.

Sodium 1,2-naphthoquinone-4-sulphonate (NQS) (Aldrich) was used to prepare the analyte solutions. It was always kept in 0.1 M hydrochloric acid (Merck) in order to ensure their stability for at least two weeks.

The following two buffer stock solutions were used to generate on-line the titrant solution. Buffer solution B1 was 0.05 M $NaH_2PO_4$ + 0.05 M $H_3BO_3$ + 0.05 M $NaHCO_3$ + 0.05 M NaOH. Buffer solution B2 was 0.05 M $NaH_2PO_4$ + 0.05 M $H_3BO_3$ + 0.05 M $NaHCO_3$ + 0.35 M NaOH (all these chemicals were obtained from Merck).

The composition of stock buffer solutions B1 and B2 was adequate to neutralize the acidity of the analyte solutions and, at the same time, to provide different buffered media in a wide range of pH. Spectral characteristics of the chemicals used in these buffer solutions are suitable to work without spectrophotometric interference in the UV–visible range chosen (from 290 to 590 nm).

### 2.2. Apparatus

A Perkin Elmer UV/Vis/NIR Lambda 19 spectrophotometer with a Hellma flow cell (10 mm path-

length and 18 μl volume) was used. Spectra were collected and translated to JCAMP ASCII files by means of Perkin Elmer software. The pH was measured with an Orion ROSS combined glass electrode connected to a Radiometer PHM84 pHmeter.

### 2.3. Continuous flow system

The continuous flow manifold used to carry out the titrations (Fig. 1) is described as follows. Solutions were pumped through standard Tygon tubing by using two peristaltic pumps P1 and P2 (Scharlau HP4). PTFE tubing and PTFE standard T-pieces were used for all connections of the manifold. The titrant solution was formed on-line in a PTFE mixing coil MC1 (2 m × 0.5 mm i.d.) from two buffer solutions B1 and B2. The analyte solution merged with that of the titrant in another PTFE mixing coil, MC2 (10 m × 0.5 mm i.d.). Flow rates of analyte and titrant solutions were kept constant and equal to 0.73 ml/min while that of B1 and B2 buffer solutions were sequentially modified during the titration.

Fig. 2 shows the evolution of flow rates when the speed of P2 is varied. The flow rate of the titrant solution is the summation of the flow rates of B1 plus B2 and remains constant during the titration since an increase of P2 speed leads to an increase in B1 but a decrease in B2. In this way, the final pH was modified by changing the ratio of B1 and B2 solutions. For each particular titration the total concentration of analyte was kept constant because as it has already been mentioned its total flow was not modified. After each variation of P2 speed, the corresponding spectrum was registered when the absorbance values achieved the steady state. pH values were experimentally measured from the waste solutions emerging from the system.

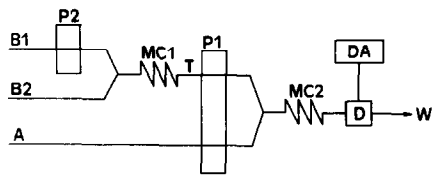


Fig. 1. Continuous flow manifold used for the spectrophotometric titrations. P1 = pump (constant speed); P2 = pump (variable speed); MC1 = mixing coil of 2 m × 0.5 mm i.d.; MC2 = mixing coil of 10 m × 0.5 mm i.d.; D = spectrophotometer; DA = data acquisition unit; B1 = buffer solution of 0.05 M $NaH_2PO_4$ + 0.05 M $H_3BO_3$ + 0.05 M $NaHCO_3$ + 0.05 M NaOH; B2 = buffer solution of 0.05 M $NaH_2PO_4$ + 0.05 M $H_3BO_3$ + 0.05 M $NaHCO_3$ + 0.35 M NaOH; T = titrant solution; A = NQS solution (in 0.1 M hydrochloric acid); W = waste. Flow rates: channel A = 0.73 ml/min; channel T = 0.73 ml/min; channel B1 = from 0.65 to 0 ml/min; channel B2 = from 0.08 to 0.73 ml/min.

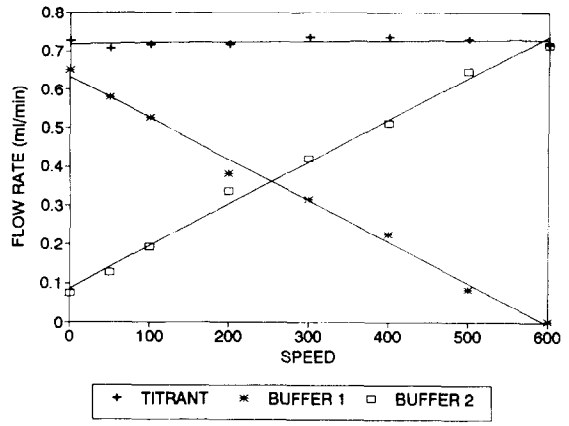


Fig. 2. Variation of flow rates of buffer B1, buffer B2 and titrant solutions with the speed of pump P2. Speed expressed in arbitrary units.

Using the continuous flow methodology proposed, solutions with different concentrations of analyte can be sequentially titrated by a simple replacement of the analyte solution which flows through channel A. This point has great importance because it guarantees to have the same pH values for all different solutions titrated in the same run (solutions are replaced before changing the pH).

### 2.4. Data treatment

Each individual continuous flow spectrophotometric titration provided a set of spectra registered at different pH values and ordered in a data matrix. Data treatment was done by means of MATLAB functions [23] and a MATLAB version of the SPFAC procedure [20]. SPFAC is aimed to the analysis of multivariate data to extract chemical information such as distribution plots with pH and unit spectra for every species present. SPFAC is a self-modelling procedure which does not require any model postulation apart from the number of species present. The whole treatment involves the following steps:

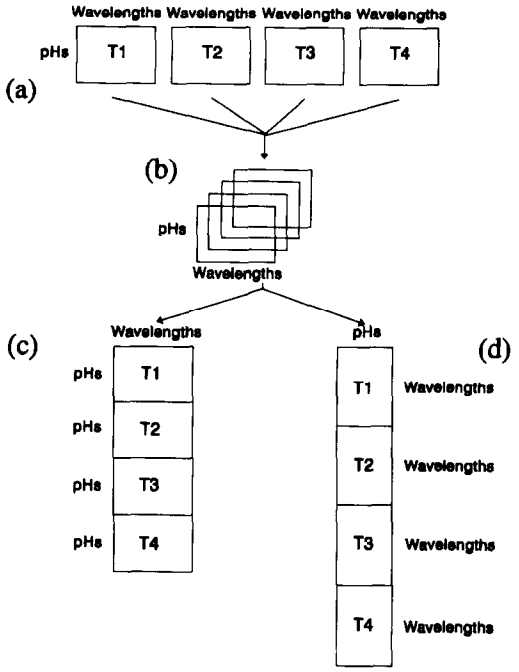


Fig. 3. Arrangements for spectra generated in the continuous flow titrations: (a) data matrices for individual titrations; (b) data arrangement keeping in common both wavelengths and pH values; (c) augmented data matrix on the wavelength direction; (d) augmented data matrix on the pH direction.

*Arrangement of data*

The spectra generated in each continuous flow titration were arranged in a data matrix **D** with a number of rows equal to the number of pH values and a number of columns equal to the number of wavelengths. The elements of the matrix are the absorbance values at each pH and wavelength (Fig. 3a). Simultaneous analysis of several titrations (Fig. 3b) was carried out setting the individual data matrices one in top of the others keeping in common the wavelengths (Fig. 3c) or the pH values (Fig. 3d).

*Determination of the number of species present in the system*

There are several methods based on principal component analysis (such as cross validation [24] and the theory of error in factor analysis [25]) which can be applied for this purpose. These techniques allow the estimation of the total number of factors including chemical species and non-chemical contributions such as instrumental drift, background noise and others. However, in general, the most important contribution to the variance is due to chemical species while the other contributions describe only a small percentage of the total variance. In many cases, the weight of these non-chemical factors can be minimized by a suitable experimental design, for instance, the instrumental drift can be avoided by subtracting a background signal before recording every spectrum. Another criterion for the determination of the number of independent species (chemical rank) is based on the experimental error, which can be estimated looking at an spectral region with no significative absorption from chemical species (the range from 550 to 590 nm in the present study).

Every chemical species is defined by two vector profiles: one concentration profile and one unit spectra profile. Principal component analysis is applied on both types of augmented matrices (Fig. 3c and d) in order to determine their chemical ranks. If the chemical ranks of these two augmented matrices are equal, the experimental data have a second order structure [26]. This involves that the unit spectra and the concentration profiles of common species in different titrations are equal. The equality in spectra is valid if there are not changes in physical properties such as solvent composition or temperature. The equality in the concentration profiles is achieved in the case of NQS acid–base equilibria since these profiles are independent of the total concentration of analyte.

*Obtaining an initial estimation of concentration profiles or unit spectra for each species*

Evolving factor analysis (EFA) [16] is an useful tool in the study of systems in evolution. EFA examines the changes in the magnitude of each principal component along the process on both forward and backward directions. According to the number of species previously deduced, the results obtained by means of EFA can be translated to an abstract representation of the concentration profiles of each species. In this work EFA has been applied to the spectrophotometric data in order to get an initial estimation of the concentration profiles which is used in the following alternating least squares procedure (ALS). ALS can also start using an esti-

mation of the unit spectra of each species. These unit spectra can be obtained by using EFA on the wavelength direction. However, if there are some selective pH zones in which mostly one species is present, the unit spectra are directly found from these zones. Fixed size moving window evolving factor analysis [17] is able to detect the one-species pH regions.

*Optimization of the concentration profiles and of the unit spectra by means of an alternating least squares procedure*

Iterative alternating least squares optimization (ALS) is applied on the compliance of Beer's law. ALS requires as input the experimental data matrix **D** obtained in the titration (or better the corresponding principal component noise-filtered reproduced data matrix [20]) and an initial estimation of the concentration profiles matrix **C** or of the unit spectra matrix **A**. In each iteration a new estimation of concentration and of spectra profiles are calculated from:

$$\mathbf{A} = \mathbf{C}^{+}\mathbf{D} \quad (1)$$

and

$$\mathbf{C} = \mathbf{D}\mathbf{A}^{+} \quad (2)$$

where $\mathbf{C}^{+}$ and $\mathbf{A}^{+}$ are the pseudoinverses of the matrices **C** and **A**, respectively. Several criteria are used to finish the iterative process: (a) when the fitting error in the reproduction of the data matrix **D** ($\mathbf{D} = \mathbf{AC}$) is lower than a certain value previously fixed; (b) when a certain number of iterations has been exceeded; or (c) when the iterations diverge for ten times consecutively.

Inherent to all curve resolution methods, final **C** and **A** matrices given by ALS can present ambiguity in both intensity and shape (rotational ambiguity) [19]. It has been shown, however, that this problem can be overcome when the system presents regions with selectivity for each species. Under selectivity conditions, the shape of the spectrum and of the concentration profiles is easily estimated.

A set of additional constraints can be applied in order to limit the number of possible solutions which eventually solve the ambiguity previously mentioned. In particular, for the NQS acid–base equilibria here studied:

(i) The concentration and unit spectra profiles are forced to be positive.

(ii) The shape of the concentration profiles is forced to be unimodal, which means that these profiles cannot have more than one peak maximum. This restriction is not applied to the unit spectra since the acid NQS species have two local absorption maxima (see Fig. 9b).

(iii) At each pH, the addition of the concentrations of all species is always constant during the titration and equal to the analytical concentration. This fact has great importance in the study of the NQS system since one of the species has a poor absorption in the spectral range used. Without this closure constraint the system keeps the intensity ambiguity.

Simultaneous analysis of several titrations helps to remove the ambiguity and improve the fitting [21]. The augmented data matrix of different titrations is built keeping in common the wavelength values. It is also possible to build the augmented data matrix keeping in common the pH values. An initial estimation of the augmented concentration matrix or of the unit spectra matrix is required. The augmented concentration matrix can be obtained from the estimations of the concentration profiles obtained in the individual analysis of each titration, arranged keeping species (columns) in common.

The ALS algorithm performs the calculations on the augmented matrices:

$$A = \begin{pmatrix} C_1 \\ C_2 \\ . \\ . \\ C_n \end{pmatrix}^{+} \begin{pmatrix} D_1 \\ D_2 \\ . \\ . \\ D_n \end{pmatrix}$$

and

$$\begin{pmatrix} C_1 \\ C_2 \\ . \\ . \\ C_n \end{pmatrix} = \begin{pmatrix} D_1 \\ D_2 \\ . \\ . \\ D_n \end{pmatrix} A^{+}$$

The presence of a second order data structure involves that common components have the same unit spectra and the same unit concentration profiles [26]. Under these circumstances all the ambiguity can be removed [19]. When the data have a second

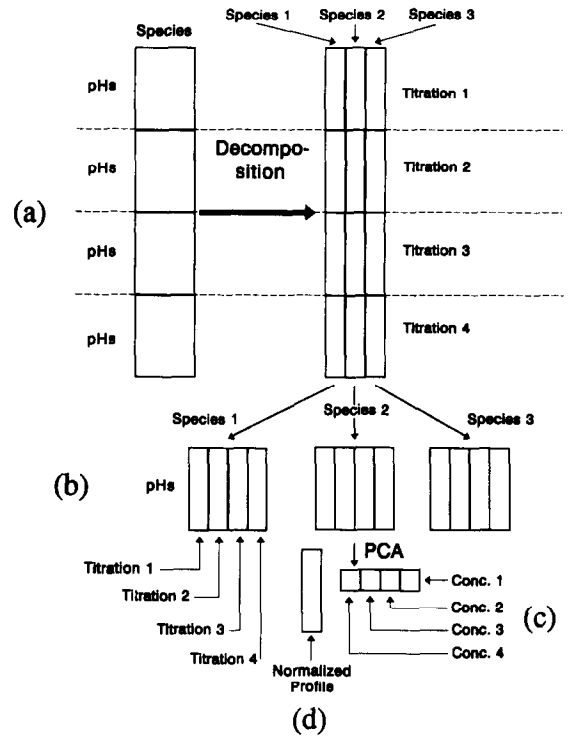


Fig. 4. Implementation of the second order constraint in the ALS algorithm.

order structure an additional constraint is forced. The way in which ALS implements this second order constraint is shown in Fig. 4. In this figure, the concentration profiles given by ALS for each species (a) are folded in a new set of matrices (b). Every one of these new matrices represents the concentration profiles for a given species in the different titrations. The principal component analysis of these matrices provides a set of loadings where the first one, suitably scaled by the corresponding scores (c), is assumed to be the concentration profile (d) input for the next iteration.

## 3. Results and discussion

The acid–base titrations were performed at four different concentrations of NQS: $1.66 \times 10^{-4}$, $3.32 \times 10^{-4}$, $4.97 \times 10^{-4}$ and $7.30 \times 10^{-4}$ M. The speed

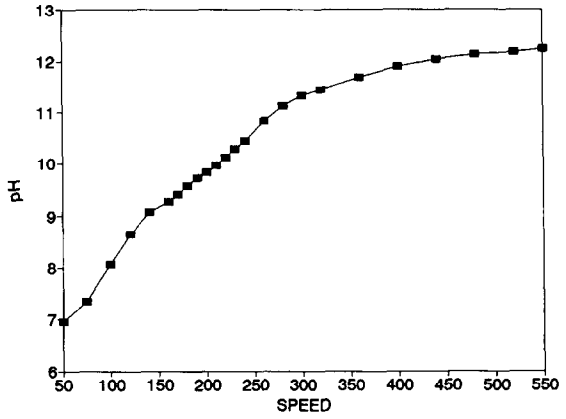


Fig. 5. Variation of pH with the speed of the pump P2. Speed expressed in arbitrary units.

of pump P2 was changed 24 times during each titration, giving 24 pH values from 6.96 to 12.24. At each pH, spectra corresponding to the four NQS solutions were registered. pH values were measured at the outlet of the system collecting small volumes of waste solutions. Fig. 5 shows the relationship between the speed of pump P2 and the experimental pH values. This Fig. 5 also indicates that the composition of the two stock buffer solutions is suitable to provide buffered media during the titration process since there are not any zones in which small speed variations produce high changes in the pH.

The NQS solution of $4.97 \times 10^{-4}$ M was taken as

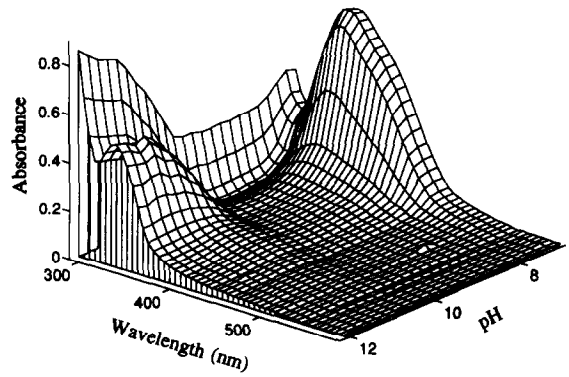


Fig. 6. Spectra obtained along an acid–base titration of a NQS solution $4.97 \times 10^{-4}$ M by using the continuous flow system of Fig. 1.

a model to develop the whole data treatment of an individual titration. Fig. 6 shows the three-dimensional plot of the experimental absorbance spectra obtained in this titration. The principal component analysis of this matrix indicates the presence of three principal components to describe almost all of the system and in consequence three chemical species are probably presents. Fig. 7a shows the eigenvalues obtained, and Fig. 7b the error in the reproduction of the experimental data matrix in absorbance units. This experimental error calculated over a non-absorbing spectral range helps to confirm the presence of these three species.

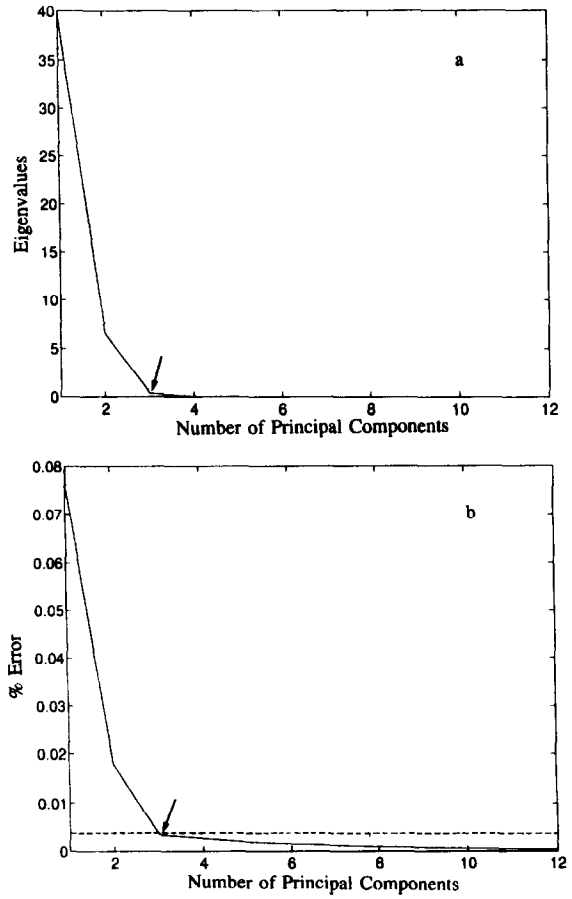


Fig. 7. Determination of the number of species present in the acid–base titration of a NQS solution $4.97\times10^{-4}$ M by using principal component analysis. (a) Eigenvalues plot. (b) Error in the reproduction of the experimental data matrix with the number of principal components considered. The dashed line corresponds to the limit of the experimental random error.

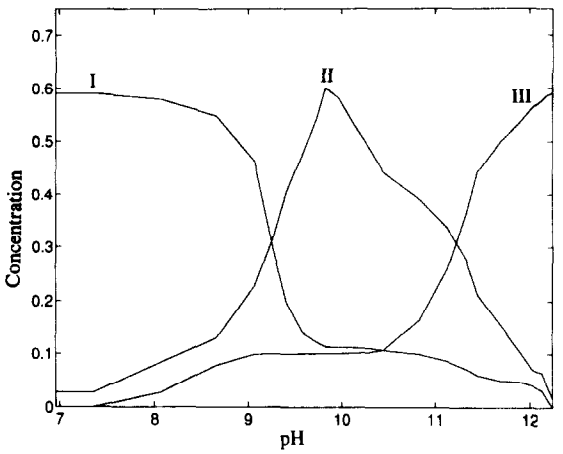


Fig. 8. Initial estimations of concentration profiles of NQS species with pH obtained using evolving factor analysis for the continuous flow titration of a NQS solution $4.97\times10^{-4}$ M. Concentration of species expressed in arbitrary units.

EFA is applied to the spectrophotometric data providing an initial estimation of the concentration profiles of the three species present noted as I, II and III (Fig. 8). The species I is the only one present in the first points of the titration (pH $<$ 7.5) as well as species III at the end (pH $>$ 11.5). Although species II overlaps with the two others, it predominates about pH 10. Fig. 9 shows the concentration profiles with pH and the unit spectra obtained by ALS.

Some possible acid–base species of quinonic compounds have been postulated in previous studies [27,28]. Fig. 10 shows the equilibria proposed for the NQS. On the basis of those studies, species II may be only an intermediary between species I and III, and, in consequence, it would be quite difficult to study it by conventional titration procedures. However, when using the continuous flow methodology proposed in this work, the different species formed on-line rapidly flow to the detector, and the results indicate that in this case, species II can be studied. This scheme of Fig. 10 agrees with the experimental results obtained from ALS since species II has lower conjugation with respect to species I and III and in consequence its spectrum presents low absorption in

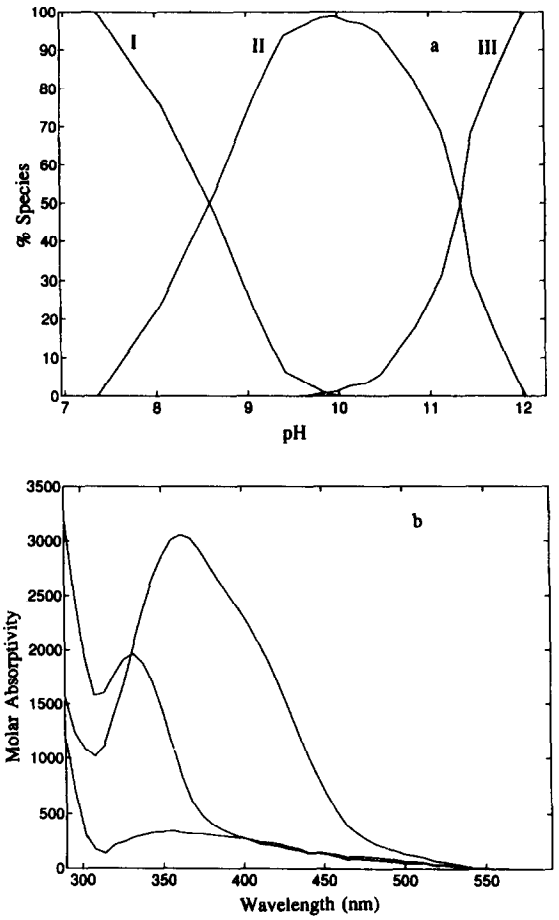


Fig. 9. Treatment of spectral data matrix corresponding to the continuous flow titration of a NQS solution $4.97\times10^{-4}$ M by means of ALS. (a) Optimum concentration profiles of species with pH. (b) Optimum unit spectra.

the range of wavelengths studied (from 290 to 590 nm).

### 3.1. Simultaneous treatment of several titrations

Fig. 11 shows the eigenvalues obtained for both augmented matrices row-wise and column-wise. It

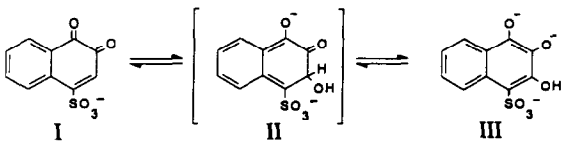


Fig. 10. Species proposed for the acid–base equilibria of NQS.

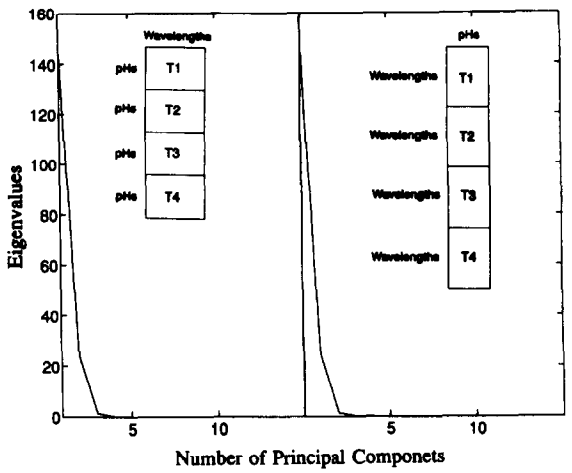


Fig. 11. Principal component analysis of both augmented data matrices on the wavelength and pH directions.

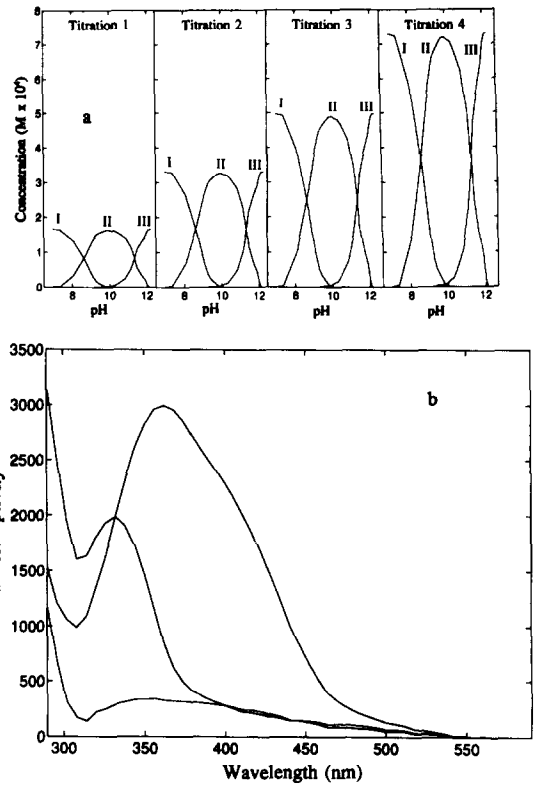


Fig. 12. Final results for the simultaneous treatment of the four NQS titrations by the ALS procedure applying the second order constraint. (a) Optimum concentration profiles with pH. (b) Optimum unit spectra.

can be seen that they do not present significant differences. Under these conditions, a second order or trilinear data structure is assumed for the experimental data.

In Fig. 12 the unit spectra (a) and the concentration profiles (b) of the different chemical species obtained in the simultaneous analysis of the spectrophotometric titrations arranged as in the augmented data matrix of Fig. 3c are given. Another global analysis of the four titrations was performed using the augmented data matrix arranged as in Fig. 3d giving unit spectra and concentration profiles with the same shapes as in Fig. 12. This proves again the particular structure of the considered experimental acid–base equilibria.

## 4. Conclusions

The continuous flow manifold developed in this work provides a robustness tool to perform titrations of stable and unstable compounds. The method has been applied to the study of the acid–base titration of 1,2-naphthoquinone-4-sulphonate although it can be easily adapted to other reaction based systems such as metal ion complexes, redox equilibria and others. The parameters affecting the reaction system (for instance buffer composition, analyte concentration or pH) can be exactly controlled. This continuous flow methodology generates rapid and reproducible multivariate data, and its coupling with a multivariate curve resolution procedure method such as SPFAC allows the resolution of chemical systems. By means of an alternating least squares procedure, the SPFAC method transforms the chemical information contained in the NQS spectrophotometric data in the distribution profiles with pH and unit spectra for every species. When several analyte solutions are sequentially titrated in the same run, spectra for each solution can be recorded at the same pH. Under these circumstances, the NQS titration data have a second order structure which involves equality in the unit spectra and in the unit concentration profiles for common species. This second order structure restriction has been added to the SPFAC procedure to remove ambiguities and improve the fitting of the results.

Further studies are in progress and focus on the study of data from derivatization and kinetics reactions where a second order structure is expected. The application of SPFAC to related flow methodologies such as flow injection analysis is also in development.

## Acknowledgements

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