UNIVERSITAT ROVIRA I VIRGILI
SEQUENTIAL INJECTION ANALYSIS USING SECOND-ORDER CALIBRATION FOR THE DEVELOPMENT OF ANALYTICAL METHODS
Verònica Gómez Cortés
ISBN: 978-84-691-0990-8/D.L: T.2293-2007

Sequential injection analysis using second-order calibration for the development of analytical methods

Doctoral Thesis

ROVIRA I VIRGILI UNIVERSITY

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ROVIRA I VIRGILI UNIVERSITY
Department of Analytical Chemistry and Organic Chemistry

Sequential injection analysis using second-order calibration for the development of analytical methods

Thesis presented by

VERÓNICA GÓMEZ CORTÉS

to receive the degree of
Doctor of the Rovira i Virgili University
Tarragona, 2007

Supervisor
Dr. M. Pilar Callao Lasmarías

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While writing this thesis I was supervised by Dr. M. Pilar Callao, and the work was undertaken in the Chemometrics, Qualimetrics and Nanosensors research group, headed by Prof. F. Xavier Rius, from the Department of Analytical Chemistry and Organic Chemistry at the Rovira i Virgili University in Tarragona, Spain.
It included a research stay in the laboratory of Prof. Víctor Cerdà at the Analytical Chemistry, Automation and Environment group of the University of Balearic Islands, Spain.

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CERTIFIES:

The Doctoral Thesis entitled: "**SEQUENTIAL INJECTION ANALYSIS USING SECOND-ORDER CALIBRATION FOR THE DEVELOPMENT OF ANALYTICAL METHODS**", presented by VERÓNICA GÓMEZ CORTÉS to receive the degree of Doctor of the Rovira i Virgili University, has been carried out under my supervision, in the Department of Analytical Chemistry and Organic Chemistry at the Rovira i Virgili University, and all the results presented in this thesis were obtained in experiments conducted by the above mentioned student.

Tarragona, November 2007

Dr. M. Pilar Callao Lasmarías

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Agraïments…, la veritat és que no sé per on ni com començar, però realment són moltes les persones que han contribuït en aquesta tesi i no voldria no deixar constància. M'agradaria donar les gràcies a totes les persones que, d'una manera o altra, han contribuït a la realització d'aquesta tesi:

En primer lugar, agradecer a mi directora de tesis, la Dra. M. Pilar Callao quien me brindó la oportunidad de realizar esta tesis doctoral, además de transmitirme sus conocimientos, ayuda y soporte durante estos cuatro años.

També voldria agraïr als membres de l'Escola d'Adoberia d'Igualada el seu assessorament i l'aplicabilitat dels meus treballs al món de la indústria.

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Els meus agraïments a tots els components del meu grup d'investigació, Quimiometria, Qualimetria i Nanosensors, els que m'han precedit, les noves incorporacions i en especial els que hem compartit més estones junts. Gràcies Enric per no deixar-me desanimar en els moments difícils i per totes les teves aportacions, tant científiques com personals a l'hora de generar-me un gran entusiasme per la investigació científica. Alberto, gracias por introducirme en el tema de trabajo y ayudarme a superar los la incertidumbre de los nuevos desafíos. A Mariano y Marta, por todas las charlas que tuvimos, científicas y de las otras. A Cristina B y a Joe, por las ayudas y los consejos que han estado siempre dispuestos a darme en la última etapa. A todos los demás, gracias por todos los momentos que hemos pasado juntos, vuestros consejos, ánimos y vuestra amistad, que ha colaborado en el trabajo diario. Un agradecimiento especial a Santi, Eulàlia y Tere por estar siempre dispuestos a solucionar todo tipo de problemas.

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També voldria fer extens el meu agraïment a tots els membres del grup de Cromatografia i aplicacions medioambientals de la Universitat Rovira i Virgili pel suport desinteressat que m'han donat durant l'última etapa de la tesi.

Asimismo, tengo muy presentes a Cristina C, Patri, Rosa y Marta S con las que he compartido muy buenos momentos durante la licenciatura y el doctorado, aun estando en áreas diferentes. Sin vosotras nada hubiera sido igual.

Finalmente querría agradecer a mi familia todo el apoyo que me han dado durante la realización de la tesis, a mi hermano y muy especialmente a Valentín, quien, sin duda ha sido la persona más implicada durante esta etapa porque siempre estuvo y está incondicionalmente, y a quien además dedico esta Tesis.

Who gives up shortly
before the finish
forgets that the way back
is much longer.
Chinese proverb

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1. Introduction and objectives

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1.1. INTRODUCTION

Since their introduction flow methods have developed rapidly and in various directions. Today, they are internationally approved, being used as routine methods for determining quite a large number of analytes, and also in sample pre-treatment for selective detectors, process control, drug dissolution testing and hyphenated separation techniques, to mention just a few further applications[1,2].

Chemometric techniques were introduced into analytical chemistry almost in parallel with flow injection analysis and, like flow methods, both their use and scope have increased[3]. Chemometric approaches are recommended both for method development in flow analysis (experimental design) and for detector signal treatment (calibration methods)[4]. However, the majority of published flow analysis papers still report method development and detector signal treatments that do not employ any chemometrics at all. Chemometrics and flow methods are two disciplines that show a strong and emerging potential to work hand in hand.

Detector systems with multi-wavelength capacity more or less necessitate the use of chemometrics, thereby, enabling multianalyte determinations. The analytical signal can be a scalar (a single absorbance measure), a vector (single absorbance measurement along time), or a data matrix (spectrum recorded along time) per each analysed sample. These data have been classified, as zero-order data when the signal is a scalar, first-order data when is a vector, and second-order data when the signal is a matrix[5]. These information-rich systems have many advantages, including, the fact that interfering contributions can be accounted for by applying multivariate calibration methods[6]. The involvement of chemometrics is clearly valuable, if not essential, for systems of this kind. Nowadays, many of the flow analysis determinations are performed by univariate calibration, using only one channel of the measured spectrum (one wavelength). First-order data and second-order data are still underused for quantitative analysis.

In this thesis we focus on the use of chemometrics in flow systems. Specifically, we focus on Sequential Injection Analysis (SIA) systems and Multivariate Curve Resolution with Alternating Least Squares (MCR-ALS)[7]. MCR-ALS[8] is a second-order calibration

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method which is relatively simple and quick compared to the analysis time. We obtained matrices of data with a sequential injection analysis (SIA) system and treated these matrices with MCR-ALS, where the concentration of the analyte of interest can be determined in an overlapped peak and selective data are not needed. We also used experimental design methodologies to optimize and select the best conditions in our system.

All the work was developed in the environmental application field. We are going to work principally with tanning wastewater samples.

1.2. OBJECTIVES OF THE THESIS

The first objective of this thesis was to study and develop analytical methods using sequential injection analysis (SIA) with a diode-array spectrophotometric detector to obtain second-order data. These data were treated with multivariate curve resolution with alternating least squares (MCR-ALS). This first objective has given rise to other equally important objectives:

a) To prepare critical overviews-reviews about working topics.

b) To study and develop an application of Sequential Injection Chromatography (SIC) using MCR-ALS. SIC is one of the current approaches with high potential in the flow analysis field.

More specifically, we developed:

1) methods to determine chromium in tanning samples by using SIA and MCR-ALS. First, our methods comprised total chromium determination and then we proposed a method for chromium speciation. This last method permits the simultaneous determination of Cr(III) and Cr(VI) in a single step.

2) methods to determine simultaneously three dyes in samples from tanning industry in the presence of interferents and in the presence of matrix effects.

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3) an equilibrium and kinetics study of the adsorption of dyes onto activated carbon and a methodology to fit a response surface and to optimize the process of adsorption of dyes onto activated carbon by using the experimental design methodology.

4) an application of sequential injection chromatography (SIC) using MCR-ALS in collaboration with the Group of Analytical Chemistry, Automation and Environment at the University of the Balearic Islands (UIB).

5) critical reviews about chromium determination and about multi-component determinations in flow analysis

1.3. STRUCTURE OF THE THESIS

This thesis is based on papers published in international journals. These papers have been edited to give a uniform format. The contents have been structured in six chapters.

Chapter 1. ***Introduction and objectives***. This chapter contains the introduction, objectives and structure of the thesis.

Chapter 2. ***Theoretical aspects.*** This chapter contains a brief description of the theoretical backgrounds that have been used during this thesis. First, we discussed the characteristics and properties of the analytes and described the historical development of flow systems. Then we focused on the components and the process that takes place in the SIA system when the reagents and sample are introduced. Then, we introduced the theoretical background of data treatment, multivariate curve resolution with alternating least squares (MCR-ALS) and described the strategy of experimental design used in this thesis. It contains screening designs and response surface methods. In this chapter we have not included theoretical aspects of the collaboration with the Group of Analytical Chemistry, Automation and Environment of the University of the Balearic Islands, which will be developed in chapter 5.
Chapter 3, 4 and 5 contain the bulk of the work carried out for this thesis. Each of these chapters contains an introduction explaining the analytical problems we are going to look at

and how they led to the scientific papers. The experimental part is presented in papers which are published in journals. Finally the overall conclusions of the chapter are presented.

Chapter 3. ***Chromium determination in tanning samples*** deals with the experimentation and methodology for developing methods to determine total chromium and its main species, Cr(III) and Cr(VI). The paper *Use of multivariate curve resolution for determination of chromium in tanning samples using sequential injection analysis*, develops a method for total chromium determination using a pH gradient inside the SIA system. The paper *Factorial design for optimizing chromium determination in tanning wastewater* presents an automatic system for total chromium determination using different experimental designs to optimize the overall process. *Chromium speciation using sequential injection analysis and multivariate curve resolution* is a paper in which the two main species of chromium are determined simultaneously in a single analysis in the presence of interferents. Next, we present a revision of *Chromium determinations and speciation since 2000*.

Chapter 4. ***Study of dyes in wastewater samples from tanning effluents.*** This chapter contains four papers with two practical objectives, determining dye concentration and studying possible strategies to eliminate them from wastewaters. *Sequential injection analysis with second-order treatment for the determination of dyes in the exhaustion process of tanning effluents* describes the developed method for determining three acid dyes in a single step. The following section presents strategies to use when a sample presents matrix effects using second-order data. This can be seen in the paper *Matrix effect in second-order data. Determination of dyes in a tanning process using vegetable tanning agents*. To solve the second objective, we studied the behaviour of dyes on activated carbon and present the results in the paper *Kinetic and adsorption study of acid dye removal using activated carbon*. Finally, in the last section, *Experimental designs for optimizing and fitting the adsorption of dyes onto activated carbon,* we described a sequential methodology to obtain a response surface and optimize the adsorption process as a way of eliminating dyes in wastewater samples from the tanning industry.

Chapter 5. ***Hyphenated techniques involving flow systems***. In this chapter we explored the possibilities of increasing the capacity of simultaneous analysis by means of flow systems and second-order calibration methods. First, we present an overview concerning *Multicomponent*

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analysis in flow systems. Second, we present an application of Sequential Injection Chromatography (SIC) using second-order data. Results are presented in the paper *Coupling of sequential injection chromatography with multivariate curve resolution-alternating least squares for enhancement of peak capacity*.

Chapter 6. ***General conclusions***. This chapter contains the general conclusions of the thesis and suggestions for further research.

The Appendix contains the list of papers and meeting presentations given by the author during the period of development of this thesis. It also contains a list of papers not contained in the thesis.

1.4. REFERENCES

[1] E.H. Hansen, Anal. Chim. Acta 600 (2007) 4.
[2] G.D. Christian, Anal. Chim. Acta 499 (2003) 5.
[3] B. Karlberg, R.Torgrip, Anal. Chim. Acta 500 (2003) 299.
[4] H. Martens, T. Naes, Multivariate Calibration, Wiley, 1989.
[5] E. Sanchez, B. R. Kowalski, J. Chemom. 2 (1988) 247.
[6] K.S. Booksh, B.R. Kowalski, Anal. Chem. 66 (1994) A782.
[7] A. Pasamontes, M.P. Callao, Trends Anal. Chem. 25 (2006) 77.
[8] R. Tauler, A. Smilde, R. Kowalski, J. Chemometrics 9 (1995) 31.

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2. Theoretical aspects

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2.1. SAMPLES AND ANALYTES

2.1.1. Tanning industry

Tanning is one of the oldest industries in Spain and has gradually evolved from its wholly traditional past to its highly technological present. The sector consists of 150 companies and employs around 4500 workers. The companies are largely concentrated in Catalunya, the Community of Valencia, Murcia and Madrid, with some companies shared out among the other 15 provinces[1].

Tanning operations include several chemical and mechanical treatments to convert a putrescible organic material into a biochemically and mechanically stable product. The main stages of the process are *tanning*, the addition of the tanning agent; *neutralization*, in which the excess of tannin and acids that have not been fixed is removed; *retanning*, which consists of treating the leather with certain products to give it particular qualities; *dyeing*, which gives the leather its colour; and *greasing*, which prevents the leather from drying out.

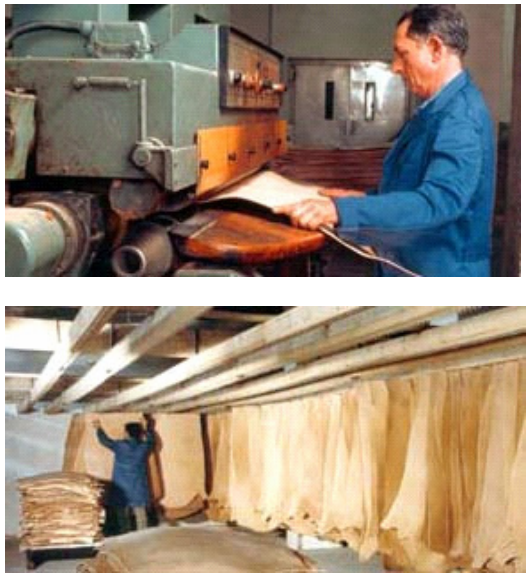


Figure 1. Stages of the leather tanning process

Two main tanning agents—chrome tanning and vegetable tanning—are used. Chrome tanning is used in most cases (70%) and vegetal tanning is used for specific preparations, such as the manufacture of shoe soles[2].

The tanning industry uses a wide spectrum of dyes to impart the desired colour to the leather matrix. About 60-80% of the dyes are adsorbed onto the leather matrix and the unspent dyes are discharged in the wastewater. The spent dye chemicals have poor biodegradability and they go through biological wastewater treatment processes without undergoing any metabolism[3].

Of all the possible components of wastewater from the tanning process, this thesis focuses on determining chromium and dyes, because of their considerable environmental impact.

2.1.2. Chromium

Chromium can take on different chemical forms depending on its oxidation state, although not all of them are stable. Cr (II), (IV) and (V) exist, but the main forms in aqueous medium are Cr (III) and Cr (VI)[4]. The hexavalent form can be found in the form of different species depending on the pH of the medium. In a basic medium, pH>6, CrO_4^{2-} predominates while in an acid medium, 2<pH<6, $Cr_2O_7^{2-}$ is in equilibrium with $HCrO_4^-$.

For a long time it was accepted that the two species Cr (III) and Cr (VI) were stable and that interconversion was limited by the high potential required to oxidize them. It was also thought that the relative kinetics of the transformation was slow. Some experimental evidence clearly shows that this is not true. In fact, when the pH increases, the potential of the chromate decreases considerably and even weak oxidants can produce large amounts of Cr (VI)[5].

Toxicological studies indicate that the degree of toxicity of the metals, chromium included, depends on the chemical form of the element[6]. Chromium (VI) is much more toxic than chromium (III). The toxic effects of Cr (VI) include an immediate cardiovascular shock, with subsequent effects on the kidney, the liver and the blood vessels[7,8]. It is

potentially a carcinogenic agent[9]. Its toxic nature is attributed to its high oxidation potential and its relatively small size, which enable the atoms to penetrate the cell membranes[10]. Chromium (III), on the other hand, is considered to be an essential micronutrient for humans and plays an important role in maintaining the levels of glucose, cholesterol and fatty acids. The amount of chromium allowed in wastewaters is very strictly controlled (0.05-0.1 mg L^{-1} depending on the type of industry). The maximum concentration of chromium (VI) allowed in drinking water is 0.05 mg L^{-1} (recommendations by the World Health Organization, WHO)[11].

Chromium is widely used in the industry in both its elemental form to form alloys and as a trivalent or hexavalent salt[12-15]. Chromium compounds enter natural waters mainly from effluents from electrodeposition, dyes, health-related institutions, refrigeration towers and the tanning industry[16].

Because of the different toxicities of Cr (III) and Cr (VI) it is important to determine both species as well as the total chromium content[17]. This is one of the main reasons for the recent development of analytical methods to differentiate the forms of chromium in the medium of interest.

2.1.3. Dyes

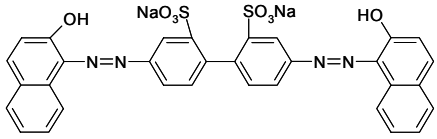
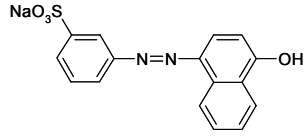
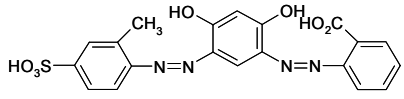
Dyes are organic substances that are soluble in basic, acidic and neutral media. Their structure is electronically unstable, which means that they can absorb energy in the visible region. Azo dyes, characterized by the presence of one or more azo groups (-N=N-), are extensively used in such consumer goods as textiles and leather. They are all obtained by chemical synthesis and none of them exist in nature.

The increased awareness of the potential risk to consumer health of exposure of such dyes resulted in European Union (EU) Directive 2002/61/EC[18]. This directive, largely based on existing German legislation, restricts the use of certain azo dyes which, on reduction, form any of 22 listed amines, in textile and leather articles that may come into direct and prolonged contact with the human skin or oral cavity. Furthermore, the European

Commission (EC)[19] emphasizes that harmonized test methods are vital for application of this directive.

Dyes need to be determined for both technological and environmental reasons. Technologically, it is important to determine the proportions of dyes and the length of time of the dyeing process needed to achieve a certain tone. Environmental interest in determining dyes is due to the fact that the biodegradability of these species is poor and that conventional wastewater treatment plants are not very efficient at eliminating them[20,21]. Some of the azo dyes can be degraded by the oxidative enzymes found in our organism to form aromatic amines that are highly toxic for ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). For this reason they are considered to be genotoxic and carcinogenic[22,23]. Table 1 shows the structure and the name of the three dyes that we have studied.

Table 1. Studied dyes

Dye	Structure
Acid Red 97	
Acid Orange 61	 * chromium complex
Acid Brown 425	 * chromium complex

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2.2. FLOW SYSTEMS

2.2.1. Historical development

The first flow analysis systems appeared in the 1950s and, since then, they have become excellent tools for chemical analysis. Flow techniques allow a high frequency of analysis and minimum handling of the sample and reagents. Another advantage of these systems is that they minimize the consumption of reagents and samples and the generation of waste[24].

The basic components of flow analysis systems are a module for pumping the liquid (peristaltic pumps, two-way piston pumps or micro pumps), a sample injection device, a set of plastic tubes (manifold) and a detector. These methods are based on sample introduction into a tube with a small diameter in which the reagents are added and mixed with the sample and then they are carried to the detector. The order in which the sample and the reagents are added, as well as the reaction time and the flow volume, are controlled automatically, giving high repetitivity of the analytical signal and sampling frequency.

The first flow technique was segmented flow analysis (SFA)[25] and was proposed by Skeegs in 1957. These systems segment the sample with air bubbles, and then carry out a cleaning cycle to avoid cross contamination between samples. The biggest drawbacks of this technique come from the presence of air bubbles that can affect the reproducibility of the system, the flow rate and the shape of the signal. For this reason, the SFA methodologies have gradually been substituted by continuous, not segmented, flow techniques.

The flow injection analysis (FIA) technique was proposed almost simultaneously by Ruzicka and Hansen in Denmark[26] and by Steward, Beecher and Hare[27] in the USA in 1975, and it is a considerable improvement on its predecessor. In FIA systems sample is introduced using an injection valve. FIA systems do not segment samples with air bubbles, so there is no need for the cleaning stage, and the tubes are narrower (the internal diameter is between 0.5 and 0.8 mm in comparison to the 2 mm of the SFA systems), so the sample volumes injected are smaller and the consumption of reagents decreases considerably. As with SFA, its greatest drawback is that it uses peristaltic pumps whose flexible tubes can be

damaged by relatively aggressive reagents (moderately concentrated acids and bases)[24]. The FIA technique is known as the first generation of flow techniques.

In 1990, Ruzicka developed the technique of sequential injection analysis (SIA) as an alternative to FIA[28]. These systems include a selection valve on its configuration. This technique is based on the sequential aspiration of volumes of sample and reagents that are mixed by diffusion in the holding coil and then, the flow is inverted and they are pumped through the reaction coil towards the detector. Among the advantages of SIA systems over FIA, of particular importance is the lower consumption of reagents and a simpler and more universal manifold. What is more, the possibility of replacing peristaltic pumps with syringes means that the SIA technique is more robust. The main disadvantage of the technique is that the sampling frequency is lower than that of FIA[29]. SIA is known as the second generation of flow systems.

Although FIA and SIA have played and continue to play important roles in the automation and miniaturization of on-line sample treatment, they have limitations in the analysis of very small volumes, the handling of chemically hazardous compounds and those operations in which the production of waste is a critical parameter. To overcome these drawbacks, in 2000 Ruzicka[30] introduced the third generation of flow techniques, Sequential Injection- Lab on Valve, SI-LOV and Micro-Sequential Injection- Lab on Valve, μSI-LOV, the components of which are all on a considerable smaller scale. The main advantage of this system is the micro-miniaturization of on-line sample pretreatments[31,32].

In recent years, new flow techniques have emerged as alternatives to conventional flow techniques. Of these, particular mention should be made of multi-commutation flow techniques, which include multi-commutated flow injection analysis (MCFIA)[33], multi-syringe flow injection analysis (MSFIA)[34], and more recently multi-pumping flow systems (MPFS)[35]. These systems enable complex analytical procedures to be carried out in a single stage.

2.2.2. Sequential injection analysis

Figure 2 shows a scheme of a basic SIA system. The principal components of the sequential injection analyzer are an automatic pump or syringe, which is used to aspirate the reagents and sample via a valve and push them towards the detector; a selection valve; a detector, which in our case is a diode-array spectrophotometer; a holding coil; a reaction coil; and finally a computer that controls the functions of each component. The lateral ports of the valve are connected to the recipients that contain the sample, the carrier and the reagents required for the analytical determination, and to the detector.

The basic configuration can also contain other components that allow pretreatments such as separations and preconcentrations (for example, liquid-liquid extraction, precipitation/coprecipitation in auxiliary reactors or solid-phase extraction in column reactors)[36].

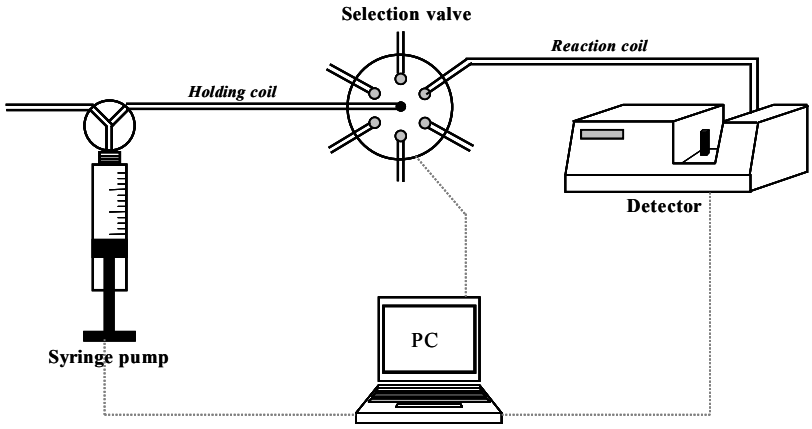

Figure 2. Scheme of a sequential injection analyser.

The process involves the following steps. First, the order in which the reagents are aspirated through the valve is selected. The syringe then aspirates the carrier, the sample and the reagents. Next, the syringe pump pushes the reagents towards the detector. In this step, the direction of flow is changed. Finally, the detector provides information about the measurements registered in a period of time and a prefixed range of wavelengths

(absorbances in our case) and once the sample has passed though the detector, it is expelled as waste.

During the process, reagents and sample are mixed through the phenomenon of dispersion. This penetration zone is the most important parameter for designing an SIA system and some studies have been published that describe the parameters that are most important for optimiation[37,38].

The type of data obtained provided by SIA (a single value, a value over time or a series of values over time) depends on the type of detector used and whether the reagents totally or partially interacted with the analyte before passing through the detector. If the reagent interacted totally, the analyte will be converted into the reaction product. However, if the reagent only interacted partially, as the solution passes though the detector, a diffusive mixture of reagent, analyte and product will be observed. This, together with the system's versatility at coupling to different types of detectors, enables data of different dimensions to be obtained. See Figure 3. This means that various chemometric tools can be used to provide chemical information about the system.

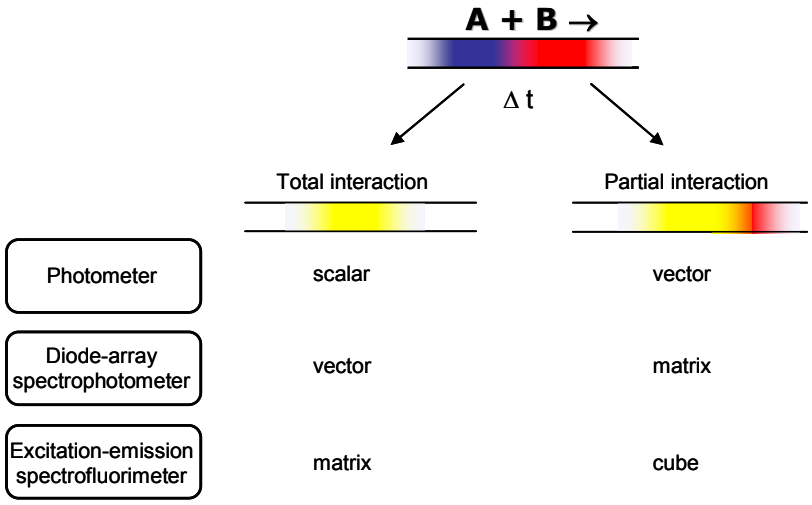

Figure 3. Different types of data that can be obtained by a SIA and different types of detector. A, Analyte; B, Reagent.

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The numerous studies that have been published using this technique show that it is useful for solving a range of analytical problems. It has been used to determine a wide variety of analytes in different matrices: food, beverages, bioprocesses, environmental matrices, pharmaceuticals, industrial processes, etc. [39-42]. Many different types of detection have been used, for example, ultraviolet-visible absorption spectrophotometry, fluorimetry, turbidimetry, atomic spectroscopy, chemiluminescence, electrochemical and radiochemical detectors, and inductively-coupled plasma mass spectrometry. Likewise, studies have been published that illustrate the use of chemometric techniques to treat data generated by SIA[43] or FIA[44,45].

2.3. SECOND-ORDER DATA TREATMENT

2.3.1. Introduction

Experimental measurements generated by analytical instruments were classified by Sánchez and Kowalski[46], as zero-order data, when there is one scalar per analyte (e.g. an absorbance at one wavelength); first-order data, when there is one vector per sample (e.g. an absorbance over time) and second-order data, when there is one matrix per sample (e.g. a spectrum over time). When the order of the data increases, the complexity of the mathematical/statistical data processing also increases. However, with higher order data, the following benefits are obtained: (i) the analyte of interest can be detected if other components (interferents) also contribute to the measured signal, and (ii) it can be quantified in the presence of these interferences, by mathematically deconvoluting the signal[47]. These abilities are summarized in Table 2.

Table 2. Capabilities of data of different orders

Data order	Detection of interferences	Quantification in the presence of non-calibrated interferences	Common type of calibration
Zero	No	No	Univariate linear regression
First	Yes	No	Multivariate calibration
Second	Yes	Yes	Second-order calibration

First-order data can be used to deal with and model the interferences. We need: (i) a series of standards with a known concentration of the analyte of interest, in which the interferences are also present, and (ii) measurements, for both the standards and the test sample, of at least as many instrumental responses as interferents that contribute to the signal. The use of first-order data to build calibration models is known as multivariate calibration. This type of calibration is widely used with spectroscopic data[48]. When a test sample contains an interferent that was not considered in the standards, a multivariate model will give biased predictions of the concentration of the analyte of interest (like univariate calibration). The presence of the non-calibrated interferents can be detected, but it is impossible to

determine what effect the interferents had on the prediction and, therefore, the inaccurate prediction cannot be corrected.

Second-order data can be used to predict the concentration of an analyte in a sample even in the presence of unknown interferents which were not present in the calibration standards. This useful property is called 'second-order advantage'[47]. Multivariate curve resolution (MCR)[49-51] is one of the chemometric techniques that is useful for treating this sort of data. It can be defined as a group of techniques that have been especially designed for studying chemical systems with the aim of isolating, resolving and quantifying the sources of variability present in a data set. Some reviews explain the state-of-the-art of curve resolution methods[52-54].

Notation

Throughout this thesis, bold uppercase letters indicate matrices (second-order data), e.g. **A**; bold lowercase letters indicate vectors (first-order data), e.g. **a**; italic lowercase letters indicate scalars (zero-order data), e.g. *a*. Transposition of a matrix or vector is symbolized by a superscripted "T", e.g. $\mathbf{A}^T$. For a given matrix **A**, the matrices $\mathbf{A}^{-1}$ and $\mathbf{A}^+$ stand for its inverse and pseudoinverse, respectively. In full rank matrices $\mathbf{A}^+=(\mathbf{A}^T\mathbf{A})^{-1}\mathbf{A}^T$.

2.3.2. Multivariate curve resolution with alternating least squares (MCR-ALS)

MCR-ALS[55] decomposes a matrix into the product of two matrices. The most common assumption in multivariate resolution methods is that the experimental data follow a linear model similar to that of the Lambert Beer law in absorption spectroscopy. In matrix form, the model can be written as:

$$\mathbf{D} = \mathbf{C}\mathbf{S}^T + \mathbf{E} \qquad (1)$$

and in graphical form it can be portrayed as in Figure 4.

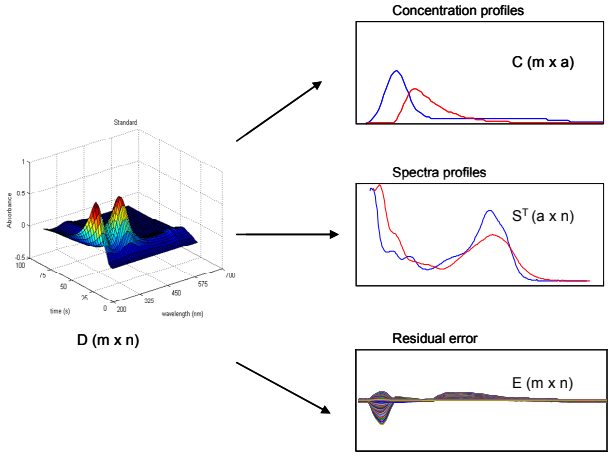

Figure 4. Decomposition of the original matrix into the product of matrices.

D is the spectral data matrix acquired at different values of a particular variable (time, pH, etc.) during a chemical reaction or process. One column in matrix **D** corresponds to the concentration profile in the direction considered (e.g. time) and each row is the spectrum at a fixed time. **C** and **S** are matrices related to the concentration and spectral profiles of the spectroscopically active chemical species in the reaction or process. **E** is the residual matrix with the data unexplained by $\mathbf{C}\cdot\mathbf{S}^T$ and it is close to the experimental error. The dimensions of these four matrices are **D** ($m \times n$), **C**($m \times a$), $\mathbf{S}^T$($a \times n$) and **E**($m \times n$), where m is the number of measurements analysed, n is the number of spectroscopic channels and a is the number of chemical species actives to the detector in the mixtures. In the Figure $a = 2$.

Generally, MCR-ALS operates in the following sequence:

1. Evaluation of the number of components that make a significant contribution to the signal response. Principal Components Analysis (PCA) is used. It expresses the relevant information contained in a data matrix **D** ($m \times n$) in a reduced number of hierarchized variables known as principal components.

2. Search for matrices of the initial estimations. Initial estimations of **C** (concentration profiles) or **S** (spectral profiles) can be evaluated with techniques based on the detection of pure variables, like *Simple-to-use Interactive Self-Modelling Mixture*, SIMPLISMA[56] or techniques based on *Evolving Factor Analysis*, EFA[57-59].

SIMPLISMA searches for the pure variables in which each compound of the system has a more specific spectral response. EFA, on the other hand, aims to study the number of species over time, pH, temperature, etc; that is to say, it aims to know the profile of these species in each stage of the reaction or process.

Matematically, SIMPLISMA is based on evaluating the relative standard deviation of the column *n*, from equation 2:

$$p_n = \frac{s_n}{\bar{x}_n + \delta} \qquad (2)$$

where s_n is the standard deviation of the column *n*, $\bar{x}$ is the average of the column *n* and δ is a correction factor that is added in order to prevent columns with a low average value (generally associated with noise) from being the purest variables. A large relative standard deviation (p_n) indicates that the column is very pure. The process continues to select the successive columns on which it imposes the additional condition that they be minimally correlated with the previous ones. The number of selected columns is the number of principal components evaluated in step 1.

Because the method evaluates the columns to obtain the initial estimates of the spectral profiles, equation 2 is applied to the transposed matrix $\mathbf{D}^T$ ($n \times m$) to determine at which times the purest, and therefore most selective, responses are obtained for each component.

3. Iterative resolution process with ALS[64,65] The alternating least squares method consists of two steps which are repeated iteratively:

a) From the estimation of spectra profiles, the concentration profiles are obtained with the least-squares resolution according to equation 3:

$$\mathbf{C} = \mathbf{D}^*(\mathbf{S}^T)^+ \qquad (3)$$

where $(\mathbf{S}^T)^+$ is the pseudoinverse matrix of $\mathbf{S}^T$ obtained from the initial estimation of SIMPLISMA and **D*** is the data matrix reconstructed from the number of significant principal components selected in the principal component analysis. **C** is the matrix of concentration profiles calculated by least squares.

b) By using least squares with equation 4, a new estimate of spectra profiles can then be obtained from the concentration profiles of the previous step:

$$\mathbf{S}^T=\mathbf{C}^+\mathbf{D}^* \qquad (4)$$

where $\mathbf{C}^+$ is the pseudoinverse matrix of **C**.

Steps a) and b) are repeated until the difference between the residual of one iteration and the next is less than a prefixed value.

If we use an initial estimate of the concentration profiles, the process is reversed. We begin with equation 4 instead of equation 3, and in step b) we use equation 3.

The result of the process of iterative resolution improves considerably if information is provided that is based on chemical knowledge of the system being studied. This information can be transmitted by using augmented matrices[60-62] and/or imposing constraints[62-65].

Matrix augmentation strategy:

If two or more matrices are available that have at least one order in common (rows or columns), they can be analysed together.

The most common ways of arranging matrices are to maintain the columns a) or to maintain the rows b) (see Figure 5). The matrix resulting from these groupings is known as the augmented matrix.

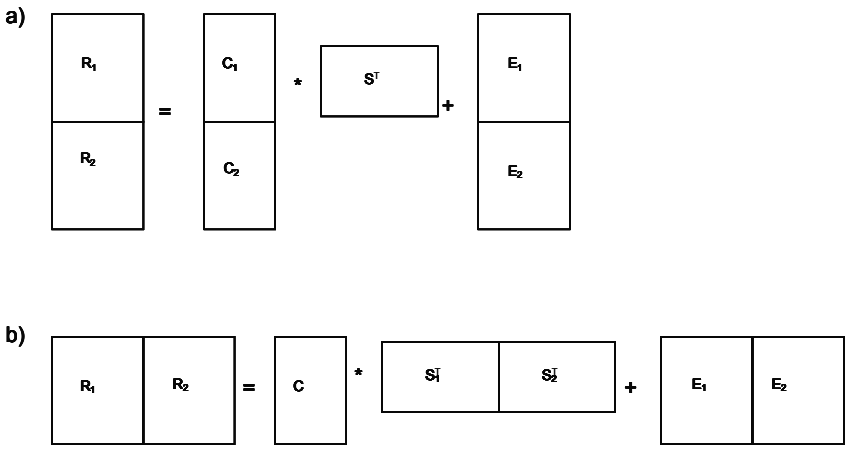

Figure 5. Arrangements of matrices and information obtained from MCR-ALS

This strategy not only improves the resolution, it is also very useful when the matrices are rank deficient: that is to say, systems in which fewer species than the ones that we actually have are resolved[66].

Constraints:

The constraints that can be applied for each iterative cycle are:

- Non-negativity. This obliges the concentrations of the chemical species and/or the values of the spectra to be positive or zero.

- Zero selectivity and concentration windows. It can be applied when zones are known to be totally selective for one of the species. This means that the concentrations or signals of the other components must be equal to zero.

- Unimodality. This is used when it is known that the profiles have a single maximum, which is common in the concentration profiles but not in the spectral profiles.

- Closed system constraint (closure). This can be applied when the sum of the concentrations of all the species involved in the reaction or the sum of some of them is forced to be constant at each stage of the reaction. Closure may be a mass balance constraint.

- Constraint on the correspondence between species. This is used when performing augmented matrix analysis of several data matrices. This constraint identifies the same species in matrices from different experiments.

- Trilinearity (three-way structure). This condition means that the profiles of one analyte in the various matrices used must be equal. Possible deviations from this parameter may lead to incorrect results.

Evaluation of the process quality:

Once the ALS optimization process is over, the profile concentrations and the spectra obtained can differ from the real ones to a greater or lesser extent because there is always a certain level of ambiguity in the results[63] so the quality of the results obtained must be evaluated.

One of the critical steps in the resolution is to choose the correct number of principal components. One way to determine whether the estimate of principal components has been corrected is to study the lack of fit (*lof*), expressed in equation 5, of the model with respect to the original:

$$lof = \sqrt{\frac{\sum_{i,j}(d_{ij} - \hat{d}_{ij})^2}{\sum_{i,j} d_{ij}^2}} \qquad (5)$$

where d_{ij} may be each of the elements of the raw data matrix or each of the elements of the matrix reconstructed from the principal components selected in the principal component

analysis (PCA), and $\hat{d}_{ij}$ are the corresponding values calculated after the optimization process (ALS). Therefore, depending on the matrix used, either $lof_{\text{ex p}}$ or lof_{pca} is obtained.

If the pure analyte is available, another way to check if the model is well fitted is to determine the correlation between the spectra obtained in the resolution process and the spectra of the pure species, expressed in equation 6:

$$r = \cos\gamma = \frac{s_i^T \hat{s}_i}{\|s_i\| \cdot \|\hat{s}_i\|} \qquad (6)$$

where γ is the angle defined by the vectors associated to the profile recovered by MCR-ALS ($\hat{s}_i$) and the real rofile (s_i), for a particular species studied *i*.

The fit improves as the selectivity (for example, wavelengths in which only one species absorbs) increases and the local rank (number of species simultaneously present in a region) decreases.

2.3.3. Second-order calibration with MCR-ALS

Second-order calibration can reconstruct the response of the analyte in the presence of unknown and uncalibrated interfering species. The pure analyte is often used as the standard while the interferences are not taken into account in the calibration stage, which is a clear advantage over first-order or multivariate calibration.

An analyte can be quantified when the ALS optimization method is simultaneously applied to data matrices of standards of known concentration and samples of unknown concentration in the form of an augmented matrix where the matrix above corresponds to the calibration standards and the matrix below corresponds to a reference standard whose concentration is affixed.

This process is carried out using the area ratios of the concentration profile resolved for matrix $\mathbf{C}_i$, obtained from *i* calibration standards, and the area of the same compound in the reference standard matrix, $\mathbf{C}_{rst}$ used as a reference[67] so that:

$$r_i = \frac{a_{st,i}}{a_{rst}} \qquad (7)$$

where $a_{st,i}$ is the area of the analyte in the calibration standard sample and a_{rst} is the area in the reference standard.

Using the r_i values a univariate linear regression is obtained of the type:

$$r_i = b_1 c_i + b_0 \qquad (8)$$

where c_i is $c_{st,i}/c_{rst}$.

$c_{st,i}$ is the concentration of the target analyte in the calibration standard, while c_{rst} is the concentration in the reference standard, and b_0 and b_1 are the parameters of the regression straight line.

The concentration of the analyte in the sample, c_{sample}, is obtained from the corresponding value r_i (r_{sample}/r_{rst}) according to the expression:

$$c_{sample} = \frac{r_i - b_0}{b_1} c_{rst} \qquad (9)$$

The resolution of a standard (calibration stage) and a sample (quantification stage) are shown schematically in Figure 6.

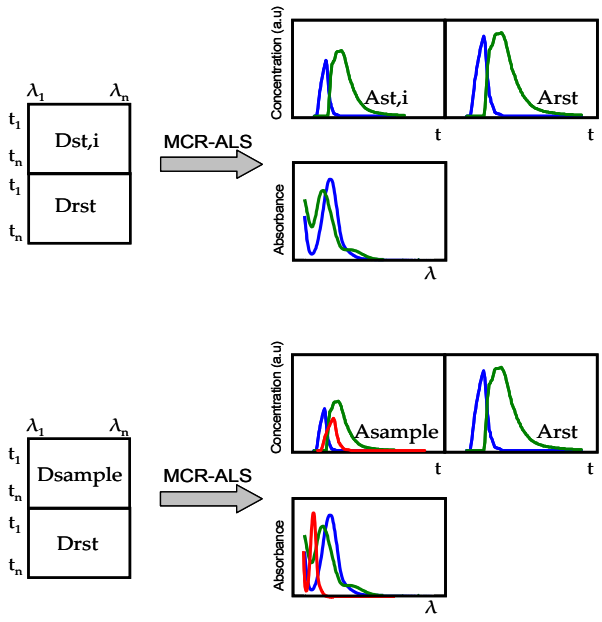

Figure 6. Resolution of a calibration standard and an unknown sample with MCR-ALS

When the aim of the resolution is quantification, the quality of the process can be measured with the error of quantification, evaluated as in equation 10:

$$Error\ (\%) = \left| \frac{a_i \times f}{a_{rst}} - 1 \right| \times 100 \qquad (10)$$

where a_i and a_{rst} are the areas of the concentration profiles obtained for each species in a sample of known concentration and in a reference standard, respectively, and f is a correction factor for the difference between the concentrations of the species in the sample and in the reference standard:

$$f = \frac{c_{rst}}{c_i} \qquad (11)$$

MCR-ALS is particularly applicable to chemical data structures that do not need the demanding requirements of other second-order methods, such as the strict trilinearity or

bilinearity of the analytes[66]. However, it must be borne in mind that the fewer variables the system of equations has, the easier it will be to obtain the correct solution.

The accuracy of the determination when second-order multivariate curve resolution is used depends on the selectivity [63], the differences between the species and the level of the second-order data structures[55,68].

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2.4. EXPERIMENTAL DESIGN

The experimental design, also called design of experiments (DOE), involves planning experiments systematically in order to extract the maximum amount of information with the least number of experiments possible using statistical tools[69]. Experimental design helps the experimenter to select an optimal experimental strategy to achieve the proposed objectives. It includes:

• The determination of the influence of the factors which, a priori, have been selected as significant in the process being studied.

• Optimization of a response by modelling it in an experimental domain to obtain a response surface.

The conclusions drawn after experimental design has been applied can only be generalized in the domain studied.

2.4.1. Screening designs

1. Full factorial design

A full factorial design is an experimental set-up consisting of all the possible combinations between the different factors and their levels. The set of all experiments with codified factor values is the design matrix. In general, a design in which k factors are studied at m levels contains m^k different experiments. Of all these experimental designs, full factorial design at two levels is the one that is most used.

A full factorial design at two levels estimates not only the effects of factors A, B and C (main factors) on the response, but also the interactions of the factors. A two-factor interaction occurs when the effect of the first factor (A) on the response is different at both levels of the second factor (B), or vice versa. A three-factor interaction means that a two-

factor interaction effect is different at the two levels of the third factor, and so on for higher order interactions.

One disadvantage of full factorial designs is that the number of experiments to be performed increases rapidly as the number of factors examined increases.

2. Fractional factorial design 2^{k-q}

If the number of factors is high, and the full experimental cost associated with a full factorial design cannot be assumed, an alternative is to perform only a fraction of the experiments of a full factorial design. This fraction should be selected so that the experimental space is mapped as well as possible and orthogonality (all factor effects uncorrelated) is preserved.

The practical set-up of a two level fractional factorial design 2^{k-q} is *via* a full factorial design for k-q factors, where k is the number of factors and q is the number that indicates the reduction (a half, a quarter, etc.). For example, for a 2^{4-1} fractional factorial design, a full factorial design for 4-1=3 factors (A, B and C) is first constructed. The fourth factor (e.g. factor D) is assigned to one of the interactions (i.e. ABC). The relationship D = ABC is called the generator. By multiplying both parts of the generator with each other, a defining relation or defining contrast (I) is produced:

I = D x ABC = ABCD

The alias of any factor or interaction is then obtained by multiplying it with the defining relation. An additional rule is that when a term appears an even number of times in the product, it disappears. The generator can determine how the factors were confounded. In the previous example, some of the confounded effects are shown:

A x ABCD = BCD
B x ABCD = ACD
……
AB x ABCD = CD

where factor A is confounded with interaction BCD, factor B is confounded with interaction ACD, factor AB is confounded with interaction CD, etc.

The main effects tend to be more important than two-factor interactions, which in turn tend to be more important than three-factor interactions and so on. However, one should be careful with this assumption, as two-factor or even three-factor interactions can be larger than non-significant main effects.

3. Study of the factor influences

The influence of the factors A on the response in an experimental design can be calculated as:

$$b_A = \frac{\sum Y(+)}{n} - \frac{\sum Y(-)}{n} \qquad (12)$$

where ΣY(+) and ΣY(-) are the sums of the responses where factor A is at its high (+1) and low (-1) level, respectively, and *n* is the number of times each factor is at the (+1) or (-1) level. Equation 12 can be applied for each factor or interaction.

There are several statistical tools for studying the significance of the estimated effects when working on screening designs[70]. These statistical tools can be classified by: (i) visual interpretation or (ii) ANOVA.

(i) A *Pareto chart* displays a frequency histogram where the length of each bar on the chart is proportional to the absolute value of its associated estimated effect or the standardized effect. A *Normal probability plot* displays a normal distribution on which the effects that are found to be part of this normal distribution cannot be considered to be significant.

(ii) One of the most important statistical techniques for deciding which factors and which interactions are significant is ANOVA, which shows the results of partitioning the variability in the selected response into separate items for each of the effects. We then test the statistical

significance of each effect by comparing the mean square with an estimate of the experimental error.

2.4.2. Response surface methods to process optimization

Response surfaces are used to build a mathematical model for the experimental response as a function of the factor values. The type of experimental design can be related to the type of function we wish to model (linear, quadratic, etc.). Normally, the type of function that relates the response to the factors is unknown, but we know it can be modelled by a polynomial function.

Typical polynomial functions are given below for two factors, x_1 and x_2, and the single response y:

$$y = b_o + b_1x_1 + b_2x_2 \quad \text{(a)}$$

$$y = b_o + b_1x_1 + b_2x_2 + b_{12}x_1x_2 \quad \text{(b)}$$

$$y = b_o + b_1x_1 + b_2x_2 + b_{12}x_1x_2 + b_{11}x_1^2 + b_{22}x_2^2 \quad \text{(c)}$$

$$y = \text{polynomial function of higher order} \quad \text{(d)}$$

where b_0, b_1, b_2, b_{12}, b_{11} and b_{22} are the coefficients of the model. Such a model contains a constant term b_0, which estimates the response when the values of all the factors are set at zero (coded factors), linear coefficients (b_1 and b_2), which describe the sensitivity of the response to the variations in the corresponding factors (equivalent to the main effects discussed earlier), cross-product coefficients (b_{12}), which are measures of the interactions between factors, and quadratic terms (b_{11} and b_{22}), which describe curvature.

When we do not know which model is valid, the experimental procedure is to: (i) begin with the simplest model and obtain its coefficients; (ii) check the validity of the model and, if it is valid, end the process; (iii) if the model is not valid, conduct more experiments to obtain the coefficients of a higher-degree polynomial and then repeat step (ii) and (iii).

If the postulated model is type (a) or type (b), a full factorial design is used. If it is type (c), one possibility is to make a central composite design. The last design contains three

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parts: a full factorial design 2^k, where k is the number of factors, a number of centre experiments and a number of star experiments with a pre-determined axial distance. The centre points are often replicated, which gives an immediate idea of experimental precision.

This response surface allows us to choose the best experimental conditions, either the optimum response or the best relationship between the response and the factor values that are most suitable for the experimenter.

2.5. REFERENCES

[1] http://www.leather-spain.com/esp/CECinfo.asp

[2] Prospects of EM Technology In Controlling Leather Industry Pollution, http://www.embiotech.org.

[3] G.M. Shau, C.R. Dempsey, K.A. Dostal, Fate of water soluble azo dyes in the activated sludge process. Environ Prot Agency, Cincinnati, OH (USA), Gov Rep Announce (US) 1988.

[4] F.A Cotton, G. Wilkinson, P.L. Gaus, Basic Inorganic Chemistry, Chichester, 1987.

[5] P. Pastore, G. Favaro, A. Ballardin, D. Danieletto, *Talanta* 63 (2004) 941-947.

[6] A. Kot, J. Nameiesnik, *Trends Anal. Chem.* 19 (2000) 69-79.

[7] E. Vassileva, K. Hadjiivanov, T. Stoychev, C. Daiev, *Analyst* 125 (2000) 693-698.

[8] P.V. Oliveira, E. Oliveira, *Fresenius J. Anal. Chem.* 371 (2001) 909-914.

[9] D.T. Gjerde, D.R. Wiederin, F.G. Smith, B.M. Mattson, *J. Chromatogr*. *A* 640 (1993) 73-78

[10] E.A. Carcea, D.B. Gomis, *Analyst* 122 (1997) 899-902

[11] A.M. Gevorgyan, S.V. Vakhnenko, A.T. Artykov*, J. Anal. Chem.* 59 (2004) 371-373.

[12] A.P. Greef, C.W. Louw, H.C. Swart, *Corros. Sci*. 42 (2000) 1725-1740.

[13] J.M. Boiano, M.E. Wallace, W.K. Sieber, J.H. Groff, J. Wang, K. Ashley, *J. Environ. Monitor*. 2 (2000) 329-333.

[14] H.J. Giba, P.S.J. Lees, P-F- Pinsky, B.C. Rooney, *Am. J. Ind. Med.* 38 (2000) 115-126.

[15] Y. Harada, M. Ohmori, F. Yoshida, R. Nowak, *Mat. Lett*. 57 (2003) 1142-1150.

[16] M.I.C. Monteiro, I.C.S. Fraga, A.V. Yallouz, N.M.M. de Oliveira, S.H. Ribeiro, *Talanta* 58 (2002) 629-633.

[17] K. Stein, G. Schwedt, *Fresenius J. Anal. Chem*. 350, 1994, 38-43.

[18] European Commission (2002) Off J Eur Commun L 243:15

[19] Second Amendment to the German Consumer Goods Ordinance (1994) Bundesgesetzblatt Part 1:1670

[20] L.H. Ahlström, A. Sabine, L. Mathiasson, *J. Sep. Sci.* 28 (2005) 2407-2412.

[21] G.A. Umbuzeiro, H.S. Freeman, S.H. Warren, D. Palma de Oliveira, Y. Terao, T. Watanabe, L.C. Claxton, *Chemosphere* 60 (2005) 55-64.

[22] H.M. Pinheiro, E. Touraud, O. Thomas, *Dyes and Pigments* 61 (2004) 121-139.

[23] K. Golka, S. Kopps, Z.W. Myslak, *Toxicology Letters* 151 (2004) 203-210.

[24] M. Miró, V. Cerdà, J.M Estela, *Trends Anal. Chem*. 21 (2002) 199-210.

[25] L.T. Skeggs, *Am. J. Clin. Pathol*. 28 (1957) 311-322.

[26] J. Ruzicka, E.H. Hansen, *Anal. Chim. Acta* 78 (1975) 145-157.

[27] K.K Steward, G.R. Beecher, P.E. Hare, *Anal. Biochem.*70 (1976) 167-173.

[28] J. Ruzicka, G.D. Marshall, *Anal. Chim. Acta* 237 (1990) 329-343.

[29] V. Cerdà, A. Cerdà, A. Cladera, M.T. Oms, F. Mas, E. Gómez, F. Bauzá, M. Miró, R. Forteza, J.M Estela, *Trends Anal. Chem*. 20 (2001) 407-418.

[30] J. Ruzicka, *Analyst* 125 (2000) 1053-1060.

[31] J. Wang, E.H. Hansen, M. Miró, *Anal. Chim. Acta* 499 (2003) 139-147.

[32] Y. Ogata, L. Scampavia, J. Ruzicka, R.C. Scout, M.H. Gelb, F. Turecek, *Anal. Chem*. 74 (2002) 4702-4708.

[33] B.F. Reis, M.F. Giné, E.A.G. Zagatto, J.L.F.C Lima, R.A. Lapa, *Anal. Chim. Acta* 293 (1994) 129-138.

[34] V. Cerdà, J.M Estela, R. Forteza, A. Cladera, E. Becerra, P. Altimira, P. Sitjar, *Talanta* 50 (1999) 695-705.

[35] R.A.S. Lapa, J.L.F.C. Lima, B.F. Reis, J.L.M. Santos, E.A.G. Zagatto, *Anal. Chim. Acta* 466 (2002) 125-132.

[36] E.H. Hansen, J. Wang, *Anal. Letters* 37 (2004) 345-360.

[37] R. Pérez-Olmos, J.C. Soto, N. Zárate, A.N. Araújo, J.L.F.C. Lima, M.L.M.F.S. Saraiva, *Food Chemistry* 90 (2005) 471-490.

[38] A. Pasamontes, M.P. Callao, *Talanta* 68 (2006) 1617-1622.

[39] J.F. van Staden, R.E. Taljaard, *Talanta* 64 (2004) 1203-1212.

[40] J.F. van Staden, R.I. Stefan, *Talanta* 64 (2004) 1109-1113.

[41] M. Miró, J.M. Estela, V. Cerdà, *Talanta* 63 (2004) 201-223.

[42] P. Solich, M. Polasek, J. Klimundova, J. Ruzicka, *Trends Anal. Chem*. 23 (2004) 116-126.

[43] A. Pasamontes, M.P. Callao, *Trends Anal. Chem*. 25 (2006) 77-85.

[44] J. Saurina, S. Hernández-Cassou, *Anal. Chim. Acta* 438 (2001) 335–352.

[45] M.M. Reis, S.P. Gurden, A.K. Smilde, M.M.C. Ferreira, *Anal. Chim. Acta* 422 (2000) 21–36.

[46] E. Sánchez, B.R. Kowalski, *J. Chemometr*. 2 (1988) 247-263.

[47] K.S. Booksh, B.R. Kowalski, *Anal. Chem*. 66 (1994) 782A-791A.

[48] H. Martens, T. Naes, Multivariate Calibration, John Wiley & Sons, 1989.

[49] J.C. Hamilton, P.J. Gemperline, *J. Chemomentr.* 4 (1990) 1-13.

[50] W. Windig, *Chemom. Intell. Lab. Syst*. 16 (1992) 1-16.

[51] E. Sánchez, B.R. Kowalski, *J. Chemometr.* 2 (1990) 247-263.

[52] A. de Juan, R. Tauler, *Anal. Chim. Acta* 500 (2003) 195-210.

[53] J. Jiang, Y. Liang, Y. Ozaki, *Chemom. Intell. Lab. Syst.* 71 (2004) 1-12.

[54] A. de Juan, R. Tauler, *Crit. Rev. Anal. Chem*. 36 (2006) 163-176.

[55] R. Tauler, *Chemom. Intell. Lab. Syst*. 30 (1995) 133-146.

[56] W. Windig, J. Guilment, *Anal. Chem*. 63 (1991) 1425-1432.

[57] H. Gampp, M. Maeder, Ch. Meyer, A.D. Zuberbuhler, *Talanta* 32 (1985) 1133-1139.

[58] H. Gamp, M. Maeder, Ch. Meyer, A.D. Zuberbuhler, *Anal. Chim. Acta* 193 (1987) 287-293.

[59] R. Tauler, E. Cassasas*, J. Chemometr*. 3 (1988) 151-161.

[60] R. Tauler, D. Barceló, *Trends Anal. Chem*. 12 (1993) 319-327.

[61] R. Tauler, A.K. Smilde, J.M. Henshaw, L.W. Burgess, B.R. Kowalski, *Anal. Chem*. 66 (1994) 3337-3344.

[62] R. Tauler, A. Izquierdo-Ridosa, E. Cassasas, *Chemom. Intell. Lab. Syst*. 18 (1993) 293-300.

[63] R. Tauler, A. Smilde, B.R. Kowalski, *J. Chemometr*. 9 (1995) 31-58.

[64] R. Tauler, E. Cassasas, *Anal. Chim. Acta* 223 (1989) 257-268.

[65] R. Tauler, E. Cassasas, A. Izquierdo-Ridosa, *Anal. Chim. Acta* 248 (1991) 447-458.

[66] A. Izquierdo-Ridorsa, J. Taurina, S. Hernández-Cassou, R. Tauler, *Chemom. Intell. Lab. Syst*. 38 (1997) 183-196.

[67] A. de Juan, S.C. Rutan, R. Tauler, D.L. Massart, *Chemom. Intell. Lab. Syst*. 40 (1998) 19-32.

[68] J. Saurina, S. Hernández-Cassou, R. Tauler, *Anal. Chim. Acta* 335 (1996) 41-49.

[69] L. Eriksson, E. Johansson, N. Kettaneh-Wold, C. Wikström, S. Wold, *Design of Experiments. Principles and Applications*, Umetrics Academy, 2000**.**

[70] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. de Jong, P.J. Lewi, J. Smeyers-Verbeke, *Handbook of Chemometrics and Qualimetrics* Part A, Elsevier, Amsterdam, 1997.

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3. Chromium determination in tanning samples

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3.1. INTRODUCTION

This chapter contains four papers. The first three describe the experimental part and methodology for developing new methods based on sequential injection analysis (SIA) and multivariate curve resolution with alternating least squares (MCR-ALS) to determine total chromium and its main species, Cr(III) and Cr(VI), in tanning samples. The methods have been applied to samples from different stages of a tanning process and several environmental wastewater samples.

The papers are sequential. That is to say, the objectives and the experiments carried out of the second and the third are based on the results and the conclusions of the first. What is more, the analytical methods proposed are separate entities and, depending on the objectives of the problem to be resolved, either one or the other can be chosen.

The first paper, entitled *Use of multivariate curve resolution for determination of chromium in tanning samples using sequential injection analysis*, lays the foundations that will enable the SIA-MCR-ALS system to be used for chromium determination. This means that, depending on the reactionability (capacity of reaction) of Cr(III), an analytical sequence must first be designed in the SIA system in order to obtain an evolving system that provides a data matrix as the signal. Other considerations, such as the need for maximum sensitivity, enabled us to know the chemical aspects of the system: the chromium (III) was off-line oxidized to Cr(VI) to increase chromium sensitivity. In order to obtain an evolving system within the SIA system, we induced a pH gradient to convert chromate into dichromate, by means of an interdiffusion process between the acid medium, used as a reagent, and the sample in the channel through the detector. In this study, we used different arrangements of matrices to check two calibration options.

The objectives of the second paper, entitled *Factorial design for optimizing chromium determination in tanning wastewater*, as the title suggests, consisted of optimizing the previous developed method in order to incorporate in a single step its main two steps (Cr(III) oxidation and Cr(VI) evolution to its two species) and automatize them in the SIA system. In this case, volumes of reagents and sample were reduced, as well as time analysis. Sample frequency was increased considerably by making several combinations: first,

determine the experimental conditions to oxidize totally Cr(III) into Cr(VI) and, at the same time, modify the experimental conditions of the analytical sequence to make them compatible with the chemical conditions after the oxidation. Since the entire process can depend on many factors, experimental designs were used so that the best conditions could be found.

Given the different toxicities of the stable species of chromium, the speciation of chromium is of great interest in many fields, particularly the environment. In the third paper, *Chromium speciation using sequential injection analysis and multivariate curve resolution*, we simultaneously determine Cr(III) and Cr(VI) in a single analysis. In the first step, and because of the lower sensitivity of Cr(III), we studied various complexant Cr(III) reagents which formed more absorbent species. We preferred the complexation reaction between Cr(III) and ethylendiamine (EDTA) because its product is highly coloured. The evolution system was obtained in the same way as when total chromium was determined, evolving Cr(VI) changing pH. However, in this case the system is more complex because three species affect the signal (Cr-EDTA; CrO_4^{2-}; $Cr_2O_7^{2-}$). The advantages of this method are clear since many fields of application often require speciation.

The aim of our work was to set up analytical methods for quickly determining chromium and we had soon provided ourselves with an extensive bibliography, which not only gave us information about the various strategies proposed for resolving these problems but also showed us the numerous ambits in which this analyte can be applied. Therefore, in the fourth paper, entitled *Chromium determination and speciation since 2000*, we describe the state-of-the-art for chromium determination and for speciation by reviewing the bibliography since 2000. We examine the detection techniques, pre-treatments and applications of the various methods. We also discuss the potential of chemometrics for determinations where there is no selectivity and pre-treatments are needed.

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3.2. PAPER

Use of multivariate curve resolution for determination of chromium in tanning samples using sequential injection analysis

V. Gómez, M.P. Callao
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Use of multivariate curve resolution for determination of chromium in tanning samples using sequential injection analysis

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Abstract

We report a method for determining total chromium in tanning samples using sequential injection analysis (SIA) with a diode-array spectrophotometric detector. With a suitable analytical sequence CrO_4^{2-} is converted to $Cr_2O_7^{2-}$ inside the tubes of the SIA system, after total oxidation of chromium(III). A data matrix is obtained and analysed by several chemometric techniques based on multivariate analysis: principal components analysis, simple-to-use interactive self-modelling mixture analysis, and multivariate curve resolution-alternating least-squares. We studied several samples from different stages of a tanning process. Two of these samples were easily oxidized but the others needed more extreme conditions. The analytical sequence prepared, which was based on obtaining a pH gradient and used H_2SO_4 as reagent, is valid and independent of the level of oxidation needed for the sample. We established a calibration model and evaluated the figures of merit. In some samples we found interferents. With this method the amounts of chromium in each sample were quantified and the results were statistically similar to those obtained by use of the reference method, atomic absorption spectrometry.

Keywords: Chromium; SIA; MCR; Tanning sample

1. Introduction

Chromium is a toxic heavy metal whose toxicity depends on its oxidation state. Cr(VI) is significantly more toxic than Cr(III). The hexavalent form of chromium can be found in different species by varying the pH of the media—CrO_4^{2-} occurs in alkaline media and $Cr_2O_7^{2-}$ in acid media. All species absorb in the UV–visible region.

The leather industries, mostly those engaged in tanning, produce many tons of potentially toxic waste because of the chromium content. Nearly 90% of all the leather produced is tanned using chromium [1]. The preferred tanner is a basic salt of trivalent chromium(III). Hexavalent chromium is not one of the raw materials used in this industry but can be produced by oxidation of Cr(III).

Methods of determination of chromium include flame atomic absorption spectrometry (FAAS) [2, 3–5], inductively coupled plasma atomic emission spectrometry (ICP–AES) [3, 6–8], adsorptive stripping voltammetry (ASV) [9, 10], and electrothermal atomic-absorption spectrometry (ET AAS) [11]. Only the colorimetric method with DPC (1,5-diphenylcarbohydrazide) has been reported for determining total Cr in the final effluent from a leather tannery, however [12].

Flow systems such as that we propose have some of the following advantages over the techniques listed above: high level of automation, high frequency of analysis, and a low consumption of reagents [13]. With a diode-array spectrophotometric detector, SIA, which was introduced by Ruzicka in 1990 [14], is an easy way to generate second-order data [15, 16]. Obtaining such data requires an evolving system, so the sample components must evolve in the reaction pipe.

In this paper we propose an SIA system with a diode array spectrophotometric detector, with second-order calibration, for determination of chromium in tanning water samples. Obtaining and treating second-order data (one matrix per sample) provides information about the presence of interferents in the sample and the analyte of interest can be quantified even when these are present [17].

The method involves oxidation of the sample, preparation of the analytical sequence, and calibration. Our procedure is first to oxidize the chromium of the samples and then to induce the conversion of CrO_4^{2-} to $Cr_2O_7^{2-}$, by means of the interdiffusion process between the acid medium and the sample when the reagents pass through the channel to the detector. When multivariate curve resolution (MCR) is applied to ordered data matrices, a profile in each order (concentrations and spectra) of the data matrix is recovered for each component in the mixture.

We applied this method to tanning samples and determined the total chromium concentration in the presence of interferents of unknown nature. We checked our results with others from atomic absorption spectrometry (AAS).

2. Experimental

2.1. Reagents and samples

Sequential injection analysis

Standards of Cr(III) and both species of Cr(VI) were prepared from appropriate amounts of $Cr(NO_3)_3$, K_2CrO_4, and $K_2Cr_2O_7$, obtained from Probus. Hydrogen peroxide and sodium hydroxide standards were also prepared from appropriate amounts of those reagents (from Prolabo). All solutions were prepared in purified water from a Milli-Q water system from Millipore.

AAS standards

Standards of Cr(III) were prepared by dilution of appropriate volumes of a 1000 mg L^{-1} standard of Cr(III) for atomic absorption analysis from Merck. All standards were acidified with 1% sulphuric acid. Standards ranged from 0–10 mg L^{-1}.

Tanning samples

These were provided by Escola d'Adoberia de Igualada (Spain) and corresponded to different steps in the tanning process:

Type A: samples 1 and 2 were obtained from waters from the initial tanning process.

Type B: sample 3 was obtained in the neutralization step, which took place after the first stage of the tanning process.

Type C: samples 4 and 5 were obtained from waters in which the second tanning step is carried out with another product, which in this case was an aldehyde.

Type D: sample 6 was obtained from waters obtained from the later dyeing step.

Type E: sample 7 was obtained from the step that involved washing the skins with products that provide skins with a waterproof effect.

The samples had to be filtered and some also had to be centrifuged because they contain solid wastes from the black dyes.

2.2. SIA manifold and measurement conditions

The sequential injection analyzer comprised a CAVRO XL 3000 stepper motor-driven syringe pump connected to a PC with an RS-232 interface; A six-position Eurosas EPS 1306 BPB automatic valve connected to the computer through a PCL-711S PC–Lab-Card; Omnifit PTFE tubing reaction coil: 70 mm × 0.8 mm; holding coil: 200 mm × 0.8 mm; an HP8452A diode-array spectrophotometer controlled by an HP-IB IEFE 488 interface for communications; a Hellma 178.711QS flow-through cell. A schematic diagram of the analyzer is shown in Figure 1.

Spectra were recorded from 275 to 650 nm in 2-nm steps.

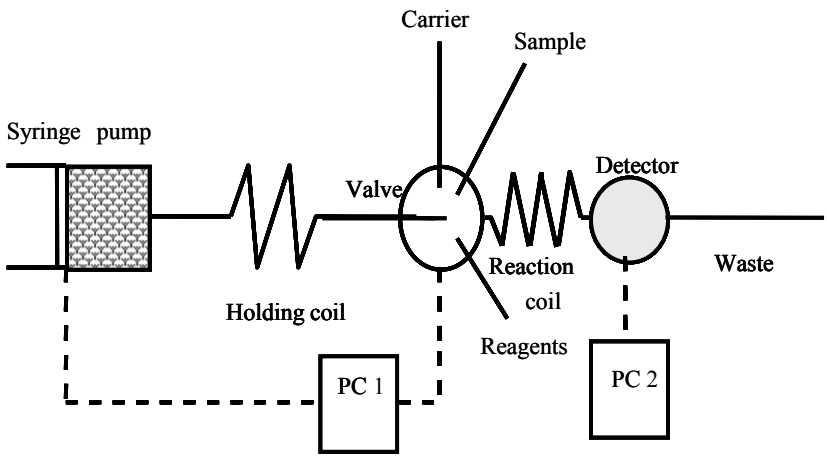

Figure 1. Schematic diagram of the sequential injection analyzer

2.3. AAS manifold

A Hitachi Polarized Zeeman AAS was used. This instrument was operated according to the manufacturer's instructions and used a flame of air and acetylene. The flows used were 2.3 L min^{-1} for the fuel and 15.0 L min^{-1} for the oxidant. The instrument works with a chromium lamp at a wavelength of 359.3 nm; the slit width was 1.30 nm.

2.4. Software

HP89531A software was used to record and store the spectra. Customized software was used to control the SIA.

All calculations related to multivariate curve resolution with alternating least squares (MCR-ALS) were performed with laboratory-written software under a Matlab 5.3 computer environment [18]. This software is available from the authors [19].

3. MCR: data treatment

The multivariate curve resolution method (MCR-ALS) involves decomposing the experimental data into two matrices: a matrix of concentrations and a matrix of spectra. The main steps in the second-order multivariate curve resolution procedure are summarized as follows [20]:

1. Analyse the rank of the individual or augmented data matrices to determine how many species are present in the sample by inspecting the singular values [21].

2. Make an initial estimate of the concentration profiles or of the pure spectra of these species. These are derived from techniques based either on detecting the ‘‘purest’’ variables [22] or on evolving factor analysis [23].

3. Perform alternating least-squares optimization of the concentration profiles or of the pure spectra obtained in the previous step.

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Several constraints can be applied during the optimization step. These depend on the nature and structure of the data. Whether initial estimations of concentrations or spectra are used, these constraints are [24]:

1. The concentration profiles and UV–visible spectra of species present in the system must be non-negative.
2. If there are areas that contain no species, the concentration profile for these species must be zero.
3. Common species in the augmented data matrices must correspond.
4. The pure spectra of common species present in different runs must be equal.
5. The concentration profiles of common species in different runs must have equal shapes.
6. When initial estimates of spectra are used, another important constraint is that the spectrum of a certain species must remain constant during the ALS optimization.

Using constraints implies improvement of the resolution conditions. Another form of improving the results could be working with augmented matrices which might have any order in common [24].

At the end of the optimization process, the performance of the model can be evaluated from lack-of-fit (*lof*) data as:

$$lof = \sqrt{\frac{\sum_{i,j}(d_{ij} - d_{ij}')^2}{\sum_{i,j} d_{ij}^2}} \qquad (1)$$

where d_{ij} are each of the elements of the raw data matrix or each of the elements of the matrix reconstructed from the principal components selected in the principal component analysis (PCA), and d_{ij}' are the corresponding values calculated after the optimization process (ALS).

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4. Calibration and quantification steps

Quantification of the analyte is possible when the proposed ALS optimization method is simultaneously applied to standard data matrices and data matrices of unknown mixtures, arranged together in an augmented data matrix.

To establish a calibration model, we used a set of calibration standards of known concentrations in the linear range and a standard solution of constant composition, which we called the reference standard.

Calibration was performed using column-wise augmented data matrices. Figure 2 shows the arrangement of the matrices. Two procedures are possible. The first involves working with as many augmented matrices as there are calibration standards (see Fig 2a, where matrices $\mathbf{D}_1$ correspond to different calibration standards). The second involves constructing a single matrix with all the calibration standards (see Figure 2b, where $\mathbf{D}_i$ corresponds to different calibration standards). In all cases the matrix on the bottom corresponds to the reference standard, the concentration of which is constant.

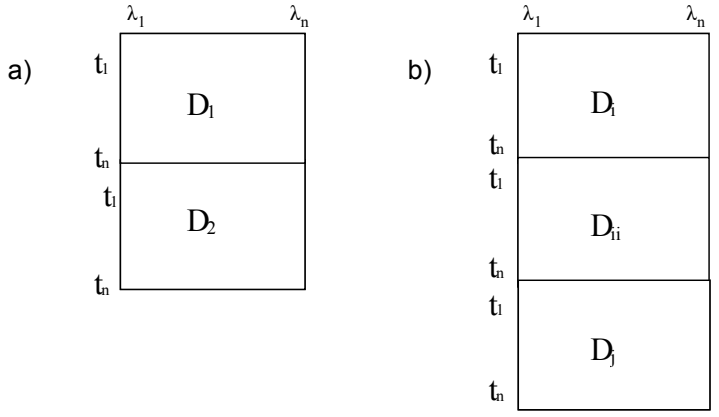

Figure 2. Arrangements of spectra generated in the various experiments corresponding to augmented matrices in the wavelength direction. a) Augmented matrices using one calibration standard and one reference standard. b) Augmented matrices using all the calibration standards and one reference standard

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After applying the ALS algorithm, we obtained the areas of the concentration profiles of all species. From these we obtained the relative area r_i of both species of chromium, CrO_4^{2-} and $Cr_2O_7^{2-}$:

$$r_i = \frac{a_i}{a_{rst}} \qquad (2)$$

where a_i and a_{rst} are the areas of the chromium species in the sample ''*i*'' and in the reference standard ''*rst*'', respectively.

To determine the value of r_i in Eq. 2, two aspects must be considered:

1. The areas for the two chromium species had to be taken into consideration because their relative magnitude depends on the pH of the sample, which is difficult to control.

2. In solutions with the same concentration of chromium, the area for dichromate was half the size of the area for chromate, so for determination of total chromium both areas must be taken into consideration, chromate and dichromate.

Therefore, a_i and a_{rst} in Eq. 2 were obtained by considering the area of chromate plus twice the area of dichromate.

The concentration of the reference standard will always be the calibration standard with the highest concentration [25]. From the values of r_i obtained from the standards a univariate linear regression was established as:

$$r_i = b_1 c_i + b_0 \qquad (3)$$

where c_i is c_{st}/c_{rst}, in which c_{st} is the concentration of the standards and c_{rst} is the concentration of the reference standard, and b_1 and b_0 are the parameters of the regression line.

The value of the concentration of the analyte in the sample, c_s, was obtained from its corresponding r_i value and the calibration parameters in accordance with the expression:

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$$c_s = \frac{r_i - b_0}{b_1} c_{rst} \qquad (4)$$

5. Results and discussion

First we explored the spectroscopic behaviour of both species of interest, chromate and dichromate. The spectra of these species are shown in Figure 3. Absorbance was linear for concentrations of Cr up to 100 mg L^{-1}.

The process for oxidation of Cr(III) to chromate was established with 100 mg L^{-1} Cr(III) standards. Oxidation was regarded as complete when the area of the peak, obtained in the resolution process from oxidation of Cr(III) was the same as that of a 100 mg L^{-1} CrO_4^{2-} standard. Our working conditions were: 5 mL H_2O_2 0.05 mol L^{-1} and 0.75 mL NaOH 0.5 mol L^{-1} in total volumes of 25 mL.

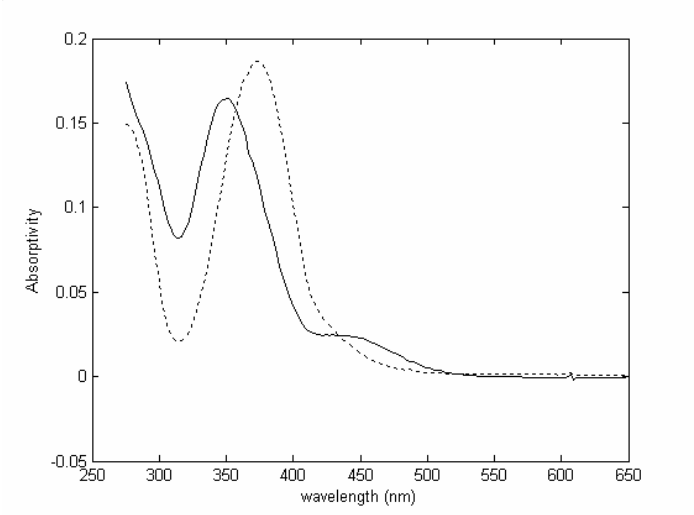

Figure 3. Spectra of CrO_4^{2-} (dotted line) at pH 7 and $Cr_2O_7^{2-}$ (continuous line) at pH 3 for a 100 mg L^{-1} solution of chromium

To establish an analytical sequence so that the chromate, CrO_4^{2-}, would change gradually into $Cr_2O_7^{2-}$ in the channel, we chose the order of aspiration of the reagent in Figure 4a: first the sulphuric acid and then the sample. For the reversed step, Figure 4b, the sample

goes through the detector in the reaction coil and a selective area of chromate could be obtained and the dichromate species then appeared.

We chose the working conditions with regard to flow rate and volume of acid and sample in accordance with operational restrictions (length of tubes, volume of syringe, etc.) and previous knowledge. The flow rate was therefore 1.5 mL min^{-1} and the volumes of the sample and sulphuric acid were the same, 0.21 mL. We optimized the acid concentration by studying several concentrations of H_2SO_4 in the 0.005–0.025 mol L^{-1} range.

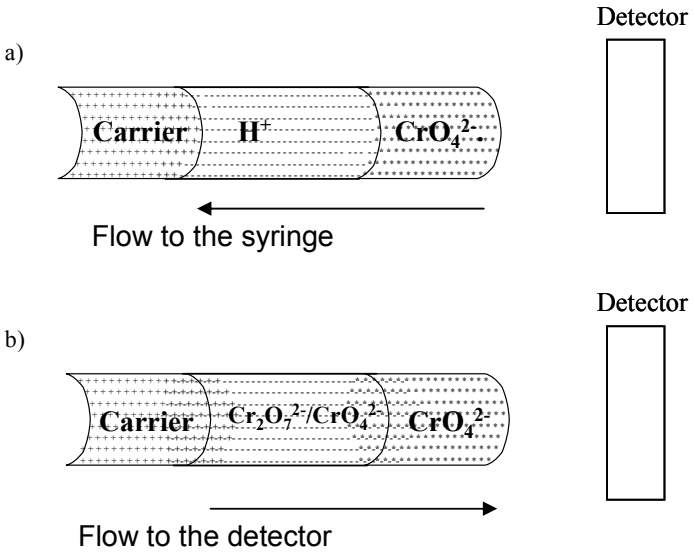

Figure 4. Schematic diagram of the process for mixing sample, reagent, and carrier in the channel of the SIA system. a) time 0, interdiffusion has not taken place, and b) time t, some interdiffusion has taken place.

We applied the curve-resolution algorithms to the data matrix obtained from these experiments. We used SIMPLISMA to estimate pure spectra and applied the ALS optimization program with the following restrictions: non-negativity for concentration profiles and spectra; unimodality for concentration profiles. For all concentrations of sulphuric acid we observed both species of Cr(VI) and, as expected, as the acid concentration increased the dichromate area increased and the chromate area decreased. This was evaluated from the areas corresponding to each species obtained during the resolution process. As an example, Figure 5 shows results from the resolution of two matrices analysed together, for

100 s using H_2SO_4 0.005 mol L^{-1} and from 100 to 200 s using extremely acidic conditions, H_2SO_4 0.025 mol L^{-1}, which explains the larger dichromate area in this case.

The relative areas of the chromium species, CrO_4^{2-} and $Cr_2O_7^{2-}$, depend on the reagent acid concentration and also on the initial pH of the sample.

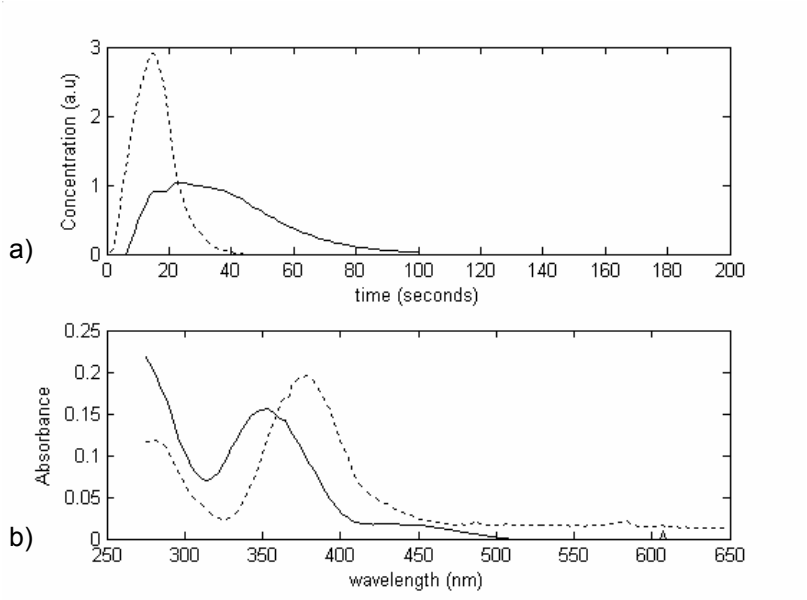

Figure 5. Normalized concentration profiles (a) and pure spectra (b) obtained by multivariate curve resolution of the analysis of an augmented data matrix, using left sulfuric acid 0.005 mol L^{-1} and right 0.025 mol L^{-1}. Dotted line: CrO_4^{2-} ; continuous line $Cr_2O_7^{2-}$. (u.a. are arbitrary units of concentration).

We considered a suitable sequence to be one that provided areas of both species of the same order of magnitude, because this would be more robust to slight changes in the initial pH of the sample. A suitable sequence was obtained with an acid concentration of 0.005 mol L^{-1}.

In the next step, we applied the proposed procedure, oxidation and SIA, to samples from the tannery. We observed chromium only in samples of type A. In the other samples we

did not observe the characteristic yellow of chromate, so we concluded that chromium was not oxidized.

For samples of type A resolution as shown in Figure 5 was obtained. If we choose two principal components we can see the two species of interest. This resolution is similar to that obtained with the standard even though the initial pH was different.

Figure 6 shows the behaviour of a sample of type D. Although in the corresponding solution we did not observe the characteristic yellow colour of CrO_4^{2-}, we applied the analytical sequence and resolved the data matrix in order to observe the spectral characteristics of the sample. As expected, because of the colour observed, we could not discern the spectra of any of the chromium species, but other components could be seen. Those presented important signals in the first wavelengths and could be overlapped with those of CrO_4^{2-} and $Cr_2O_7^{2-}$.

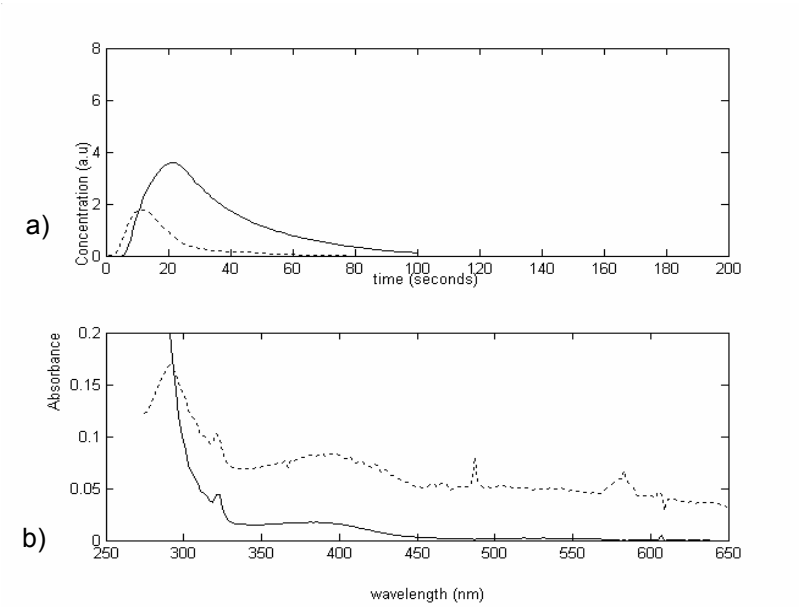

Figure 6. Normalized concentration profiles (a) and pure spectra (b) obtained by multivariate curve resolution from analysis of a sample of type D. (u.a. are arbitrary units of concentration).

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In both cases we checked the resolution by adding one more component to those already evaluated. The spectrum of this component was the same as one of the existing components, so the number of relevant components was established. We considered the fit of the models to the experimental data, evaluated by lack of fit, to be satisfactory. In the type D sample we observed no Cr(III) even though it produced a signal in the UV–visible. This was because of the low sensitivity for these species in the UV–visible.

From these results we concluded that, in these latter samples, which we were not able to oxidize, there was a more potent reducing agent than chromium, because the hydroxide peroxide content was consumed in another reaction. We therefore treated the samples in more extreme reactant conditions. We achieved total oxidation with the same NaOH conditions and H_2O_2 5 mol L^{-1}.

Figure 7, obtained from the type D sample, shows the presence of chromate and also, therefore, the presence of dichromate. If we compare this resolution with that from the sample we were unable to oxidize (Figure 6), we can see that, because of the high peroxide concentrations, one of the two initial components has been converted into the other, and the species related to chromium has appeared.

We should also mention that the sequence chosen for the SIA system was the same as before, so only variation of the oxidation conditions was required, because conversion of chromate into dichromate was readily achieved under the same conditions.

We performed the calibration by following the two schemes shown in Figure 2. The results were similar. We eventually used the first configuration, because it was easier to calculate.

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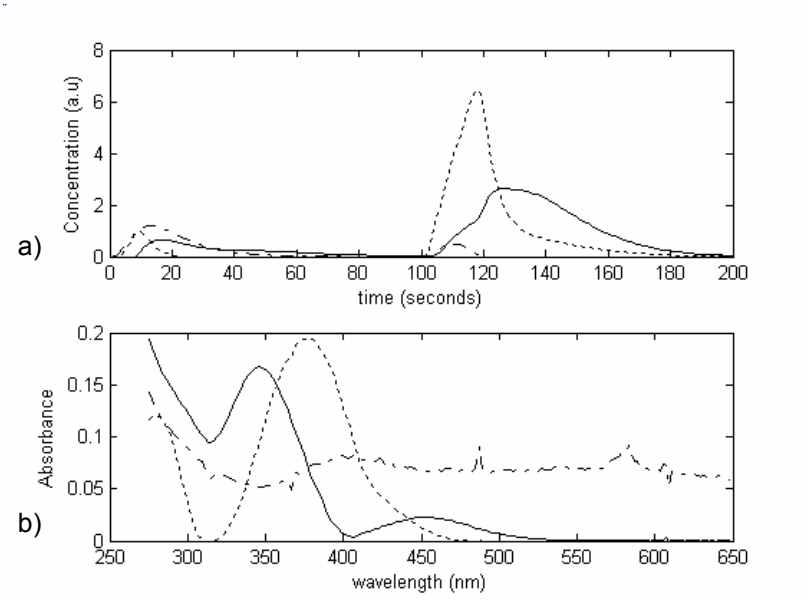

Figure 7. Normalized concentration profiles (a) and pure spectra (b) obtained by multivariate curve resolution from analysis of an augmented matrix with the sample of type D oxidized under extreme conditions and a standard of 100 mg L^{-1} chromium and selecting three relevant components. Dots: CrO_4^{2-}; continuous line: $Cr_2O_7^{2-}$; broken line: interference.

The statistical data and figures of merit are shown in Table 1. Ideally, the slope should be unity and the intercept should be zero because, when the relationship between the concentrations is unity, the relationship between the areas should be unity and this proportionality should always be maintained. Our results were close to the ideal situation and passed a joint comparison test of intercept and slope with regard to a slope of unity and an intercept of zero [26].

The limit of detection was estimating by considering the uncertainty of the calibration model in accordance with the recommendations of the AOAC [26, 27]. Therefore, we present two values, the critical value x_c, which considers only the probability of error α (α=0.05), and the detection level x_d which considers probabilities of error α and β (α=β=0.05).

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Table 1. Statistical parameters and figures of merit of the linear calibration obtained from MCR-ALS.

Working range	10 - 100 mg L^{-1}
Intercept	$7{,}79 \cdot 10^{-2}$
Slope	0,9348
Correlation coefficient	0,9978
n	8
x_c	4 mg L^{-1}
x_d	8 mg L^{-1}
Standard deviation of the residuals	$2{,}07 \cdot 10^{-2}$
Standard deviation of intercept	$2{,}51 \cdot 10^{-2}$
Standard deviation of the slope	$1{,}45 \cdot 10^{-2}$

We followed the same procedure to analyse the tanning samples. We augmented the matrix corresponding to the sample with that of the reference standard, following the scheme in Figure 2a, where $\mathbf{D}_1$ is the matrix of the sample. Depending on the samples, the resolution indicated two or three principal components corresponding to the two species of chromium and an interferent.

Table 2 shows the results from our analysis of the tanning samples by the reference method, AAS [27], and the proposed method. The uncertainty is associated with the calibration line for an α level of 0.05 and later application of the corresponding dilution factors. We can see that the uncertainties were either similar or lower for the proposed method. These results were attributed to the dilution factors, which are greater for the AAS method.

Table 2. Concentration of chromium by two methods: the AAS and the SIA method

Tanning samples	nº runs		Concentration (mg L^{-1})	
	AAS	SIA	AAS	SIA
1	3	3	1020 ± 50	980 ± 40
6	3	6	43 ± 2	38 ± 1,3
7	3	5	20 ± 1,7	19,1 ± 0,8
2	3	4	1450 ± 90	1170 ± 50
3	3	4	440 ± 18	420 ± 19

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To compare these results, we performed linear regression to represent the values from the reference method (AAS) on the x-axis and the values from the proposed method on the y-axis and carried out a joint test of slope and intercept with respect to a slope of unity and an intercept of zero [28]. The experimental F value was 5.25 and the F value for a 95% level of confidence was 16.04, which indicates that the values were statistically similar.

6. Conclusions

SIA with diode-array spectrophotometric detection enables us to quantify chromium in tanning samples. After oxidation of the samples, we achieved a suitable analytical sequence to obtain a data matrix for each sample. Chemometric methods can then be applied to obtain the concentration profiles and the pure spectra of the components, which absorb in this region of the UV–visible. We can quantify this analyte of interest from the relationship between the response and that from a reference standard of known concentration, whether or not there are interferents in the sample. The results obtained with this method are statistically similar to those from the reference method (AAS).

The advantages of using this system of injection analysis are that automation is easy, the frequency of analysis is high (25 samples h^{-1}, taking into consideration all the cleaning steps), and the consumption of the reactants is low.

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References

[1] K. Stein, G. Schwedt, *Fresenius J Anal. Chem*. 350 (1994) 38.

[2] R.M. Cespón-Romero, M.C. Yebra-Biurrun, M.P. Bermejo-Barrera, *Anal. Chim. Acta* 327 (1996) 37.

[3] American Public Health Association, Standard methods for the examination of water and wastewater, (1998) 20th edn. Washington, USA, pp 3–66.

[4] A. Sahuquillo, J.F. López-Sánchez, R. Rubio, G. Rauret, V. Hatje, *Fresenius J Anal. Chem*. 351 (1995) 197.

[5] B. Pasullean, C.M. Davidson, D. Littlejohn, *J. Anal. At. Spectrom*. 10 (1995) 241.

[6] J.L. Manzoori, F. Shmirani, *J. Anal. At. Spectrom*. 10 (1995) 881.

[7] A.G. Cox, C.W. McLeod, *Microchim. Acta* 109 (1992)161.

[8] Z.S. Horváth, A. Lásztity, I. Varga, E. Mészáros, A. Molnár, *Talanta* 41 (1994) 1165.

[9] J. Wang, J. Lu, K. Olsen, *Analyst* 117 (1992) 1913.

[10] J. Golimowski, P. Valenta, H.W. Nürnberg, *Fresenius J. Anal. Chem*. 322 (1985) 315.

[11] M.I.C. Monteiro, A.K. Avila, R. Neumann, *Anal. Chim. Acta* 428 (2001) 265.

[12] M.I.C. Monteiro, I.C.S. Fraga, A.V. Yallouz, N.M.M. de Oliveira, S.H. Ribeiro, *Talanta* 58 (2002) 629.

[13] G.D. Christian, *Anal. Chim. Acta* 499 (2003)5.

[14] J. Ruzicka, G.D. Marshall, G.D. Christian, *Anal. Chem*. 62 (1990) 1861.

[15] J. Saurina, S. Hernández-Cassou, R. Tauler, *Anal. Chim. Acta* 335 (1996) 41.

[16] A. Izquierdo-Ridorsa, J. Saurina, S. Hernández-Cassou, R. Tauler, *Chemometr. Intell. Lab. Syst*. 38 (1997) 183.

[17] A.K. Smilde, R. Tauler, J. Saurina, R. Bro, *Anal. Chim. Acta* 398 (1999) 237.

[18] Matlab, The Mathworks, South Natick, MA, USA

[19] R. Tauler, A. de Juan, Multivariate Curve Resolution home page, http://www.ub.es/gesq/mcr/mcr.htm.

[20] J. Saurina, S. Hernández-Cassou, R. Tauler, *Anal. Chim. Acta* 312 (1995) 189.

[21] J. Saurina, S. Hernández-Cassou, R. Tauler, *Anal. Chim. Acta* 335 (1996) 41.

[22] J.M. Phalp, A.W. Payul , W. Winding, *Anal. Chim. Acta* 318 (1995) 43.

[23] E.R. Malinowski, *Factor analysis in chemistry*, (1991) 2nd edn. Wiley, New York.

[24] A. Izquierdo Ridorsa, J. Saurina, S. Hernández-Cassou, R. Tauler, *Chemometr. Intell. Lab. Sys*. 38 (1997)183.

[25] A. Pasamontes, M.P. Callao, *Anal. Chim. Acta* 515 (2004) 159.

[26] D.L. Massart, B.G.M. Vandeginste, L.C.M. Buydens, S. de Jong, P.J. Lewi, J. Smeyers-Verbeke, *Handbook of chemometrics and qualimetrics Part A*. (1997) Elsevier, Amsterdam.

ISBN: 978-84-691-0990-8/D.L: T.2293-2007

[27] *Reagent chemicals: American Chemical Society specifications,* 7th edn. (1986) American Chemical Society, Washington.

[28] G.T. Wernimont *Use of statistics to develop and evaluate analytical methods*. (1987) AOAC, Arlington.

3.3. PAPER

Factorial design for optimizing chromium determination in tanning wastewater

V. Gómez, A. Pasamontes, M.P. Callao

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Factorial design for optimizing chromium determination in tanning wastewater

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Abstract

A totally automatized procedure for determining chromium by sequential injection analysis (SIA) linked to multivariate curve resolution with alternating least squares (MCR-ALS) is proposed. With this system Cr(III) is oxidized to chromate (Cr(VI)) and this form is then converted to dichromate in order to obtain second-order data. The experimental design method was used to establish the best conditions. The identification of the most influential factors was validated using ANOVA tests. We used this method to successfully analyse total chromium in several aqueous tannery samples from various steps of a tanning process. The results from this method and those from the atomic absorbance spectroscopy (AAS) method are comparable. Sample frequency was 30 samples h^{-1}.

Keywords: Chromium; Tanning samples; Sequential injection analysis; Second-order data; Experimental design

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1. Introduction

Tanning agents bring about permanent stabilization of the skin matrix against biodegradation. This industry has gained a negative image in society with respect to its pollution potential and therefore is facing a severe challenge. The unit processes that cause tanners the most difficulty with regard to perceived environmental impact are unhairing and chrome tanning [1]. Basic chromium sulphate (BCS) is a tanning agent, which is employed by 90% of the tanning industry. Chromium content in wastewater tanning samples ranges between 0.5 and 30 g L^{-1} depending on the treatment [2]. Chromium in its trivalent form is an essential trace element when present at the micro level [3], whereas the same when present in excess is proven to be a potential soil, surface water, groundwater, and air contaminant under specific conditions [4]. Considering the large amounts applied and the low biodegradability of such chemicals, tannery wastewater treatment represents a serious environmental and technological problem [5]. For economic reasons, and also to ensure compliance with environmental regulations, it is interesting to determine the chromium content at the various stages of the tanning processes.

The reference method for determining chromic oxide [6] usually takes up to several hours. Several alternatives [7–9] have been reported for the determination of total chromium in final effluent from a leather tannery, but these processes are slow and laborious and require large volumes of reagents. Some methods based on spectroscopy and multivariate calibration [10] are promising alternatives to the reference method. However, multivariate calibration is costly to implement and must be valid over time.

In this paper we propose to determine chromium using sequential injection analysis (SIA) and chemometric tools of second-order data treatment. SIA was developed by Ruzicka and co-workers in 1990 [11–13]. These systems are versatile and efficient for automatization and miniaturization, the frequency of analysis can be high and the consumption of samples and reagents is low. Thanks to these advantages, SIA has been used in a wide range of applications, for example in environmental, food, pharmaceutical and bioprocessing fields.

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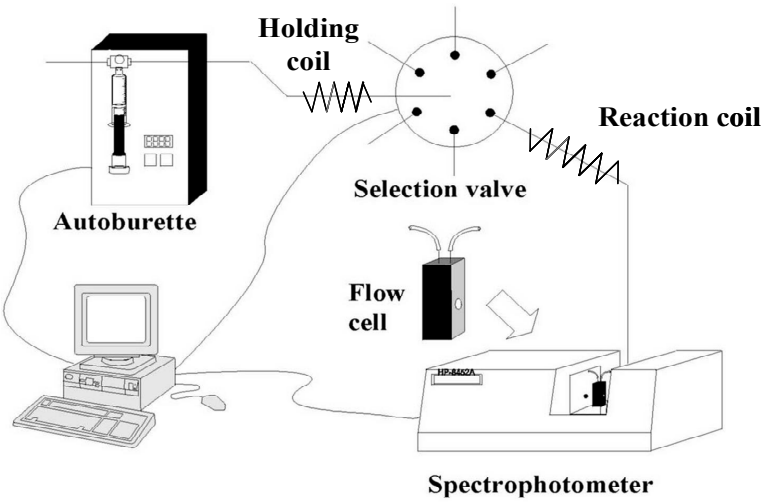

Figure 1. Scheme of a sequential injection analyzer. Dotted lines are computer connections.

The basic configuration of a SIA system is shown in Figure 1. The valve selects the carrier, the sample and reagent volumes to be sequentially aspirated towards the holding coil. The flow is then reversed and the valve is switched to direct the processed sample towards the detector. During this process, the sample and reagents are mixed by interdiffusion. By the time they reach the detector, the sample and the reagents will have reacted either partially or totally. If they have reacted partially, the signal that reaches the detector depends on the measurement times (t_1, t_2, t_3, etc.). If the detector used is a UV–visible diode–array detector (or any other multivariate detector) that obtains absorbance at multiple wavelengths ((λ_1, λ_2,…, λ_n), and the signal is picked up at different times, a data matrix (also called second-order data) is obtained. The columns of this data matrix represent the wavelengths and the rows represent the times at which the measurements were taken.

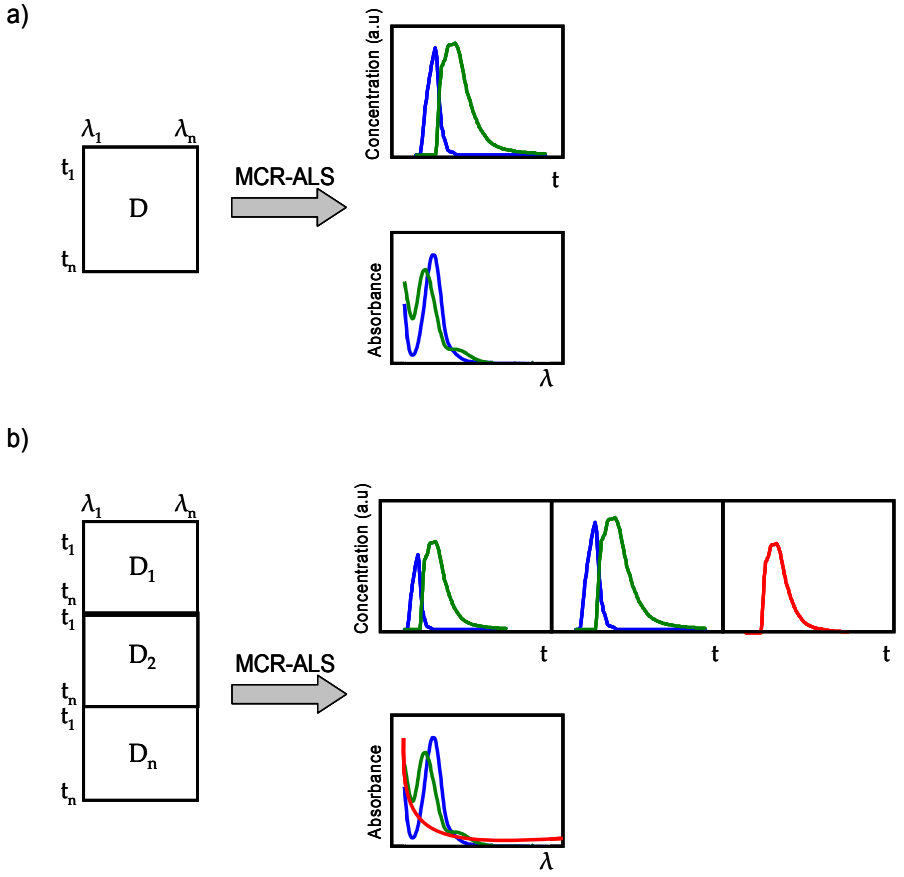

Figure 2. MCR-ALS procedure working with a single matrix of data (a) or with an augmented matrix (b) maintaining the order of the columns.

The aim of second-order data chemometric techniques as multivariate curve resolution with alternating least squares (MCR-ALS) [14,15] is to decompose the raw data matrix into the product of two matrices. This process is shown in Figure 2a. One matrix provides information about concentration profiles and the other provides information about the spectra profiles of all the components in the system. Working with this type of data has the "second-order advantage" [16], which permits to identify and quantify the components of a sample when unknown interferents are present.

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In this paper we pretend to determine total chromium in tanning samples introducing reagents and sample sequentially into the SIA system as is shown in Figure 3. The global process is made up of two steps:

$$Cr(III) + H_2O_2 + NaOH \longrightarrow CrO_4^{2-} \xrightarrow{H_2SO_4} CrO_4^{2-}/Cr_2O_7^{2-}$$

In the first stage, Cr(III) should react totally. The introduction of H_2SO_4 should produce a pH gradient that allows the partial conversion of CrO_4^{2-} to $Cr_2O_7^{2-}$ so that, by measuring the absorbance at different times and different wavelengths, a data matrix is obtained. In the literature we found reports of the oxidation of Cr(III) in basic medium with H_2O_2 using FIA systems [17] and of the determination of Cr(VI) either using an SIA system with a total reaction [18,19] or with the generation of a system in evolution [20]. However, we have found no reports of joint studies. Advantages of our proposed method are that the consumption of the reagents and the sample frequency are lower.

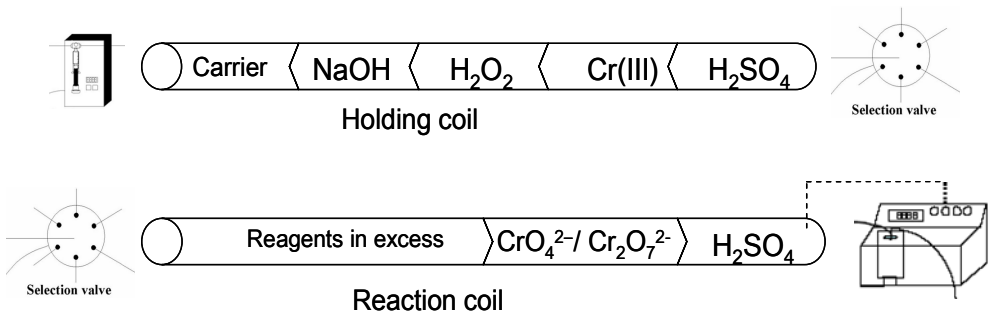

Figure 3. Scheme of the process for mixing sample, reagents and carrier in the channel of the SIA system.

Since the entire process for the total oxidation of Cr(III) and partial conversion of CrO_4^{2-} into $Cr_2O_7^{2-}$ can depend on many factors – such as the concentrations and volumes of the reagents, the waiting time (time before the solution is switched towards the detector), the flow and the sample volume – we propose applying experimental designs in order to optimize the global process. The identification of the most influential factors was validated using ANOVA tests.

2. Materials and methods

2.1. Reagents and samples

Reagents

All chemicals and solvents were of analytical grade and used without further purification unless otherwise mentioned. Stock solutions of chromium (III) and both species of chromium (VI) – chromate and dichromate – were prepared separately by dissolving the accurate weight of the respective reagents, $Cr(NO_3)_3$, K_2CrO_4 and $K_2Cr_2O_7$ from Probus S.A., in water. Hydrogen peroxide and sodium hydroxide standards were prepared from the required amounts of these reagents, obtained from Prolabo, and then dissolved in purified water. Water was used as carrier (from a Milli-Q water system from Millipore).

Tanning samples

The tannery wastewater used was the effluent of a mechanical and chemical industrial wastewater treatment plant in Igualada (Spain). The samples belonged to different steps in the tanning process: the initial tanning process, the neutralization step, the second dyeing step, the latter dyeing step, the cleaning process before the second dyeing step, and the bathing step with waterproof products. Each step produces samples with different compositions and chromium contents.

2.2. SIA manifold and software

The equipment used for the SIA operations comprised a stepper motor-driven syringe pump (CAVRO XL 3000) used as a liquid driver and a 6-position automatic valve (Eurosas EPS 1306 BPB) used as a reagent or sample selector. As a detector, a diode–array spectrophotometer (HP8452A, Hewlett-Packard, Waldbronn, Germany) equipped with a flow-through cell (Hellma 178.711QS) was used. All tubing was Omnifit PTFE of 0.8 mm id. The lengths of the holding and reaction coils were 2 and 0.7 m, respectively. The spectra were registered from 290 to 650 nm in 2 nm steps.

The syringe pump, automatic valve and data acquisition provided by the spectrophotometer were performed by a personal computer (PC) via an RS-232 interface, PCL-711S PC-Lab-Card and HP-IB IEEE 488 interface for communications, respectively.

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The data provided by the spectrophotometer were acquired and controlled using HP89531A software. Instrumentation was controlled by customized software.

All calculations related to multivariate curve resolution with alternating least squares (MCR-ALS) were performed with laboratory-written software under a MATLAB 5.3 computer environment [21]. This software is available from the authors [22].

2.3. Data treatment

We applied the MCR-ALS method to augmented data matrices. Augmented data matrices are the result of an arrangement of matrices with one or two orders in common as in Figure 2b. We use augmented data matrices to add extra information to the system in order to eliminate possible rank deficiency and break rotational ambiguity. Moreover, when one of the matrices corresponds to a reference standard with a known concentration, it is possible to quantify an analyte by establishing calibration curves that relate the quotients of areas from the concentration profiles with the quotients of concentrations. We obtained augmented data matrices by maintaining the columns (absorbance at each wavelength) in common as in Figure 2b. Normally, augmented data matrices are built arranging a matrix of the experiment in the study ($\mathbf{D}_1$), a matrix of a standard of chromium ($\mathbf{D}_2$) and a matrix with the contribution of the reagents ($\mathbf{D}_3$). The obtained results show the concentration profiles of the different initial matrices and a spectral profile containing the species absorbent in the media.

In the oxidation optimization step, the augmented data matrices were made up of: 1) a matrix obtained from the process of chromium (III) oxidation, 2) a matrix obtained from a standard of CrO_4^{2-} and 3) a matrix with the contribution of the reagents used in the oxidation. The data obtained from the concentration profiles was used in the following equation to obtain the percentage of oxidation:

$$\%\text{Oxidation} = \frac{a_1}{a_2} \cdot \frac{c_2}{c_1} \cdot 100 \qquad (1)$$

where a_1 and a_2 are the areas of CrO_4^{2-} obtained from matrix 1 and 2, respectively and c_1 and c_2 are the corresponding concentrations.

In the calibration step we obtained data matrices applying the whole analytical process (oxidation and acidification) to different Cr(III) standards of known concentration. The resolution was performed using column-wise augmented data matrices formed with 1) each one of the calibration standards, 2) a matrix obtained from a constant reference standard and 3) a matrix with the contribution of the reagents, all obtained under the same experimental conditions. The area obtained in the resolution process is the signal used to establish the following calibration model:

$$r_i = b_1 c_i + b_0 \qquad (2)$$

where r_i is the relative area (quotient of area between the calibration standard and the reference standard) and c_i is the relative concentration (quotient of concentration between the calibration standard and the reference standard).

In the prediction step we use the expression (2) and the experimental r_i obtained from the augmented data matrix formed as in calibration step where matrix 1 is get from the sample.

3. Results and discussion

We began our study of the oxidation process by selecting the factors whose influence on the on-line process is the most unknown: the waiting time and the concentration of the reagents (sodium hydroxide and hydrogen peroxide). We did a first study – experiments corresponding to full factorial design of experiments, 2^3 – to determine the effects of these three factors. We called this set of experiments *Design 1*. The results can be seen in Table 1. The first three columns show the factors, their codified name and the interval studied, respectively. The last columns show the effects of these three factors and of their interactions and the F value obtained after an ANOVA test was applied. We can see that relevant factors are: the concentration of sodium hydroxide and the interaction between the concentration of NaOH and the concentration of H_2O_2. Due to the positive effect of the NaOH concentration and the negative effect of its interaction with the H_2O_2 concentration, the best results are obtained at high levels of NaOH concentration and low levels of H_2O_2 concentration.

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Table 1. Effects (%response) from experimental Design 1 and Design 2 applied to the chromium standards

Factor	Codified name	Range	Effect (%)	F
DESIGN 1				
$[H_2O_2]$[a]	A	0,5-1	-0.09	0.02
[NaOH][b]	B	0,1-0,5	23.13	1375.30
time[c]	C	0-20	1.76	7.98
	AB		-5.66	82.35
	AC		0.85	1.86
	BC		-1.27	4.17
	ABC		0.26	0.17
Maximum obtained response = 71.6				
DESIGN 2				
Vsample[d]	A	0,125-0,208	-9.15	2131.16
[NaOH][b]	B	0,5-1	8.40	1799.61
flow[e]	C	0,5-1,5	0.66	11.00
	AB		-1.41	50.97
	AC		0.45	5.17
	BC		0.56	8.00
	ABC		-0.06	0.09
Maximum obtained response = 98,6 F(0,05, 1, 8)= 7,57				

[a] Hydrogen peroxide concentration in mol L^{-1}

[b] Sodium hydroxide concentration in mol L^{-1}

[c] Waiting time in seconds

[d] Sample volume in mL

[e] Flow in mL min^{-1}

To check whether the concentration range of hydrogen peroxide was unnecessarily high, we studied the responses at lower concentrations than the proposed minimum. The results are shown in Figure 4 (line a). From these results we considered a suitable concentration to be 0.15 M.

Due to the best response obtained with *Design 1* was 71.6%, to achieve total oxidation of chromium (III), we proposed another full factorial design (*Design 2*) in which we expanded the concentration domain of sodium hydroxide and studied other factors, such as the flow and sample volume, that could affect the response. The effects and the ANOVA test of this design can be seen in Table 1 (*Design 2*). These results show that all the effects were important, especially the volume of the sample and the concentration of NaOH, the

interaction of these two factors and, to a lesser extent, the interaction between flow and the concentration of NaOH. These results were expected because the conversion of Cr(III) to Cr(VI) took place in an alkaline medium, so augmenting the NaOH will increase the velocity of the reaction, at least up to a certain concentration of NaOH. Our best experiment had oxidation degrees of almost 100%. In this experiment the sample volume was low and the NaOH concentration and flow were high.

After achieving total oxidation of the Cr(III) standard, the next step was the oxidation of the Cr(III) in the samples because they are known to contain other species, such as dyes or waterproof products. We studied two samples—one from an initial step in the tanning process and one from a more complex step i.e. the latter dyeing step. Under the best conditions obtained for the standards with *Design 2*, we obtained an oxidation of 62.8% for the initial tanning step sample and an oxidation of 39.9% for the latter dyeing step sample.

Table 2. Experimental domain and effects (% response) of full factorial design for the oxidation of two types of samples.

Factor	Codified name	Range	Effect (%)		F	
			Initial tanning step	Latter dyeing step	Initial tanning step	Latter dyeing step
Vsample[a]	A	0,083-0,125	-10.85	-12.37	518.53	308.51
V H_2O_2[b]	B	0,025-0,042	0.09	0.39	0.04	0.30
[NaOH][c]	C	1-1,4	8.13	11.49	291.54	266.53
	AB		0.47	0.04	0.97	0.00
	AC		-1.31	-2.74	7.56	15.15
	BC		-0.36	0.82	0.58	1.37
	ABC		0.48	-0.31	1.00	0.19

F(0,05, 1, 8)= 7,57
Maximum response for the initial tanning step sample = 93,5%
Maximum response for the latter dyeing step sample = 64,3%

[a] Sample volume in mL

[b] Hydrogen peroxide volumen in mL

[c] Sodium hydroxide concentration in mol L^{-1}

Because of the positive effect of the sodium hydroxide concentration and the negative effect of the sample volume, we worked with a full factorial design and changed the domain of these variables (the sample volume and the concentration of sodium hydroxide) in order to increase the oxidation of the samples. We also considered a new variable—the volume of hydrogen peroxide (which may influence the pH of the sample) because of the

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change in the volume in which the sodium hydroxide must spread itself around the sample. Under the best conditions, percentage oxidation was almost total for the initial tanning sample but not for the latter dyeing step sample. Our experimental design and results are shown in Table 2.

Although the effects of the factors may suggest that we could improve the results by increasing the concentration of sodium hydroxide and decreasing the sample volume, we also studied the effect of the concentration of hydrogen peroxide because the sample could contain other reducing agents that consume reagent. Figure 4 (line b) shows that the percentage of oxidation is higher to 90% for a concentration of 1.3 M. We then checked the effect of waiting time on the oxidation of the tanning samples and found that a waiting time of 60 s slightly improved the results, obtaining values of almost 100%.

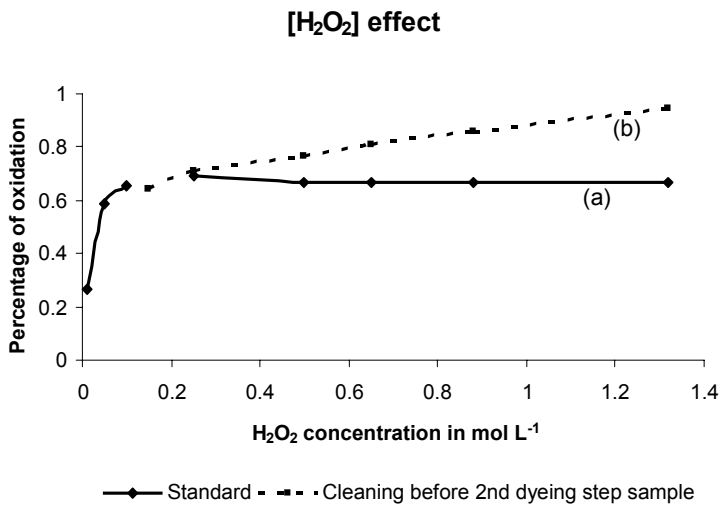

Figure 4. Effect of hydrogen peroxide concentration on oxidation: a) the chromium (III) standard and b) cleaning before second dyeing step sample.

Once total oxidation conditions were achieved (NaOH concentration: 1.4 M, H_2O_2 concentration: 1.3 M, sample volume: 0.083 mL, H_2O_2 volume: 0.042 mL, waiting time: 60 s and flow: 1.5 mL min^{-1}), the next step was the optimization of the global process as described in Figure 3. We performed a full factorial design in which we studied the concentration of

sulphuric acid between 0.05 and 0.1 M and the acid volume between 0.083 and 0.17 mL. We achieved a suitable conversion of chromate into dichromate working with an acid concentration of 0.05 M and an acid volume of 0.083 mL. A resolution of a sample from the bathing step with waterproof products is shown in Figure 5. These corresponded to the two species of interest (chromate and dichromate), one species related to the reagents and some interferents.

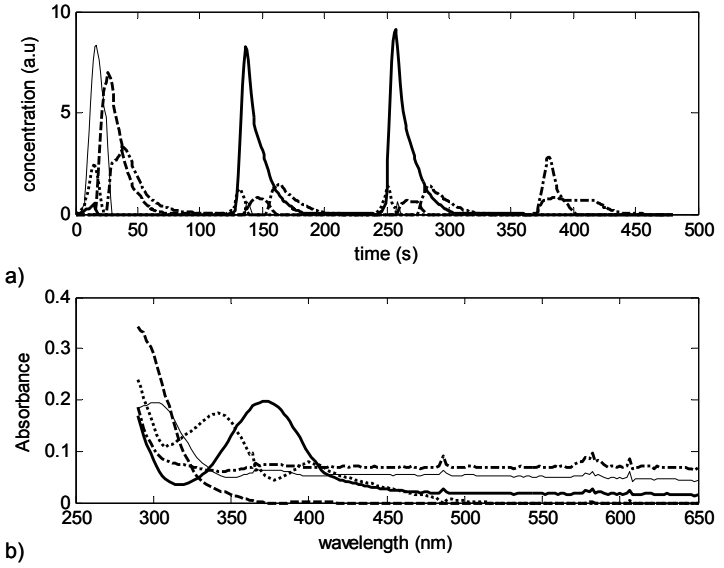

Figure 5. Normalized concentration profiles (a) and pure spectra (b) obtained by multivariate curve resolution of the analysis of an augmented data matrix: [bathing with waterproof products sample; standard matrix of oxidized Cr(III); a matrix of a reference standard of Cr(VI) in the same conditions as Cr(III); the reagent matrix]. (—) CrO_4^{2-}; (…) $Cr_2O_7^{2-}$; (—) unknown species of the sample. The other contributions are from the reagents. (a.u. are arbitrary units of concentration).

To quantify the total chromium present in tanning samples, we then plotted a calibration line as in Eq. 2 using a set of calibration standards of Cr(III) of known concentrations in the established linear range (from 5 to 50 mg l^{-1}). The statistical parameters and figures of merit are shown in Table 3. When we checked the regression line with an ANOVA test of regression, it passed the test. Ideally, the slope should be one and the intercept should be zero because, when the relationship between the concentrations is one, the

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relationship between the areas should also be one and this proportionality should always be maintained. Our results were close to the ideal situation and passed a joint comparison test of intercept and slope for a slope of 1 and an intercept of 0 [23].

Table 3. Statistical parameters and figures of merit of the linear calibration obtained from MCR-ALS.

Working range	5-50 mg L^{-1}
Intercept	0.0754
Slope	0.8797
Correlation coefficient	0.98720
n	14
Standard deviation of the residuals	0.0452
Standard deviation of intercept	0.0243
Standard deviation of the slope	0.041
ANOVA test	
Fcalculated	0.000043
Ftabulated (0.05, 5, 6)	6.978

When we analyzed the tanning samples we obtained the results shown in Table 4, which also shows the chromium concentration obtained with the atomic absorption spectrometry (AAS) method [20]. When the chromium concentration increased, the discrepancy between the results obtained by the AAS method and those obtained by the SIA method and the uncertainty of the results also increased. This was because, before the analysis, these samples were diluted, so when we presented the results for the original samples, they were magnified by the dilution factor.

Table 4. Concentration of chromium obtained by two methods: AAS and SIA

	Concentration (mg L^{-1})	
Tanning samples	AAS	SIA
Initial tanning step	1000±100	1020 ± 50
Neutralization step	370±20	440 ± 18
Second dyeing step	24.4±1.0	24.1 ± 1.7
Latter dyeing step	45±2	43 ± 2
Cleaning before 2ond dyeing step	25.0±1.0	25.0 ±1.7
Bathing with waterproof products	22.7±0.9	20 ± 1,7

We performed a joint test of intercept and slope to compare the two methods. From this test, the F statistic was 0.95. For a 95% confidence level and the stipulated degrees of freedom the tabulated F was 8.43. Our results are therefore comparable to those of the AAS method. The sample frequency for SIA measurements was at least approximately 30 samples h^{-1}.

4. Conclusions

We have developed an automated method for determining chromium in waste tanning samples. This method, which does not require sample pre-treatment, integrates two necessary steps – the oxidation and evolution of the two Cr(VI) species – into one single process.

The experimental design method is a useful technique for finding suitable experimental conditions.

The results for samples from various stages of the tanning process are comparable to those obtained by AAS. The method is quick, the consumption of the reagents, which are in common use, is low and sample frequency is high.

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The authors would like to thank the Spanish Ministry of Education, Culture and Sports (Project BQQ2003-01142) for economic support, the AGAUR, of the Catalan Government, for providing Verónica Gómez with a doctoral fellowship and the College of Industrial Engineering of Igualada (Spain) for supplying the tanning samples.

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References

[1] T. Ramasami, J.R. Rao, N.K. Chandrababu, K. Parthasarathi, P.G. Rao, P. Saravanan, R. Gayatri, K.J. Sreeram, *J. Soc. Leather Technol. Chem.* 83 (1999) 39.

[2] V. Suresh, M. Kanthimathi, P. Thanikaivelan, J.R. Rao, B.U. Nair, *J. Clean. Prod*. 9 (2001) 483.

[3] L. Friberg, G.F. Nordberg, U.B. Work, *Handbook of toxicity of metals*, Elsevier Medical Press, North Holland, 1980.

[4] J.R. Rao, R. Gayatri, R. Rajaram, B.U. Nair, T. Ramasami, *Biochim. Biophys. Acta* 1472 (1999) 595.

[5] C.D. Iaconi, A. Lopez, R. Ramadori, A.C.D. Pinto, R. Passino, *Water Res*. 36 (2002) 2205.

[6] IUC-8, *J. Soc. Leather Technol. Chem*. 82 (1998) 200.

[7] S. Balasubramanian, V. Pugalenthi, *Talanta* 50 (1999) 457.

[8] A. Walsh, O'Halloran, *Water Res*. 30 (1996) 2393.

[9] M.I.C. Monteiro, I.C.S. Fraga, A.V. Yallouz, N.M.M. de Oliveira, S.H. Ribeiro, *Talanta* 58 (2002) 629.

[10] M.D. Borràs, J.M. Morera, A. Rius, R.M. Romero, *J. Soc. Leather Technol. Chem*. 84 (2000) 79.

[11] J. Ruzicka, G.D. Marshall, *Anal. Chim. Acta* 237 (1990) 329.

[12] T. Gubelli, G.D. Christian, J. Ruzicka, *Anal. Chem*. 63 (1991) 2407.

[13] G.D. Christian, *Anal. Chim. Acta* 499 (2003) 5.

[14] A. de Juan, Y. van der Heyden, R. Tauler, D.L. Massart, *Anal. Chim. Acta* 346 (1997) 307.

[15] R. Tauler, A. Izquierdo, E. Cassasas, *Chemometr. Intell. Lab. Syst*. 18 (1993) 293.

[16] K.S. Booksh, B.R. Kowalski, *Anal. Chem*. 66 (1994) A782.

[17] J.E.T. Andersen, *Anal. Chim. Acta* 361 (1998) 125.

[18] Y. Luo, S. Nakano, D.A. Holman, J. Ruzicka, G.D. Christian, *Talanta* 44 (1997) 1563.

[19] P.C.C. Oliveira, J.C. Masini, *Analyst* 123 (1998) 2085.

[20] V. Gómez, M.P. Callao, *Anal. Bioanal. Chem*. (2005) 328.

[21] Matlab, The Mathworks, Sourth Natick, MA, USA.

[22] R. Tauler, A. de Juan, Multivariate Curve Resolution homepage, http:// www.ub.es/gesq/mcr/mcr.htm.

[23] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. de Jong, P.J. Lewi, J. Smeyers-Verbeke, *Handbook of Chemometrics and Qualimetrics. Part A*, Elsevier, 1997.

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3.4. PAPER

Chromium speciation using sequential injection analysis and multivariate curve resolution

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Chromium speciation using sequential injection analysis and multivariate curve resolution

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Abstract

In this paper we develop a suitable method for the speciation of chromium in tanning and environmental water samples. We use sequential injection analysis (SIA) with a diode array detector and chemometric tools such as multivariate curve resolution with alternating least squares (MCR-ALS) to determine Cr(III) and Cr(VI) species. Although Cr(III) is an absorbent species, its sensitivity is much lower than that of Cr(VI). To increase its sensitivity, therefore, it was complexed with ethylenediaminetetraacetic acid (EDTA).

This method involves generating a pH gradient in the system reactor that converts dichromate into chromate in such a way that, when the sample reaches the detector, selective areas are observed and a data matrix is obtained. Applying MCR enables Cr(III) and Cr(VI) to be successfully determined simultaneously in tanning and environmental wastewater samples.

Keywords: Chromium speciation; Sequential injection analysis; Multivariate curve resolution; Tanning and environmental samples

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1. Introduction

Chromium is found in several chemical forms with oxidation numbers that range from zero (free metal) to six (chromate and dichromate). Only the trivalent (III) and the hexavalent (VI) forms are stable enough to be found in the environment [1]. Because of the different toxicities of Cr(III) and Cr(VI) (the former, an essential micronutrient [2] and the latter, a high toxic element [3]), it is important to determine them separately as well as to determine the total chromium content [4]. This difference in toxicity is one of the main reasons for the enormous recent development of analytical methods for differentiating the various forms of chromium in the medium of interest [5–13].

Several possibilities for the speciation of Cr(III) and Cr(VI) in aqueous solutions exist. One is to complex and selectively extract both Cr(III) and Cr(VI) before instrumental analysis [14–20]. The other is to analyze one of the two species and the total chromium and then calculate the concentration of the other species by subtraction [21–24]. Recent studies [25–27] have proposed retaining both species of chromium on different sorbents and then eluting them specifically.

In this study we propose an analytical method for the simultaneous speciation of chromium in a single process using sequential injection analysis (SIA) with a UV–visible detector array and second-order data treatment.

SIA, developed by Ruzicka and Marshall in 1990 [28] has great potential for on-line measurements. It is a simple technique and sample manipulations can be easily automated. It is also highly versatile, both in terms of instrumental configuration and in terms of the type of data it can provide, which ranges from zero-order data (a scalar) to second-order data (a matrix) [29]. If second-order data have been obtained by a suitable treatment, with multivariate curve resolution-alternating least squares (MCR-ALS) it is possible to resolve the various species present in a sample and conduct the joint analysis in the presence of interferents without having to apply physical separation steps for both species [30,31]. In a previous paper [32] we proposed a method based on the same instrumental configuration and data treatment to determine total chromium. In the present paper we determine both species of chromium (Cr(III) and Cr(VI)) simultaneously in a single step. The advantages of this method

are clear since many fields of application, for example, the environment, require speciation in many cases.

Though Cr(III) is an absorbent species in the UV–visible region, its sensitivity is low. Cr(III) has a strong tendency to form hexacoordinate octahedral complexes with ligands such as water, ammonia, urea, ethylenediaminetetraacetic acid (EDTA) [15,33,34] and other organic ligands containing oxygen, nitrogen or sulphur [35]. A previous complexation of Cr(III) with EDTA is therefore proposed in order to increase its sensitivity. Most complexation kinetics are slow [36], so several studies have been conducted to speed up the reaction [37,38]. In this paper we determine the best experimental conditions for this step. To do so, we consider these conditions to be compatible with the subsequent process, which will be conducted in the SIA system. We also developed the SIA experimental conditions that provide the optimal responses (e.g. spectra correlation, model fit and error quantification).

Using second-order calibrations, we determined the concentrations of Cr(III) and Cr(VI), as well as several figures of merit in wastewater samples from the leather-tanning process. This process only uses Cr(III) at concentrations in the order of those in this study. However, because of the storage and subsequent treatment of the samples, we thought this strategy could be used to check for the appearance in the samples of any Cr(VI), which is a toxic species. We also applied this method to analyze industrial wastewater. Although these samples did not contain chromium at the levels studied, they were of interest because we were able to test whether the method is applicable to other types of samples.

2. Experimental

2.1. Procedure

The procedure used is illustrated in Figure 1. In a first step, the Cr(III) is complexed with EDTA to increase its sensitivity.

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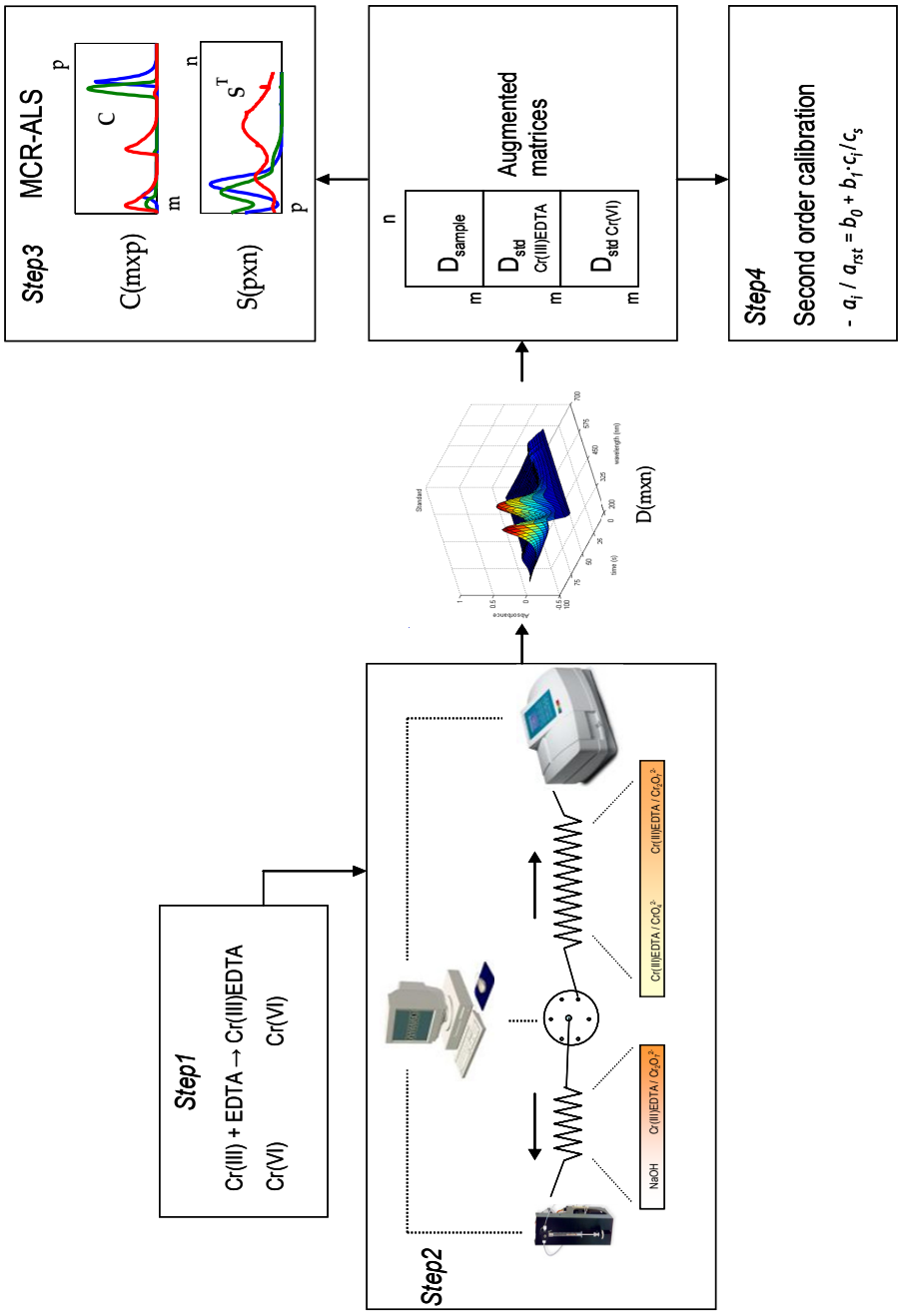

Figure 1. Scheme of the proposed process for the speciation of Cr(III) and Cr(VI).

The second step involves the sequential aspiration of sodium hydroxide (NaOH) and sample and their subsequent expulsion towards the detector, which generates a pH gradient such that Cr(VI) evolves from $Cr_2O_7^{2-}$ to CrO_4^{2-} [32] in the following order: first $Cr_2O_7^{2-}$, then a mixture of $Cr_2O_7^{2-}$ and CrO_4^{2-}, and finally CrO_4^{2-}.

The Cr(III)–EDTA species is present throughout the time the sample passes through the detector. The results correspond to a data matrix **D** ($m \times n$), where m are the times and n are the wavelengths at which the signal is recorded.

In the third step, the data are treated using MCR-ALS [39]. The aim of the MCR-ALS method is the bilinear decomposition of the experimental data set **D** in order to obtain matrices **C** and $\mathbf{S}^T$ (which have a real chemical significance), according to Eq. 1:

$$\mathbf{D} = \mathbf{C}\mathbf{S}^T + \mathbf{E} \qquad (1)$$

where matrix **C**($m \times p$) has column vectors corresponding to the profiles of concentration of the p pure components that are present in matrix **D**. The row vectors of matrix $\mathbf{S}^T$($p \times n$) correspond to the spectra of the p pure components, and **E** is the matrix of the residuals.

Specifically, MCR-ALS is an iterative method that, at each cycle, calculates new matrices **C** and $\mathbf{S}^T$. In this process, a set of constraints [40] is introduced. These constraints derive from chemical knowledge of the system, so that the value of **E** is minimum. For example, the concentration profiles or spectra could not be negative, so the non-negative constraint was applied. In practical applications of MCR-ALS, it is common to use augmented matrices. These matrices are formed by arranging individual matrices and keeping one order in common. Working with augmented matrices can also solve the rotational ambiguity problem and break rank deficiency [41–43].

In Figure 1 (step 3), the vectors of matrices **C** and $\mathbf{S}^T$ are graphically superimposed. This figure for the concentration profile shows information about three augmented matrices whose columns have been kept in common: a sample containing Cr(III) and Cr(VI), a standard of Cr(III) and a standard of Cr(VI) in which the two species in evolution appear ($Cr_2O_7^{2-}$ and CrO_4^{2-}).

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To evaluate the quality of the resolution process, we considered the following parameters:

- the lack of fit (*lof*) of the model, calculated from the following expression:

$$lof = \sqrt{\frac{\sum_{i,j}(d_{ij} - d'_{ij})^2}{\sum_{ij} d_{ij}^2}} \qquad (2)$$

where d_{ij} are each of the elements of the raw data matrix and d'_{ij} are the corresponding values calculated after the optimization process (ALS).

- the errors associated with the concentration profiles of Cr(III) and Cr(VI) obtained in the resolution process with augmented matrices (sample with known concentration + reference standard), calculated from (3):

$$\%\ \text{Error} = \left|\frac{a_1 \times f}{a_2} - 1\right| \cdot 100 \qquad (3)$$

where a_1 and a_2 are the areas of the concentration profiles obtained for each species in the sample and the reference standard, respectively, and f is a correction factor for the difference between the concentrations of the species in the sample and in the reference standard:

$$f = \frac{c_{st}}{c_s}$$

- the correlation coefficient of the spectra obtained in the resolution step (**x**) and the pure spectra (**y**), calculated from (4), for the three species of interest: the chromate, the dichromate and the Cr–EDTA complex.

$$r = \sqrt{\frac{ss_{xy}^2}{ss_{xx} ss_{yy}}} \qquad (4)$$

where ss is the sum of squared values of a set of *n* points.

Under experimental conditions that provide suitable values for these parameters, a second-order calibration is established. As we can see for step 4 in Figure 1, the signal used is the ratio of the areas of the various standards (a_i) (obtained from the resolution of the augmented matrices) to the area of the reference standard (a_{rst}).

The regression lines obtained are validated using the ANOVA test [44] and root mean square error (RMSE), which is calculated from:

$$\text{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - y_i')^2} \qquad (5)$$

where y_i is the reference value for the *i*th sample, y'_i the predicted value for the same sample and *n* is the number of samples.

2.2. Reagents and samples

In all analyses we used analytical grade chemicals. These were $Cr(NO_3)_3$ and $K_2Cr_2O_7$ from PROBUS S.A, EDTA, NaOH and H_2SO_4 from PROLABO and purified water from a Milli-Q water system from MILLIPORE, USA. Standard buffer solutions of pH 4 and 7 (Hamilton) were used to calibrate the pH-meter.

Stock solutions of Cr(III) and Cr(VI) were prepared by dissolving $Cr(NO_3)_3$ or $K_2Cr_2O_7$ in a known volume of purified water.

2.2.1. Tanning samples

The samples were taken from various steps in the tanning process: the initial tanning step (A1, A2), the step after the neutralization step (A3), the second dyeing step (A4), the bathing step with waterproof products (A5), and the bathing step with a tanning agent other than chromium (A6, A7).

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Each step produces samples with different compositions and different Cr(III) contents. Sample A8, from the initial tanning process, was spiked with Cr(VI).

2.2.2. Environmental samples

The samples were taken from various procedures: wastewater from chemical manufacturers (B1), metal furniture manufacturers (B2 and B5), manufacturers of lacquered aluminium (B3) and an industrial wastewater treatment plant (B4). At first, no chromium was observed in these samples when analyzed. All samples were spiked with different contents of Cr(III) and Cr(VI).

2.3. Instrumental and software

The SIA-based flow system used to determine chromium is shown in step 2 in Figure 1. This system comprised a Cavro XL 3000 stepper motor-driven syringe pump equipped with a six-port multi-position automatic selection valve (Eurosas EPS 1306 BPB), and a Hewlett-Packard 8452A spectrophotometer with a Hellma 178.713QS flow-through cell. All tubes to connect the various components of the flow system were made of Omnifit PTFE with 0.8mm (i.d.). The lengths of the holding and reaction coils were 2 and 0.7 m, respectively. The syringe pump, the automatic valve and the acquisition of data provided by the spectrophotometer were controlled by a personal computer (PC) via an RS-232 interface, a PCL-711S PC- Lab- Card and an HP-IB IEEE488 interface for communications, respectively.

The spectra were recorded from 290 to 700 nm in 2 nm steps. As each sample passed through the detector, 100 measurements, one after every 0.7 s, were taken. The data were acquired and monitored by the spectrophotometer using the HP89531A software. The instrumentation was controlled by customized software. A Crison pH-meter was used to measure the pH of the samples.

All calculations for multivariate curve resolution with alternating least squares (MCR-ALS) were performed with laboratory-written software under a MATLAB 5.3 computer environment [45]. This software is available from the authors[46].

3. Results and discussion

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Figure 2 shows the spectra of the species that may be present in the system. These are both species of Cr(VI), CrO_4^{2-} and $Cr_2O_7^{2-}$, and the Cr(III)–EDTA complex. We have included the spectrum of Cr(III) to illustrate its lower sensitivity.

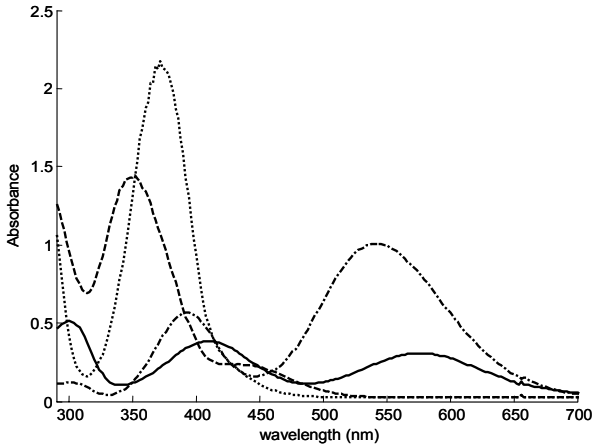

Figure 2. Spectra of chromium solutions in a stopped flow system: (—) Cr(III) of 1000 mg L^{-1}; Cr(VI): (· · ·) CrO_4^{2-} 25 mg L^{-1}, (- - -) $Cr_2O_7^{2-}$ 50 mg L^{-1}; (-·-·) Cr(III)–EDTA 250 mg L^{-1}.

We first studied how parameters such as the pH of the sample, the time the solution was heated and the proportions of the reagents affect the formation of the Cr(III)–EDTA complex.

Figure 3 plots absorbance at 540 nm (maximum absorbance of Cr–EDTA) against the reaction time for different pHs. The pH was adjusted by adding sulphuric acid or sodium hydroxide. The interval studied was from 1.83 to 4.12. When the pH is above 4.12, the Cr(III) may precipitate in the form of hydroxides. We did not study pH below 1.83 because of the results observed. The reaction took place on a heating plate at a temperature of 90 ºC. We can see that the pH affected both the speed of the reaction (slow at low pHs) and the quantity of the complex obtained. By extrapolating the results, we can conclude that, in the 2.5–4 pH range, the complex with maximum absorbance was obtained in 5 min.

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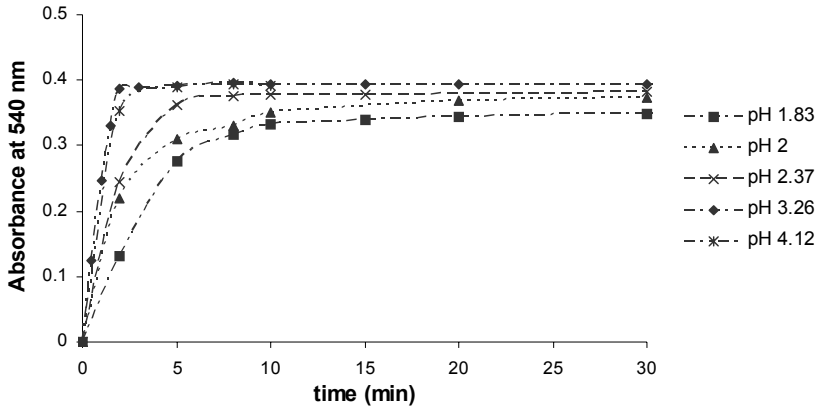

Figure 3. Absorbance at 540 nm against time for a solution of Cr(III) and EDTA with a molar ratio of 1:3.

We then studied how the concentration of EDTA affects the kinetics of the reaction. We analyzed three solutions with Cr–EDTA molar ratios of 1:3, 1:2 and 1:1, respectively. The pH of each solution was 3.7 and the working temperature was 90 ºC. Figure 4 plots absorbance at 540 nm against time. Although at longer times the complex was totally formed even at a ratio of 1:1, we decided to work at a ratio of 1:3. Since the excess EDTA was higher, this ensured total conversion also in the other cases.

In view of these results, we chose the following reaction conditions for the complex formation: a pH range for the complex forming reaction of 2.5–4, a Cr:EDTA molar ratio of 1:3, and a reaction time of 5 min.

To study the stability of the Cr(III)–EDTA complex, a complex of 250 mg L^{-1} was formed. This complex was measured daily with the DAD detector in the 290–700 nm range. The complex was stable for at least 30 days.

3.1. Analytical sequence

The aim of this step (step 2 in Figure 1) was to find a sequence (some values of the variables) for producing a pH gradient that enables the two species of Cr(VI) to appear sequentially and the Cr(III)–EDTA complex to appear superimposed between these two species. In this step we considered the following factors: initial pH, sodium hydroxide

concentration and sample volume. Another variable that can affect the result is the flow rate. We did not consider this variable, however, because in practice its range of values is rather narrow: a flow rate of over 2mL min^{-1} can produce bubbles and a flow rate of under 1mL min^{-1} lengthens the residence time too much and leads to concentration profiles that are too wide. To determine the effects of the above parameters, we developed a full factorial design of 2^3 experiments, which has the experimental domain shown in Table 1. The experiments were conducted with standards of Cr(III) (100 mg L^{-1}) and Cr(VI) (50 mg L^{-1}) after complexation of Cr(III) with EDTA under the conditions outlined previously. We set the flow rate at 1.5mL min^{-1} and the NaOH volume at 0.167 mL.

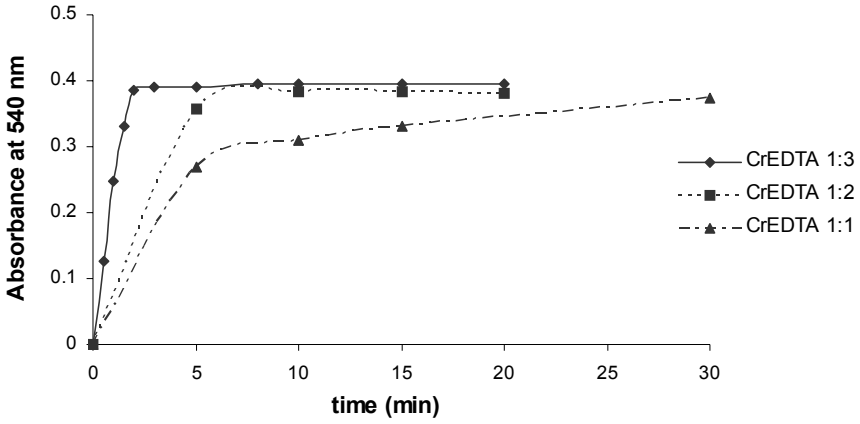

Figure 4. Absorbance at 540 nm against time for different molar ratios of Cr(III)–EDTA.

Data matrices were analyzed using MCR-ALS (step 3 in Figure 1). We worked with column-augmented matrices, formed with a matrix of each experiment, and two matrices of standards, one of a complex Cr(III)–EDTA and another of Cr(VI) evolving from dichromate to chromate, as is shown in Figure 1. Some constraints were applied to the resolution process, e.g. unimodality for the concentration profiles (**C**) and non-negativity for both the concentration profiles and the spectra (**C** and **S**).

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Table 1. Experimental domain of the full factorial design applied to the Cr(III)-EDTA-Cr(VI) solution

Exp. Nº	[NaOH][a]	Vm[b]	pH
1	0.01	0.08	3
2	0.1	0.08	3
3	0.01	0.25	3
4	0.1	0.25	3
5	0.01	0.08	4.5
6	0.1	0.08	4.5
7	0.01	0.25	4.5
8	0.1	0.25	4.5

[a]Sodium Hydroxide concentration in mol L^{-1}

[b]Sample volume in mL

The responses considered were lack of fit, errors of Cr(III) and Cr(VI) and the correlation coefficients of the spectra of the species present in the system, calculated from Eqs. 2–4, respectively.

Our results are shown in Table 2. We studied the significance of the estimated effects using Yates' method and the ANOVA test [44]. We concluded that no factor was important for the $Cr_2O_7^{2-}$ or CrO_4^{2-} correlation coefficient because there was little difference between their values and these values were very good. We also observed that the other responses were not affected by pH in the range considered, which is suitable for complexing Cr(III) with EDTA. NaOH concentration and sample volume, however, affected all the responses in the same way, i.e. the results were better for lower concentrations of NaOH and higher volumes of sample. We also found a strong interaction between these factors. As a result of this interaction, experiments 2 and 6 were clearly the worst in all cases.

Table 2. Responses obtained from the 2^3 experimental design

Experiment number	*lof*	Error Cr(III)	Error Cr(VI)	$r\ CrO_4^{2-}$	$r\ Cr_2O_7^{2-}$	r Cr(EDTA)
1	4.5	12.4	7.9	0.9995	0.9998	0.9815
2	5.1	39.8	19.5	0.9992	0.9998	0.9683
3	3.9	4.0	5.1	0.9994	0.9998	0.9861
4	3.5	0.2	8.1	0.9993	0.9997	0.9769
5	4.4	4.9	14.5	0.9995	0.9998	0.9812
6	5.1	37.4	21.9	0.9992	0.9998	0.9714
7	3.7	1.5	8.9	0.9994	0.9998	0.9844
8	3.5	0.7	10.9	0.9993	0.9997	0.9748

In case the results were dependent on the concentration of Cr(VI), we repeated the experiments at a low concentration (10 mg L^{-1}). The results of these new experiments were in total agreement with the previous ones.

3.2. Calibration step

To establish the calibration curves, we studied how various quantities of Cr(III) can affect the response of Cr(VI), and vice versa. We used augmented matrices as explained before, with each standard of calibration, a reference standard matrix of Cr(III)–EDTA and a reference standard matrix of Cr(VI) evolving between chromate and dichromate. We had as many resolution processes (MCR-ALS runs) as calibration standards. We used profile areas to construct the curves (step 4 of Figure 1). a_i and a_{rst} are the areas of each calibration standard and of the reference standard, respectively, and are calculated from each resolution process. c_i and c_s are the concentrations. For Cr(VI) we used the sum of the areas of chromate and dichromate.

Standard Cr(III) solutions containing 5, 25, 50, 75, 100 mg L^{-1} and standard Cr(VI) solutions of 5, 10, 25, 40, 50 mg L^{-1} were prepared, subjected to the above procedure and analyzed in the SIA system. For each standard, several quantities of the other species (see the first column of Table 3) were added to the other. For example, we had five solutions containing 5 mg L^{-1} of Cr(III), which also contained 5, 10, 25, 40 and 50 mg L^{-1} of Cr(VI), and the same for the rest of the solutions.

The reference standards were 100 and 50 mg L^{-1} of Cr(III) and Cr(VI), respectively.

The next three columns in Table 3 show the calibration parameters obtained at the various concentrations of the other species and for the overall calibration line, i.e. when all the data are considered. For each species we had five calibration curves, one at each of the five levels of the other species considered. The overall line was obtained by considering the data (a_i/a_{rst}) from all the standards. The table also includes the *F*-value, which is used to compare the variance between the individual regression lines and the overall regression line, and the corresponding *t-statistic*, or *t*' [44], which is used to compare the slopes of these curves.

Table 3. Calibration parameters for the determination of Cr(III) and Cr(VI)

[Cr(VI)][a]	b_0[b]	b_1[c]	r[d]	F	t	
Calibration curves for Cr(III)						
5	-0.0414	1.1433	0.9936	1.21	0.57	
10	-0.0637	1.1579	0.9850	0.7	0.42	
25	-0.0483	1.1059	0.9890	1.09	0.03	
40	0.0114	1.0488	0.9789	0.62	0.38	
50	0.0178	1.0284	0.9918	1.71	0.67	
Global	-0.0246	1.0966	0.9724			
[Cr(III)][a]	b_0[b]	b_1[c]	r[d]	F	t	t'
Calibration curves for Cr(VI)						
5	0.0813	0.9236	0.9943	0.95	1.13	
25	0.0842	0.9288	0.9879	0.43	0.84	
50	0.0377	1.0499	0.9998	28	1.06	2.05
75	0.0175	1.0685	0.9995	6.19	0.865	2.14
100	0.0209	1.0413	0.9997	15.6	2.22	2.08
Global	0.0483	1.0024	0.9945			

$F(\alpha=0.05, 48, 8)= 2.5$
t $(\alpha=0.05, 8)= 2$
[a] Cr(III) or Cr(VI) concentration in mg L^{-1}
[b] intercept
[c] slope
[d] correlation coefficient

The curves showed that the concentration of the other chromium oxidation state present in the medium had no effect: Cr(III) can be determined in this range of concentrations even when there were different amounts of Cr(VI), and vice versa.

Table 4. Figures of merit for calibrations of Cr(III) and Cr(VI)

	Cr(III)	Cr(VI)
Working range (mg L^{-1})	5-100	5-50
slope	1.10	1.00
intercept	-0.03	0.05
r	0.9855	0.9945
n	50	50
lod (mg L^{-1})	7.7	2.2
RMSE	0.0644	0.0362
Standard deviation of the residuals	0.0657	0.0369
Standard deviation of the slope	0.0274	0.0152
Standard deviation of intercept	0.0168	0.0095

We decided to work with the calibration curve formed with all the data. Table 4 shows the figures of merit for these calibrations. In accordance with the recommendations of the AOAC [44,47], we estimated the limit of detection by considering the uncertainty of the calibration model.

3.3. Sample analysis

We used the proposed method to determine Cr(III) and Cr(VI) in tanning and environmental samples (samples A and B, respectively, in Table 5).

Table 5. Cr(III) and Cr(VI) concentration of tanning (A) and environmental (B) samples obtained by the SIA method

	SIA method (mg L^{-1})		Known (mg L^{-1})	
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
A1	27	n.d	24	-
A2	67	n.d	78	-
A3	98	n.d	115	-
A4	20	n.d	20	-
B1	18	29	20	30
B2	65	39	40	40
B3	95	20	80	20
A5	1460	16	1450	15
A6	1404	n.d	1020	-
A7	284	n.d	440	-
B4	n.d	20	15	-
B5	68	21	80	20

n.d means non-detected

Figure 5 shows the results obtained when one of the samples is analyzed. The tanning samples contained Cr(III) in different proportions, so we previously diluted them in order to be in the working range of the calibration curves. As they were found not to contain Cr(VI), this was added to them. Environmental samples were spiked with both Cr(III) and Cr(VI).

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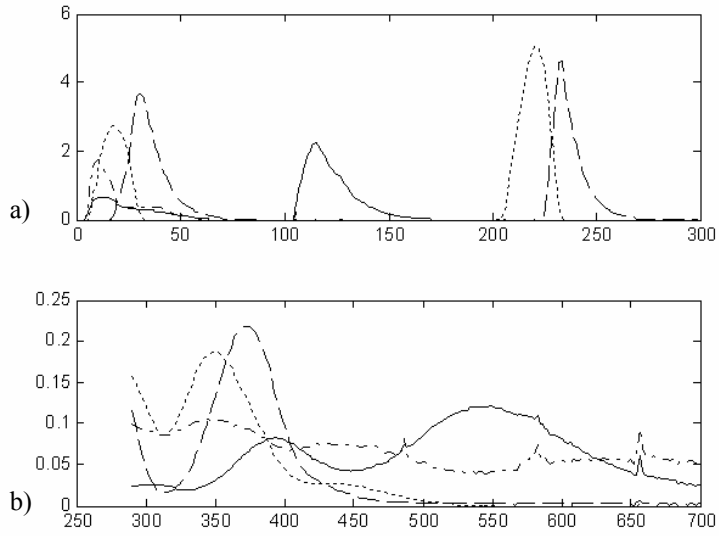

Figure 5. Normalized concentration profiles (a) and pure spectra (b) obtained by multivariate curve resolution of the analysis of an augmented matrix form with a sample, a reference standard of Cr(III)–EDTA and a reference standard of Cr(VI). (—) Cr(III)–EDTA, (· · ·) $Cr_2O_7^{2-}$, (- - -) CrO_4^{2-} and (-·-) absorbing interferences (a.u. are arbitrary units for concentration).

The accuracy of the SIA system was validated by comparing the results with those from a well-established method such as AAS or by the addition of chromium. Using the elliptic joint confidence region (EJCR) test [48], statistical comparison was carried out between the results of the proposed SIA system and the reference values. The critical values of the Snedecor–Fisher statistic at a 95% confidence level were 5.456, 10.65 and 4.687 for Cr(III), Cr(VI) and total chromium, respectively. *F*-values of 1.20, 1.82 and 1.89 were obtained respectively. This indicates that the point (1, 0) lies within the EJCR, so the ellipses for both analytes and total chromium include the theoretically expected values of (1, 0), and the method is therefore reliable. The relative standard error of the method is about 15% for Cr(III) and 7% for Cr(VI) calculated from Eq. 5. In general, the absolute error is bigger in the more concentrated samples. This is because we have applied dilution factors and these dilution factors magnify this error.

4. Conclusions

The method we have developed consisted of a simple flow system for determining Cr(III) and Cr(VI) simultaneously.

Cr(III) sensitivity increased by forming a complex between Cr(III) and EDTA. The best conditions for this reaction were achieved.

The speciation of Cr(III) and Cr(VI) can be carried out very effectively using a SIA system linked to multivariate curve resolution with alternating least squares. The advantages of these systems are the high frequency of analysis (every few seconds), the low consumption of the reagents (in the order of microlitres), and the fact that common reagents can be used.

The main shortcoming of this method are the limits of detection (8 mg L^{-1} for Cr(III) and 2mg L^{-1} for Cr(VI)), which means that analyzing other types of samples with lower quantities of chromium would require preconcentration steps.

This method was successfully used in tanning and environmental water samples.

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References

[1] J. Kotas, Z. Stasicka, *Fresenius J. Anal. Chem*. 107 (2000) 263.

[2] D.T. Gjerde, D.R. Wiederin, F.G. Smith, B.M. Mattson, *J. Chromatogr.* 640 (1993) 73.

[3] E.A. Carcea, D.B. Gomis, *Analyst* 122 (1997) 899.

[4] K. Stein, G. Schwedt, *Fresenius J. Anal. Chem*. 350 (1994) 38.

[5] M.J. Marqués, A. Salvador, A.E. Morales-Rubio, M. de la Guardia, *Fresenius J. Anal. Chem.* 362 (1998) 239.

[6] M.J. Marqués, A. Salvador, A.E. Morales-Rubio, M. de la Guardia, *Fresenius J. Anal. Chem*. 367 (2000) 601.

[7] C. Cámara, R. Cornelis, P. Quevauviller, *Trends Anal. Chem.* 19 (2000) 189.

[8] J. Kotás, Z. Statiska, *Environ. Pollut.* 107 (2000) 263.

[9] R.J.C. Brown, M.J.T. Milton, *Trends Anal. Chem.* 24 (3) (2005) 266.

[10] J. Feldmann, *Trends Anal. Chem*. 24 (3) (2005) 228.

[11] J.F. Van Staden, R.I. Stefan, *Talanta* 64 (2004) 1109.

[12] A.K. Das, M. de la Guardia, M.L. Cervera, *Talanta* 55 (2001) 1.

[13] M. Pettine, S. Capri, *Anal. Chim. Acta* 540 (2005) 231.

[14] M. Sperlimg, S. Xu, B. Welz, *Anal. Chem*. 64 (1992) 3101.

[15] Y. Inoue, T. Sakai, H. Kumagai, *J. Chromatogr. A* 706 (1995) 127.

[16] V. Orescanin, L. Mikelic, S. Lulic, M. Rubcic, *Anal. Chim. Acta* 527 (2004) 125.

[17] M.S. El-shahawi, S.S.M. Hassan, A.M. Othman, M.A. Zyada, M.A. El- Sonbati, *Anal. Chim. Acta* 534 (2005) 319.

[18] C.R. Dockery, J.E. Pender, S.R. Googe, *Appl. Spectrosc*. 59 (2005) 252.

[19] R.K. Sharma, A. Goel, *Anal. Chim. Acta* 534 (2005) 137.

[20] A.C. Sahayam, G. Venkateswarlu, S.C. Chaurasia, *Anal. Chim. Acta* 537 (2005) 267.

[21] L.V. Mulaudzi, J.F. van Staden, R.I. Stefan, *Anal. Chim. Acta* 467 (2002) 51.

[22] J. Chwastowska, W. Skwara, E. Sterlinska, L. Pszonicki, *Talanta* 66 (2005) 1345.

[23] F. Shemirani, M. Rajabi, *Fresenius J. Anal. Chem*. 371 (2001) 1037.

[24] P.G. Krishna, J.M. Gladis, U. Rambabu, T. Prasada Rao, G.R.K. Naidu, *Talanta* 63 (2004) 541.

[25] T. Sumida, T. Ikenoue, K. Hamada, A. Sabarudin, M. Oshima, S. Motomizu, *Talanta* 68 (2005) 388.

[26] A.A. Menegario, P. Smichowski, G. Polla, *Anal. Chim. Acta* 546 (2005) 244.

[27] S.Q. Memon, M.I. Bhanger, M.Y. Khuhawar, *Anal. Bioanal. Chem*. 383 (2005) 619.

[28] J. Ruzicka, G.D. Marshall, *Anal. Chim. Acta* 237 (1990) 329.

[29] A. Pasamontes, M.P. Callao, *Trends Anal. Chem*. 25 (2006) 77.

[30] J. Saurina, S. Hernández-Cassou, R. Tauler, *Anal. Chim. Acta* 335 (1996) 41.

[31] A. Izquierdo-Ridorsa, J. Saurina, S. Hernández-Cassou, R. Tauler, *Chemom. Intell. Lab. Syst*. 38 (1999) 183.

[32] V. Gómez, M.P. Callao, *Anal. Bioanal. Chem.* 382 (2005) 328.

[33] S.B. Roychowdhury, J.A. Koropchak, *Anal. Chem*. 62 (1990) 484.

[34] U. Patnaik, J. Muralidhar, *Talanta* 42 (1995) 553.

[35] J. Kotas, Z. Stasicka, *Environ. Pollut*. 107 (2000) 263.

[36] W.R. Seitz, W.W. Suydam, D.M. Hercules, *Anal. Chem.* 44 (1972) 957.

[37] A.C. Spínola, J.C. Rosa, A.L. Carvalho, S.L. Costa, M.G. Andrade, L.S. Gomes, *Quím. Nova* 22 (1999) 2.

[38] E.I. Morosanova, M.A. Kozlov, N.M. Kuz'min, *J. Anal. Chem*. 55 (2000) 182.

[39] R. Tauler, A. Smilde, R. Kowalsky, *J. Chemometrics* 9 (1995) 31.

[40] R. Tauler, A. Izquierdo-Ridorsa, E. Cassasas, *Chemom. Intell. Lab. Syst*. 18 (1993) 293.

[41] A. de Juan, E. Casassas, R. Tauler, in: R.A. Meyers (Ed.), *Encyclopedia of Analytical Chemistry*, John Wiley and Sons Ltd., Chichester, 2000, pp. 9800–9837.

[42] A. Izquierdo-Ridorsa, J. Saurina, S. Hernández-Cassou, R. Tauler, *Chemom. Intell. Lab. Syst.* 38 (1997) 183.

[43] C. Ruckebusch, L. Duponchel, J.P. Huvenne, J. Saurina, *Anal. Chim. Acta* 515 (2004) 183.

[44] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. de Jong, P.J. Lewi, J. Smeyers-Verbeke, *Handbook of Chemometrics and Qualimetrics Part A*, Elsevier, Amsterdam, 1997.

[45] Matlab, The Mathworks, South Natick, MA, USA.

[46] R. Tauler, A. de Juan, Multivariate Curve Resolution Homepage, http://www.ub.es/gesq/mcr/mcr.htm.

[47] W.G. Rohrbough, et al., *Reagent Chemicals American Chemical Society Specifications*, seventh ed., American Chemical Society, Washington, 1986.

[48] A.G. González, M.A. Herrador, A.G. Asuero, *Talanta* 48 (1999) 729.

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3.5. PAPER

Chromium determination and speciation since 2000

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Chromium determination and speciation since 2000

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Abstract

Chromium is introduced into the environment by effluents in several industries. It is important to control this element since it is both toxic and carcinogenic. As its toxicity depends on its state of oxidation, it is especially interesting to determine its most abundant species, Cr(III) and Cr(VI).

In this overview, we describe the requirements for chromium determination and speciation and review the analytical methods that have been used in these studies. Focusing in particular on developments since 2000, we examine the features of detection techniques, pre-treatments and applications of the various methods.

Keywords: Chromium; Determination; Preconcentration; Speciation

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1. Introduction

Chromium is a metallic element that exists primarily in the mineral chromite, which is present in soils, waters, rocks, fauna and flora, and volcanic dust and gases. Chromium occurs mainly as a result of human activities through production of waste water in metal smelting, electroplating, tanning, metallurgy and dyestuff industries. After processing, chromium occurs in several chemical species:

- metallic chromium (chromium 0), which is mainly found in alloys, such as stainless steel, but also in chrome-plated objects. It is the supreme additive, endowing alloys or materials with new properties, such as a resistance to corrosion, wear, temperature and decay, as well as strength, hardness, permanence, hygiene and color.

- trivalent chromium (chromium III), which exists in natural waters in hydrolyzed $Cr(H_2O)_4OH_2^+$ form and complexes, and even adsorbed on colloidal matter. It is an essential micronutrient in the body and combines with various enzymes to transform sugar, protein and fat. It is also used in a number of commercial products, including dyes, paint pigments and salts for leather tanning.

- hexavalent chromium (chromium VI), which is found as CrO_2^{4-} , $HCrO_4^-$ or $Cr_2O_7^{2-}$, depending on the pH of the medium. It is known to be carcinogenic and mutagenic and it induces dermatitis. It occurs in a range of compounds used in industrial processes, such as chrome plating.

Cr(VI) and Cr(III) enter the environment as a result of effluent discharged from industries and cooling-water towers. Chromium can also enter drinking water supply systems via corrosion inhibitors used in water pipes and containers or via contamination of underground water leaching from sanitary landfill.

Chromium is an analyte of interest to the above industries and in the environment because, like other metals, it is not biodegradable. Once it enters the environment, its toxicity is determined to a large extent by its chemical form (e.g., Cr(VI) is much more toxic than

Cr(III)). Changes in the oxidation state of an element can have a profound effect on bioavailability and toxicity.

In this article, we review the analytical methods for determining chromium (total Cr, Cr(III), Cr(VI) and both) that have been published since 2000. We examine several aspects related to the determination method (i.e. type of sample, detection method, and speciation and pre-concentration techniques). We survey the state-of the art and the future prospects for on-line methods.

2. Analytical focus

Table 1 lists the studies published in the various bibliographic sources in our review, indicating the types of sample, the concentrations of chromium per sample, the species of chromium determined, the detection techniques used and the bibliographic sources.

Chromium is determined in many fields. The types of sample analyzed are mainly aqueous solutions, both natural water (from several sources, including bottled water, tap water, seawater and river water) and industrial waters (also from several sources but mainly from the electroplating and tanning industries or waters of environmental interest). There are solid samples from sediments, soils, metallurgical materials or foods. Chromium has also been determined, though to a lesser extent, in samples from other sources (e.g., atmosphere, biological material, and oils). Figure 1 shows the types of samples analyzed in the publications that we have reviewed.

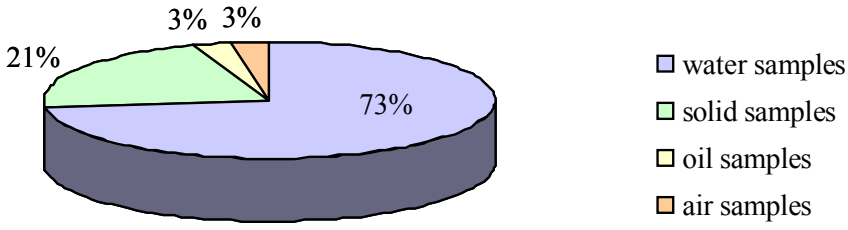

Figure 1. Papers published on chromium determination according to type of sample analyzed.

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Table 1. Most relevant analytical methods for chromium determination

Matrix	Cr content	Analyte	Detection technique	Ref
natural water	ng/l	Cr(III) Cr(VI)	AAS	1,2
natural water	µg/L	Cr(III) Cr(VI)	AAS	3-22
natural water	mg/L	Cr(III) Cr(VI)	AAS	23,24
natural water	µg/L	Cr(III) Cr(VI)	ICP-AES	25
natural water	ng/L	Cr(III) Cr(VI)	ICP-MS	26
natural water	µg/L	Cr(III) Cr(VI)	ICP-MS and AAS	27,28
natural water	0.3-6 g/L	Cr(III) Cr(VI)	ICP-MS	29
natural water	µg/L	Cr(III) Cr(VI)	ICP-MS	30
natural water	µg/L	Cr(III) Cr(VI)	IC-ICP-MS	31
natural water	µg/L	Cr(III) Cr(VI)	ICP-OES	32-33
natural water	µg/L	Cr(III) Cr(VI)	ICP-AES	34
natural water	µg/L	Cr(III) Cr(VI)	Spectrophotometry	35
natural water	mg/L	Cr(III) Cr(VI)	Spectrophotometry	36-37
natural water	g/L	Cr(III) Cr(VI)	Spectrophotometry	38
natural water		Cr(III) Cr(VI)	CE-DAD detection	39
natural water	µg/L	Cr(III) Cr(VI)	HPLC-DAD detection	40-42
natural water	µg/L	Cr(III) Cr(VI)	CCSV-DTPA	43
natural water	µg/L	Cr(III) Cr(VI)	Laser induced breakdown spectroscopy (LIBS)	44
natural water	µg/L	Cr(III) Cr(VI)	Fluorimetry	45
natural water	µg/L	Cr total	AAS	46-50
natural water	≥20 µg/L	Cr total	AAS	51
natural water	µg/L	Cr total	SF-ICP-MS	52
natural water		Cr total	ICP-OES	53
natural water	µg/L	Cr total	Chemiluminescence	54-55
natural water	ng/l	Cr(VI)	AAS	56
natural water	µg/L	Cr(VI)	AAS	57-63
natural water	µg/L	Cr(VI)	HPLC-ICP-MS	64
natural water	µg/L	Cr(VI)	Spectrophotometry	65-66
natural water	few mg/L	Cr(VI)	Spectrophotometry	67-69
natural water	µg/L	Cr(VI)	Fluorimetry	70
natural water	µg/L	Cr(VI)	IC-UV-vis detection	71
natural water	µg/L	Cr(VI)	AdCSV	72
natural water	≥30 mg/L	Cr(VI)	AdCSV	73
natural water	µg/L	Cr(III)	AAS	74
natural water	mg/L	Cr(III)	AAS	75
natural water	µg/L	Cr(III)	GC-FAAS	76
natural water	mg/L	Cr(III)	Spectrophotometry	77
natural water	3 µg/L	Cr(III)	Potentiometry	78
natural water	few mg/L	Cr(III)	Enzimatic amperometry	79

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SEQUENTIAL INJECTION ANALYSIS USING SECOND-ORDER CALIBRATION FOR THE DEVELOPMENT OF ANALYTICAL METHODS
Verònica Cerdà Cortés
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Table 1. Continued.

Matrix	Cr content	Analyte	Detection technique	Ref
natural water	µg/L	Cr(III)	Chemiluminescence	80
environmental atmospheric		Cr total	AAS	81
environmental atmospheric	mg/L	Cr total	ICP-AES	82
environmental atmospheric	30 µg/L	Cr(VI)	FPLC-AAS	83
environmental atmospheric	ng/m3	Cr(VI)	ICP-MS	84
environmental atmospheric	few mg/L	Cr(VI)	IC-spectrophotometry	85
wastewater	µg/L	Cr(III) Cr(VI)	AAS	86
wastewater	few mg/L	Cr(III) Cr(VI)	AAS	87-88
wastewater	µg/L	Cr(III) Cr(VI)	ICP-AES	34
wastewater		Cr(III) Cr(VI)	HPLC-DAD	90
wastewater	mg/L	Cr(III) Cr(VI)	Spectrophotometry	91
wastewater	≤ 1 mg/L	Cr total	Spectrophotometry	92
wastewater	µg/L	Cr total	Chemiluminescence	93
wastewater	µg/L	Cr(VI)	AdSV	72
wastewater	few mg/L	Cr(VI)	Spectrophotometry	94
wastewater	mg/L	Cr(VI)	Fluorimetry	95
wastewater	µg/L	Cr(VI)	IC-spectrophotometry	96
dye plant wastewater	≤1 mg/L	Cr(III) Cr(VI)	AAS	97
electroplating wastewater	g/L	Cr(III) Cr(VI)	AAS	98
electroplating wastewater	30 mg/L	Cr(III) Cr(VI)	AAS and DPAdCSV	99
electroplating wastewater	mg/L	Cr(III) Cr(VI)	Spectrophotometry	100
electroplating wastewater	≥100 µg/L	Cr(III) Cr(VI)	EDXRF	101
electroplating wastewater	≤10 mg/L	Cr(VI)	Fluorimetry	102
electroplating wastewater	mg/L	Cr(III)	ICP-AES	103
electroplating wastewater		Cr(III)	Potenciometry	104
tanning wastewater		Cr(III) Cr(VI)	AAS	105
tanning wastewater		Cr(III) Cr(VI)	CE-UV detection	106
tanning wastewater	µg/L	Cr(III) Cr(VI)	CAdSV	107
tanning wastewater	mg/L	Cr(III) Cr(VI)	spectrophotometry	108
tanning wastewater	40-700 µg/L	Cr total	AAS	109
tanning wastewater	mg/L	Cr total	AAS	110
tanning wastewater	40-700 µg/L	Cr total	Spectrophotometry	109
tanning wastewater	mg/L	Cr total	Spectrophotometry	111-112
tanning wastewater	few mg/L	Cr(III)	Enzimatic amperometry	78
biological	µg/L	Cr(III) Cr(VI)	AAS	21
biological	µg/L	Cr(III) Cr(VI)	ICP-OES	113
biological	µg/L	Cr(III) Cr(VI)	ICP-MS	30
biological	µg/L	Cr total	AAS	114-117
biological	mg/L	Cr total	AAS	118

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Table 1. Continued.

Matrix	Cr content	Analyte	Detection technique	Ref
biological	µg/L	Cr total	CAdSV	117
biological	few µg/L	Cr total	AAS	119-120
biological	mg/L	Cr total	GC-FID	121
biological	µg/L	Cr(VI)	AAS	59
biological	mg/L	Cr(VI)	AdSV	122
biological	few mg/L	Cr(III)	Enzimatic amperometry	79
pharmaceuticals	≤1 mg/L	Cr(III) Cr(VI)	Spectrophotometry	123
pharmaceuticals	µg/L	Cr(III) Cr(VI)	Fluorimetry	45
pharmaceuticals	g/L	Cr total	Spectrophotometry	124
pharmaceuticals	mg/L	Cr(III)	Spectrophotometry	125
solid samples	µg/g	Cr(III) Cr(VI)	Electrophoretically mediated microanalysis (EMMA)	126
solid samples	mg/L	Cr total	AAS	127
solid samples	µg/L	Cr(VI)	AAS	128
solid samples	mg/L	Cr(VI)	ICP-MS	129
solid samples	mg/kg	Cr(VI)	Spectrophotometry	130
solid samples	µg/L	Cr(VI)	Spectrophotometry	131
cement		Cr(VI)	HPLC-ICP-MS / FPLC-AAS / Spectrophotometry	132
cement	mg/L	Cr(VI)	AAS	133
alloy and steel samples	mg/L	Cr total	AAS	117
alloy and steel samples	mg/L	Cr(III)	AAS	75
alloy and steel samples	0,10%	Cr(III)	AAS	134
alloy and steel samples	mg/L	Cr(VI)	Spectrophotometry	135
alloy and steel samples		Cr(III)	Potentiometry	136
automative industry	mg/L - g/L	Cr(III) Cr(VI)	ICP-MS / HPLC-ICP-MS	137
automative industry	mg/L - µg/L	Cr(III) Cr(VI)	Spectrophotometry	37
soil and sediments	few mg/L	Cr(III) Cr(VI)	AAS	87
soil and sediments	mg/L	Cr(III) Cr(VI)	AAS	138
soil and sediments	mg/L	Cr(III) Cr(VI)	CE	139
soil and sediments	µg/L	Cr total	AAS	140
soil and sediments	mg/L	Cr total	AAS	141-142
soil and sediments	200 µg/L	Cr(VI)	AAS	143
soil and sediments	mg/L	Cr(VI)	Spectrophotometry	144
Foods	µg/L	Cr total	AAS	145-146
Foods	few mg/L	Cr total	ICP-MS	147-148
Foods	µg/L	Cr(VI)	Potentiometry	149
Foods		Cr(III)	Potentiometry	136
Foods	mg/L	Cr(III)	AAS	75
oil samples	≥100 mg/L	Cr total	AAS	150
oil samples	10-500 mg/L	Cr total	XRF - ICP-MS	151

Table 1. Continued.

Matrix	Cr content	Analyte	Detection technique	Ref
oil samples	µg/L	Cr(VI)	AdSV	73
plants	µg/L	Cr(VI)	AAS	152
TiO2 powder	≤10 mg/L	Cr total	AAS	153
reference materials		Cr(III) Cr(VI)	IC-ICP-MS	154
different types		Cr(III) Cr(VI)	Raman spectroscopy	155

The concentration of chromium in most of the samples is of the order of µg L^{-1}. The atmospheric samples contained the lowest concentrations (i.e. of the order of ng m^{-3}) [84]. In water samples, the concentration ranged from ng L^{-1} in some consumer waters [1,2] to over mg L^{-1} or up to g L^{-1} [38,98] in natural waters or in river/reservoir waters with factories nearby [98,137]. Higher chromium concentrations, of the order of mg L^{-1}, have been found in biological samples [121,122], pharmaceutical samples [124,125], industrial samples, solid samples and oils. The highest concentrations, up to g L^{-1}, are found in industrial samples (e.g., tanning [111], electroplating [98–100,102,103], automation [137] and cement [132,133]).

The biological and chemical properties of Cr(III) and Cr(VI) differ significantly[156]. Cr(VI) compounds are about 100 times more toxic than Cr(III) compounds. This is because of their high oxidation potentials and the ease with which they penetrate biological membranes. Speciation analysis of Cr(III) and Cr(VI) is therefore of great importance and much research has been devoted to this area [23], although, depending on the type of sample, we expect to observe one or other of these species, or both.

During the 1990s, some papers reported on metal speciation in general, using procedures such as hyphenated techniques [157], ion chromatography [158], and flow techniques [159,160]. There were some reviews of chromium speciation for different types of samples, such as tanning wastewater [161,162], solids [163] or environmental [164]. Total chromium determination was also reviewed using EPA protocols [165], and, in 2005, a review of Cr(III) determination in solid samples was published [166].

As Figure 2 shows, practically half of all chromium determination studies have focused on speciation.

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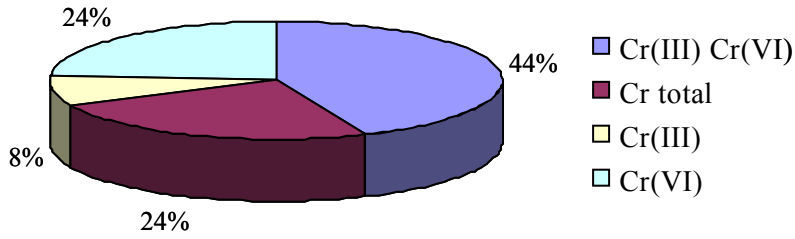

Figure 2. Papers published on chromium determination according to species determined

3. Sample-pretreatment techniques

Previous treatments for determining chromium had two objectives: speciation and preconcentration. Many pre-treatment techniques can achieve both these objectives.

The speciation study of chromium is an important challenge for analytical communities in environmental, clinical and biological research and in the control of wastewaters, natural waters and drinking waters. Table 2 shows the speciation techniques used and the limits of detection (*lod*) obtained in the articles reviewed in this study.

The strategies for achieving speciation are varied. One strategy is to use selective reagents for one species and then apply a technique, such as HPLC [42,90], electrophoresis [106,139] or stripping voltammetry [107], to distinguish between the species.

Liquid-liquid extraction (LLE) is usually based on complexation of one of the species, which is selectively extracted by a solvent. Another species is then determined using the same method after the residual chromium contained in the sample solutions has been reduced or oxidized [36]. Cloud-point extraction (CPE) is based on phase separation, which occurs in aqueous solutions of non-ionic surfactants that become turbid when heated to a temperature known as the cloud-point temperature (CPT), thus forming a two-phase system.

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Table 2. Procedures for chromium speciation

Separation	*lod*	Ref
cloud point extraction	10 ng L^{-1}	[3]
cloud point extraction	0.17-5.2 μg L^{-1}	[7,18,21,33,41]
on-line cloud point extraction	0.2 μg L^{-1}	[45]
liquid extraction	1.9-7.5 μg L^{-1}	[12,36,42]
solid extraction	0.2-30 ng L^{-1}	[1,4,9,13,14,38,43]
on-line solid extraction	3 - 20 ng L^{-1}	[6,17,20,26]
solid extraction	0.03-7.7 μg L^{-1}	[5,8,16,24,25,27,29,34,86,97,98]
solid extraction	0.04 -0.09 mg L^{-1}	[11,22,91]
on-line solid extraction	0.45-1.5 μg L^{-1}	[15,30,32]
on-line solid extraction	40 - 80 μg L^{-1}	[23]
selective reactions	4.5 ng L^{-1}	[107]
selective reactions	0.15-3 μg L^{-1}	[37,89,99,101,106]
selective reactions	0.1 -7 mg L^{-1}	[90,108,139]
on-line selective reactions	0.26-0.32 mg L^{-1}	[126]
on-line selective reactions	1-10 μg L^{-1}	[35]
ionic exchanger	5-12 ng L^{-1}	[154]
ionic exchanger	0.05 - 20 μg L^{-1}	[10,28,39,44,87,88,100]
selective coprecipitation	0.4-0.6 μg L^{-1}	[105]
bidirectional electrostacking	6 - 5 ng L^{-1}	[2]

During the formation of the two phases, hydrophobic complexes can be entrapped ‘‘in situ’’ in the surfactant phase. Mere centrifugation and decanting of the aqueous phase can easily separate the two phases. This benign LLE method has several advantages (e.g., it is inexpensive and safe and has a high concentration factor). This method has been used to form hydrophobic Cr(III) [45] or Cr(VI) [3,21] complexes.

There are many speciation strategies based on solid extraction. One is to use an adsorbent that retains the two species and then to use independent elution under selective conditions [14]. Another is to use functionalized sorbents that selectively retain one species while the other species remains in solution [38,98] or to use a common sorbent that presents selectivity in function of the conditions of the medium (e.g. the pH) and then to use selective elution under different conditions [90]. Another option for the speciation of chromium is the simultaneous retention of Cr(III) and Cr(VI) using a dual-column system [34,167]. The dual column can be constructed with the same sorbents or with different sorbents.

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Despite increasingly sensitive analytical instrumentation, the determination of chromium at trace levels usually requires previous pre-concentration. Only a few, very sensitive instrumental methods (e.g., ICP-MS, ET-AAS and stripping voltammetry) can directly determine low concentration total Cr [107].

Table 3. Preconcentration techniques for chromium determination

Analyte	Preconcentration	*lod*	Ref
Cr total	on-line cloud point extraction	0.5ng L^{-1}	[93]
Cr total	cloud point extraction	1.29 μg L^{-1}	[53]
Cr(VI)	cloud point extraction	3 ng L^{-1}	[56]
Cr(VI)	liquid extraction	μg L^{-1}	[128,132]
Cr(III)	liquid extraction	3 μg L^{-1}	[103,134]
Cr total	solid extraction	15 ng L^{-1}	[49]
Cr total	solid extraction	8 - 20 μg L^{-1}	[92,121]
Cr(VI)	solid extraction	0.02 -2 μg L^{-1}	[59,61,65,71,85,95]
Cr(III)	solid extraction	1 - 7.4 μg L^{-1}	[74,75,125,136]
Cr total	on-line solid extraction	2.59 μg L^{-1}	[48]
Cr(VI)	on-line solid extraction	8.8 ng L^{-1}	[57]
Cr(VI)	on-line solid extraction	0.03 μg L^{-1}	[63]
Cr(VI)	on-line solid extraction	0.8 μg L^{-1}	[58]
Cr(III)	solid phase microextraction	2 μg L^{-1}	[76]
Cr(VI)	selective reagents	3 ng L^{-1}	[73]
Cr total	selective reagents	20 ng L^{-1}	[52]
Cr total	selective reagents	0.05- 3 μg L^{-1}	[118,124,148]
Cr(VI)	selective reagents	μg L^{-1}	[62,68,70,77,94,130]
Cr(VI)	ionic exchanger	1.5 μg L^{-1}	[83,129,143]
Cr total	coprecipitation	0.3 μg L^{-1}	[50]
Cr total	chemical modifier	0.05-0.67 μg L^{-1}	[47,51,114,116,119,120]
Cr total	chemical modifier	3.4 μg L^{-1}	[140]
Cr total	chemical modifier	20-60 μg L^{-1}	[141,142]
Cr total	microwave digestion	1.76 mg L^{-1}	[151]
Cr(VI)	microwave assisted extraction electrokinetic flow analysis	0.012 - 0.12 mg L^{-1}	[144]
Cr(VI)	(EKFA)	10 ng L^{-1}	[60]
Cr(VI)	membranes	5 -14 μg L^{-1}	[66,96,122]

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Techniques for pre-concentrating chromium are basically the same as those for speciation except that, for preconcentration, the process does not need to be selective with regard to the two species. Table 3 shows the studies that have used preconcentration techniques, the techniques used, the species determined and the *lod* obtained. The lowest *lod* were obtained using solid extractions [49,56,93] or selective reactions [73].

Preconcentration methods involving solid sorbents are considered to be simpler, quicker and better able to obtain a high enrichment factor than LLE systems. Solid sorbent techniques (e.g., activated alumina, tributyltin chloride immobilized on C18 cartridges, ion-exchange columns and diphenylcarbazide immobilized on silica) have been used for on-line preconcentration of Cr(VI) from water. Because of their high tolerance of interfering ions and high salt content in the samples, hydrophobic materials are more attractive than chelating or cation exchange resins for assaying the ultratrace metal by generating a non-charged complex [63].

4. Analytical techniques

Figure 3 shows the instrumental techniques used to determine chromium in the studies we reviewed for this paper (see Table 1).

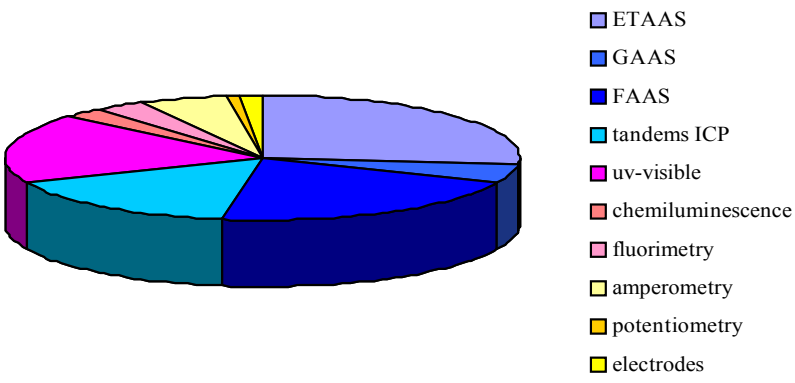

Figure 3. Analytical methods for chromium determination according to the detection technique used

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We can see that optical techniques were used in 90% of these studies and that the most common optical technique was spectroscopy (FAAS, ETAAS, ICP), which was used in 65% of the studies. Spectrophotometric techniques (UV-visible absorption, fluorimetry or chemiluminescence) were used in 25% of the studies.

4.1. Spectroscopic methods

Spectroscopic methods (e.g., FAAS, GF-AAS, ETAAS and the ICP-tandems) can be used to determine total chromium. If the aim is to determine one or both of the chromium species, these techniques must be accompanied by a pretreatment to provide the selectivity of one species with respect to the other. The *lod* can range from ng L^{-1} to μg L^{-1}, depending on the preconcentration technique used. If no preconcentration technique is used, the *lod* are mg L^{-1} for FAAS and μg L^{-1} for the other techniques.

For concentrated samples, techniques such as AES and FAAS are recommended because they can provide a large linearity range. For samples with low levels of chromium, techniques such as GF-AAS and ICP-AES may be suitable. GF-AAS can achieve very low *lod*, but the relative standard deviations are higher than for ICP-AES [19].

4.2. Spectrophotometric methods

Spectrophotometric methods can be used for selective determination of the different chromium species using reagents in order to form absorbing species that present selectivity in the response. The most common method for determining Cr(VI) in aqueous solutions is based on the reaction of diphenylcarbazide (DPC) with Cr(VI) at a pH of 1.0 ± 0.3 [65–69].

4.3. Chemiluminescence methods

Chemiluminescence methods can be used to determine total chromium at ng L^{-1} levels. Fluorimetry is mainly used to analyze Cr(VI) at levels of detection up to ng L^{-1}.

4.4. Electrical methods

With regard to electrical methods, we should highlight amperometry and voltammetry, which achieve *lod* of the order of μg L^{-1} without preconcentration and *lod* of ng L^{-1} when using selective adsorbents, masking agents or formation of complexes.

4.5. Chromatographic systems

Chromatographic systems (e.g., HPLC, GC and IC) are used in many studies both to separate and to reduce the consumption of reagents and sample. In some cases, these techniques can be used for simultaneous determination of the two most important chromium species – Cr(III) and Cr(VI) – in a single step [83,143].

5. Concluding remarks and future outlook

The papers that we reviewed indicated that there were three main areas in which research into chromium determination can develop: (i) new reagents for optimizing the sample-pretreatment processes, both for speciation and for preconcentration; (ii) automation of the sample pre-treatment process; and, (iii) incorporation of signal-treatment techniques (chemometric techniques) as an alternative to some sample pretreatments.

5.1. New reagents for preconcentration and speciation

There is a wide range of research into the study of new sorbents for effectively separating Cr(III) and Cr(VI) and determining them at trace levels. Macrocyclic compounds (e.g., crown ethers and calixarenes) have been reported for the chromatographic separation of chromium [168].

The use of vegetal materials (e.g., biosorbents) for Cr(III) adsorption is well documented [169]. Microorganisms (e.g., yeast, bacteria and fungi) and materials of plant origin have been proposed for the pre-concentration and speciation of trace metals, since they can accumulate metals present in liquid media. Bag et al. [22] used Saccharomyces cerevisiae immobilized on sepolite for separation and speciation of Cr(III) and Cr(VI). A plant biomass (Garcinia cambogia) has also been used for chromium removal and speciation [170].

5.2. Automation of the pre-treatment process

During the pre-treatment step, there is a risk of interconversion between Cr(III) and Cr(VI) because of oxidation or reduction of the chromium species. Speciation methods that can be used under dynamic conditions with minimum sample manipulation are therefore

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needed in order to stop this significantly affecting the distribution of the chromium species [65] and prevent interconversion between Cr(III) and Cr(VI).

Recent studies have focused on designing flow through, multiple-step, dynamic, fractionation procedures, mostly involving microcolumn extractions aimed at imitating field conditions more correctly than their batch counterparts [128].

The simplicity of the flow analysis manifold – flow injection analysis (FIA), sequential injection analysis (SIA), micro-sequential injection lab-on-valve (μSI-LOV) – and its low maintenance makes it an ideal tool for pre-treatment. The manifold allows appropriate unit operations to be executed by incorporating packed column reactors, reaction coils, digestion or extraction units, or dialysis modules. Also, the use of a syringe pump readily and reproducibly allows automatic microfluidic handling and operation at variable flow rates [167].

Compared with traditional batch-mode sequential extraction and currently available flow-through sequential extraction schemes with off-line detection and quantification, on-line methods have several advantages, including minimization of readsorption/redistribution, greater accuracy, higher speed, lower sample and reagent consumption (few tenths of microliters), and lower risk of contamination or analyte loss. Several recent publications have used flow techniques for speciation and preconcentration analysis but this is a field that remains open to research.

5.3. Incorporation of chemometric techniques

Most of the proposed instrumental methods are relatively complex because steps have to be taken to achieve selectivity and prevent the effects of interferents. As multivariate data can be obtained and chemometric techniques are available to evaluate them, such techniques can be applied to the determination of chromium in several fields. Their main field of application is in the determination of complex samples, which, because of their high chromium content, do not require a preconcentration step. In such cases, the concentration can be derived directly from a multivariate instrumental signal without the need for pre-treatment.

Multivariate calibration techniques enable calibrations to be made in the presence of interferents, provided the calibration samples contain these interferents (i.e. provided their composition is similar to that of the problem samples). These techniques have been applied in several studies, both with linear multivariate calibrations [46,54,55,135,138,171] and non-linear multivariate calibrations, such as neuron networks [172].

A recent trend in the field of chemometrics is the development of second-order methods, which enable calibrations to be made in the presence of interferents without the calibration samples having to take into account information about interferents (i.e. calibrations can be made with standards). Second-order calibrations have recently been successfully developed both for the determination of total chromium and for speciation [108,111,112].

References

[1] F. Shemirani, M. Rajabi, *Fresenius J. Anal. Chem*. 371 (2001) 1037.
[2] Y. Heb, M.L. Cervera, A. Pastor, M. de la Guardia, *Anal. Chim. Acta* 447 (2001) 135.
[3] X. Zhu, B. Hu, Z. Jiang, M. Li, *Water Res*. 39 (2005) 589.
[4] G. Zhu, S. Li, *Analyst* 126 (2001) 1453.
[5] J. Chwastowska, W. Skwara, E. Sterlínska, L. Pszonicki, *Talanta* 66 (2005) 1345.
[6] R.A. Gil, S. Cerutti, J.A. Gásquez, R.A. Olsina, L.D. Martinez, *Talanta* 68 (2006) 1065.
[7] F. Shemirani, S.D. Abkenar, A.A. Mirroshandel, M.S. Niasari, R.R. Kozania, *Anal. Sci*. 19 (2003) 1453.
[8] L.P. Eksperiandova, I.I. Fokina, A.B. Blank, N.N. Grebenyuk, *J. Anal. Chem*. 57 (2002) 194.
[9] E. Vassileva, K. Hadjiivanov, T. Stoycheva, C. Daieva, *Analyst* 125 (2000) 693.
[10] M.T.S. Cordero, E.I.V. Alonso, A.G. de Torres, J.M.C. Pavon, *J. Anal. At. Spectrom*. 19 (2004) 398.
[11] A. Tunc, A.R. Türker, *Talanta* 57 (2002) 1199.
[12] G.Z. Tsogas, D.L. Giokas, A.G. Vlessidis, N.P. Evmridis, *Spectrochim. Acta., Part B* 59 (2004) 957.
[13] B.N. Parodi, G. Polla, L. Valiente, P. Smichowski, *At. Spectrosc*. 26 (2005) 102.

[14] A.C. Sahayam, *Anal. Bioanal. Chem*. 372 (2002) 840.

[15] H.W. Sun, W.J. Kang, S.X. Liang, J. Ha, S.G. Shen, *Anal. Sci*. 19 (2003) 589.

[16] M. Yaman, *J. Anal. Chem*. 58 (2003) 456.

[17] R.G. Wuilloud, G.M. Wuilloud, J.C.A. de Wuilloud, R.A. Olsina, L.D. Martínez, *At. Spectrosc*. 23 (2002) 44.

[18] E.K. Paleologos, C.D. Stalikas, S.M. Tzouwara-Karayanni, G.A. Pilidis, M.I. Karayannis, *J. Anal. At. Spectrom.* 15 (2000) 287.

[19] M.J. Marqués, A. Salvador, A. Morales-Rubio, M. de la Guardia, *Fresenius J. Anal. Chem*. 367 (2000) 601.

[20] X.B. Long, M. Miró, E.H. Hansen, *J. Anal. At. Spectrom*. 20 (2005) 1203.

[21] E.K. Paleologos, C.D. Stalikas, M.I. Karayannis, *Analyst* 126 (2001) 389.

[22] H. Bag, A.R. Turker, M. Lale, A. Tunceli, *Talanta* 51 (2000) 895.

[23] M.J. Marqués, A. Morales-Rubio, A. Salvador, M. de la Guardia, *Talanta* 53 (2001) 1229.

[24] S. Yalçin, R. Apak, *Anal. Chim. Acta* 505 (2004) 25.

[25] T. Sumida, A. Sabarudin, M. Oshima, S. Motomizu, *Anal. Sci*. 22 (2006) 161.

[26] S. Hirata, K. Honda, O. Shikino, N. Maekawa, M. Aihara, *Spectrochim. Acta, Part B* 55 (2000) 1089.

[27] M.V.B. Krishna, K. Chandrasekaran, S.V. Rao, D. Karunasagar, J. Arunachalam, *Talanta* 65 (2005) 135.

[28] J.W. Ball, R.B. McCleskey, *Talanta* 61 (2003) 305.

[29] B. Wen, X.Q. Shan, J. Lian, *Talanta* 56 (2002) 681.

[30] Y.C. Sun, C.Y. Lin, S.F. Wu, Y.T. Chung, *Spectrochim. Acta, Part B* 61 (2006) 230.

[31] C. Sarzanini, M.C. Bruzzoniti, *Trends Anal. Chem*. 20 (2001) 304.

[32] A.A. Menegário, P. Smichowski, G. Polla, *Anal. Chim. Acta* 546 (2005) 244.

[33] B.N. Parodi, G. Polla, L. Valiente, P. Smichowski, *At. Spectrosc*. 26 (2005) 89.

[34] T. Sumida, T. Ikenoue, K. Hamada, A. Sabarudin, M. Oshima, S. Motomizu, *Talanta* 68 (2005) 388.

[35] D.G. Themelis, F.S. Kika, A. Economou, *Talanta* 69 (2006) 615.

[36] W. Chen, G. Zhoug, Z. Zhou, P. Wu, X. Hou, *Anal. Sci.* 21 (2005) 1189.

[37] M. Kaneko, M. Kurihara, S. Nakano, T. Kawashima, *Anal. Chim. Acta* 474 (2002) 167.

[38] H. Filik, M. Dogutan, R. Apak, *Anal. Bioanal. Chem*. 376 (2003) 928.

[39] M. King, M. Macka, B. Paull, *Anal. Lett*. 37 (2004) 2771.

[40] I. Ali, H.Y. Aboul-Enein, *Chemosphere* 48 (2002) 275.

[41] A.N. Tang, D.Q. Jiang, Y. Jiang, S.W. Wang, X.P. Yan, *J. Chromatogr. A* 1036 (2004) 183.

[42] J.S. Wang, K. Chiu, *Anal. Sci*. 20 (2004) 841.

[43] Y. Li, H. Xueb, *Anal. Chim. Acta* 448 (2001) 121.

[44] C.R. Dockery, J.E. Pender, S.R. Goode, *Appl. Spectrosc*. 59 (2005) 252.

[45] E.K. Paleologos, C.D. Stalikas, S.M. Tzouwara-Karayanni, M.I. Karayannis, *Anal. Chim. Acta* 436 (2001) 49.

[46] M. Felipe-Sotelo, J.M. Andrade, A. Carlosena, D. Prada, *Anal. Chem*. 75 (2003) 5245.

[47] M.I.C. Monteiro, A.K. Avila, R. Neumann, *Anal. Chim. Acta* 428 (2001) 265.

[48] S. Saracoglu, M. Soylak, L. Elci, *Anal. Lett.* 35 (2002) 1519.

[49] M.N. Amin, H. Okada, S. Itoh, T. Suzuki, S. Kaneco, K. Ohta, *Fresenius J. Anal. Chem*. 371 (2001) 1130.

[50] T. Minami, Y. Sohrin, L. Ueda, *Anal. Sci*. 21 (2005) 1519.

[51] L.A. Pereira, I.G. Amorim, J.B.B. da Silva, *Talanta* 64 (2004) 395.

[52] L. Yang, Z. Mester, L. Abranko, R.E. Sturgeon, *Anal. Chem*. 76 (2004) 3510.

[53] J. Li, P. Liang, T.Q. Shi, H.B. Lu, *At. Spectrosc*. 24 (2003) 169.

[54] P. Campins-Falcó, L.A. Tortajada-Genaro, S. Meseguer-Lloret, F. Bosch-Reig, *Anal. Bioanal. Chem.* 374 (2002) 1223.

[55] L.A. Tortajada-Genaro, P. Campins-Falcó, J. Verdú -Andrés, F. Bosch-Reig, *Anal. Chim. Acta* 450 (2001) 155.

[56] J. Nan, X.P. Yan, *Anal. Chim. Acta* 536 (2005) 207.

[57] W. Som-Aum, S. Liawruangrath, E.H. Hansen, *Anal. Chim. Acta* 463 (2002) 99.

[58] A.N. Anthemidis, G.A. Zachariadis, J.S. Kougoulis, J.A. Stratis, *Talanta* 57 (2002) 15.

[59] A.N. Anthemidis, G.A. Zachariadis, J.A. Stratis, *Talanta* 58 (2002) 831.

[60] L. Yang, Y. He, W. Gan, M. Li, Q. Qu, X. Lin, *Talanta* 55 (2001) 271.

[61] A.C. Sahayam, G. Venkateswarlu, S.C. Chaurasia, *Anal. Chim. Acta* 537 (2005) 267.

[62] R. Karosi, V. Andruch, J. Posta, J. Valgo, *Microchem. J*. 82 (2006) 61.

[63] X. Long, M. Miró, E.H. Hansen, *Anal. Chem*. 77 (2005) 6032.

[64] Y. Martínez-Bravo, A.F. Roig-Navarro, F.J. López, F. Hernandez, *J. Chromatogr. A* 926 (2001) 265.

[65] Y.M. Scindia, A.K. Pandey, A.V.R. Reddy, S.B. Manohar, *Anal. Chem*. 74 (2002) 4204.

[66] Y.M. Scindia, A.K. Pandey, A.V.R. Reddy, S.B. Manohar, *Anal. Chim. Acta* 515 (2004) 311.

[67] L.A. Tortajada-Genaro, P. Campins Falcó, F. Blasco-Gómez, F. Bosch-Reig, *Analyst* 125 (2000) 777.

[68] V. Andruch, M. Telepcaková, I.S. Balogh, N. Urbanová, *Microchim. Acta* 142 (2003) 109.

[69] M.A.S. Pressman, J.H. Aldstadt, *Microchem. J.* 74 (2003) 47.

[70] D. Xiao, K.M. Wang, W.X. Xiao, *Analyst* 126 (2001) 1387.

[71] D.H. Thomas, J.S. Rohrer, P.E. Jackson, T. Pak, J.N. Scott, *J. Chromatogr. A* 956 (2002) 255.

[72] A. Safavi, N. Maleki, H.R. Shahbaazi, *Talanta* 68 (2006) 1113.

[73] M. Grabarcyk, L. Kaczmarek, M. Korolczuk, *Electroanalysis* 16 (2004) 1503.

[74] S. Baytak, A.R. Türker, *Microchim. Acta* 149 (2005) 109.

[75] S. Baytak, A.R. Türker, *Talanta* 65 (2005) 938.

[76] T.H. Ding, H.H. Lin, C.W. Whang, *J. Chromatogr. A* 1062 (2005) 49.

[77] K.G. Kumar, R. Muthuselvi, *J. Anal. Chem*. 61 (2006) 28.

[78] M.B. Gholivand, F. Raheedayat, *Electroanalysis* 16 (2004) 1330.

[79] O. Domínguez Renedo, M.A. Alonso Lomillo, M.J. Arcos Martinez, *Anal. Chim. Acta* 521 (2004) 215.

[80] Y. Xu, F.G. Bessoth, J.C.T. Eijkel, A. Manz, *Analyst* 125 (2000) 677.

[81] J. Sneddon, C.O. Noble, M.V. Smith, J.N. Beck, *Spectrosc. Lett*. 37 (2004) 151.

[82] I. Boevski, N. Daskalova, I. Havezov, *Spectrochim. Acta Part B* 55 (2000) 1643.

[83] R. Milacic, J. Scancar, J. Tusek, *Anal. Bioanal. Chem*. 372 (2002) 549.

[84] Y. Li, N.K. Pradhan, R. Foley, G.K.C. Low, *Talanta* 57 (2002) 1143.

[85] E.H. Borai, E.A. El-Sofany, A.S. Abdel-Halim, *Trends Anal. Chem*. 21 (2002) 741.

[86] S. Melaku, R. Cornelis, F. Vanhaecke, R. Dams, L. Monees, *Microchim. Acta* 150 (2005) 225.

[87] A.S. Stasinakis, N.S. Thomaidis, T.D. Lekkas, *Anal. Chim. Acta* 478 (2003) 119.

[88] D.M. Adriá-Cerezo, M. Llobat-Estellés, A.R. Maurí-Aucejo, *Talanta* 51 (2000) 531.

[89] P. Bermejo-Barrera, P. Herbello-Hermelo, M.C. Barciela-Alonso, A. Bermejo-Barrera, *At. Spectrosc.* 24 (2003) 22.

[90] S. Cathum, C.E. Brown, W. Wong, *Anal. Bioanal. Chem*. 373 (2002) 103.

[91] S.Q. Memon, M.I. Bhanger, M.Y. Khuhawar, *Anal. Bioanal. Chem*. 383 (2005) 619.

[92] M. Llobat-Estellés, A.R. Maurí-Aucejo, M.D. López-Catalán, *Fresenius J. Anal. Chem*. 371 (2001) 358.

[93] E.K. Paleologos, A.G. Vlessidis, M.I. Karayannis, N.P. Evmiridis, *Anal. Chim. Acta* 477 (2003) 223.

[94] W. Gan, L. Yang, Y. He, R. Zeng, M.L. Cervera, M. de la Guardia, *Talanta* 51 (2000) 667.

[95] S.K. She, Y.Y. Zhou, L. Zhang, L.Y. Wang, L. Wang, *Spectrochim. Acta Part A* 62 (2005) 711.

[96] R. Ganeshjeevan, R. Chandrasekar, S. Yuvaraj, G. Radhakrishnan, *J. Chromatogr. A* 988 (2003) 151.

[97] P. Hashemi, J. Boroumanda, M.R. Fathi, *Talanta* 64 (2004) 578.

[98] M. Dogutan, H. Filik, I. Tor, *Talanta* 59 (2003) 1053.

[99] J.K. Kiptoo, J.C. Ngila, G.M. Sawula, *Talanta* 64 (2004) 54.

[100] M.S. El-Shahawi, S.S.M. Hassan, A.M. Othman, M.A. Zyada, M.A. El-Sonbati, *Anal. Chim. Acta* 534 (2005) 319.

[101] V. Orescanin, L. Mikelic, S. Lulic, M. Rubcic, *Anal. Chim. Acta* 527 (2004) 125.

[102] S.S.M. Hassan, A.A. Abdel-Shafi, A.H.K. Mohammed, *Talanta* 67 (2005) 696.

[103] Y.K. Agrawal, K.R. Sharma, *Talanta* 67 (2005) 112.

[104] M.R. Ganjali, M. Emami, M. Salavati-Niasari, M. Yousefi, *Anal. Lett*. 36 (2003) 2735.

[105] K. Prasad, R.S. Praveen, T.P. Rao, P. Gopikrishna, G.R.K. Naidu, *At. Spectrosc*. 26 (2005) 173.

[106] E. Pobozy1, M. Knell, K. Kilian, R. Kataky, M. Trojanowicz, *Electrophoresis* 24 (2003) 2259.

[107] A. Bobrowski, B. Bás, J. Dominik, E. Niewiara, E. Szalínska, D. Vignati, J. Zarebski, *Talanta* 63 (2004) 1003.

[108] V. Gómez, M.S. Larrechi, M.P. Callao, *Anal. Chim. Acta* 571 (2006) 129.

[109] M.I.C. Monteiro, I.C.S. Fraga, A.V. Yallouz, N.M.M. de Oliveira, S.H. Ribeiro, *Talanta* 58 (2002) 629.

[110] I. López-García, B. Merino Meroño, N. Campillo, M. Hernández- Córdoba, *Anal. Bioanal. Chem*. 373 (2002) 98.

[111] V. Gómez, M.P. Callao, *Anal. Bioanal. Chem*. 382 (2005) 328.

[112] V. Gómez, A. Pasamonetes, M.P. Callao, *Microchem. J*. 83 (2006) 98.

[113] R.A. Gil, S. Cerutti, J.A. Gasquez, R.A. Olsina, L.D. Martinez, *Spectrochim. Acta. Part B* 60 (2005) 531.

[114] K.L. Alves Lelis, C. Gonçalves Magalhaes, C. Aparecida Rocha, J.B. Borba de Silva, *Anal. Bioanal. Chem.* 374 (2002) 1301.

[115] M.V. Aguilar, C.J. Mateos, M.C. Martinez Para, *J. Trace El. Med. Biol*. 16 (2002) 221.

[116] S.N. Ignatova, A.B. Volynskii, *J. Anal. Chem*. 56 (2001) 1015.

[117] L. Husákova, A. Bobrowski, J. Srámkova, A. Królicka, K. Vytras, *Talanta* 66 (2005) 999.

[118] M.A. Taher, *Fresenius J. Anal. Chem*. 368 (2000) 421.

[119] T.W. Lin, S.D. Huang, *Anal. Chem*. 73 (2001) 4319.

[120] P.V. Oliveira, E. Oliveira, *Fresenius J. Anal. Chem*. 371 (2001) 909.

[121] V. Arancibia, M. Valderrama, K. Silva, T. Tapia, *J. Chromatogr. B* 785 (2003) 303.

[122] L. Soko, E. Cukrowska, L. Chimuka, *Anal. Chim. Acta* 474 (2002) 59.

[123] L.V. Mulaudzi, J.F. van Staden, R.I. Stefan, *Anal. Chim. Acta* 467 (2002) 51.

[124] H.D. Revanasiddappa, T.N. Kiran Kumar, *Talanta* 60 (2003) 1.

[125] S. Lapanantnoppakhun, S. Kasuwas, L. Ganranoo, J. Jakmunee, K. Grudpan, *Anal. Sci*. 22 (2006) 153.

[126] F. Priego-Capote, M.D. Luque de Castro, *J. Chromatogr. A* 1113 (2006) 244.

[127] F. Laborda, M.P. Górriz, J.R. Castillo, *Talanta* 64 (2004) 631.

[128] X. Long, M. Miró, E.H. Hansen, *Analyst* 131 (2006) 132.

[129] G.M.M. Rahman, H.M.S. Kingston, T.G. Towns, R.J. Vitale, K.R. Clay, *Anal. Bioanal. Chem.* 382 (2005) 1111.

[130] M. Korolczuk, M. Grabarczyk, *Talanta* 66 (2005) 1320.

[131] M. Pettine, S. Capri, *Anal. Chim. Acta* 540 (2005) 239.

[132] J. Scanar, R. Milacic, F. Seby, O.F.X. Donard, *J. Anal. At. Spectrom*. 20 (2005) 871.

[133] S.S. Potgieter, N. Panichev, J.H. Potgieter, S. Panicheva, *Cem. Concr. Res*. 33 (2003) 1589.

[134] S. de Paula Eiras, U. Martins Custodio, L.A. Pavanin, *Talanta* 59 (2003) 621.

[135] H. Abdollahi, *Anal. Chim. Acta* 442 (2001) 327.

[136] R. Kumar Sharma, A. Goel, *Anal. Chim. Acta* 534 (2005) 137.

[137] F. Séby, M. Gagean, H. Garraud, A. Castetbon, O.F.X. Donard, *Anal. Bioanal. Chem*. 377 (2003) 685.

[138] N. Köleli, *Chemosphere* 57 (2004) 1473.

[139] Z. Chen, R. Naidu, A. Subramanian, *J. Chromatogr. A* 927 (2001) 219.

[140] L. Almeida Pereira, I. Amorim, J. Bento Borba da Silva, *Talanta* 68 (2006) 771.

[141] M. Felipe-Sotelo, A. Carlosena, J.M. Andrade, M.J. Cal-Prieto, E. Fernández, D. Prada, *Microchem. J.* 81 (2005) 217.

[142] O. Acar, *Microchim. Acta* 151 (2005) 53.

[143] R. Milacic, J. Scancar, *Analyst* 125 (2000) 1938.

[144] S. Morales-Muñoz, J.L. Luque-García, M.D. Luque de Castro, *Anal. Chim. Acta* 515 (2004) 343.

[145] C.J. Mateos, M.V. Aguilar, M.C. Martínez-Para, *J. Agric. Food Chem*. 51 (2003) 401.

[146] M.H. Canuto, H.G. Luna Siebald, G. Magela de Lima, J.B. Borba Silva, *J. Anal. At. Spectrom.* 18 (2003) 1404.

[147] F. Cubadda, S. Giovannangeli, F. Iosi, A. Raggi, P. Stacchini, *Food Chem*. 81 (2003) 463.

[148] D. Hammer, M. Nicolas, D. Andrey, *At. Spectrosc*. 26 (2005) 203.

[149] I. Svancara, P. Foret, K. Vytras, *Talanta* 64 (2004) 844.

[150] J.L. Burguera, R.M. Avila-Gómez, M. Burguera, R. Antón de Salager, J.L. Salager, C.L. Bracho, M. Burguera-Pascu, C. Burguera-Pascu, R. Brunetto, M. Gallignani, Y. Petit de Peña, *Talanta* 61 (2003) 353.

[151] M. Pouzar, T. Cernohorsky, A. Krejcová, *Talanta* 54 (2001) 829.

[152] N. Panichev, K. Mandiwana, M. Kataeva, S. Siebert, *Spectrochim. Acta Part B* 60 (2005) 699.

[153] X. Zhu, B. Hu, L. Wang, S. Li, Z. Jiang, *Fresenius J. Anal. Chem*. 371 (2001) 497.

[154] H. Gürleyük, D. Wallschläger, *J. Anal. At. Spectrom*. 16 (2001) 926.

[155] S. Kikuchi, K. Kawauchi, M. Kurosawa, H. Honjho, T. Yagishita, *Anal. Sci*. 21 (2005) 197.

[156] C. Barnowski, N. Jakubowski, D. Stuewer, *J. Anal. At. Spectrom.* 12 (1997) 1155.

[157] L.A. Ellis, D.J. Roberts, *J. Chromatogr. A* 774 (1997) 3.

[158] M.J. Shaw, P.R. Haddad, *Environ. Int*. 30 (2004) 403.

[159] J.F. van Staden, R.I. Stefan, *Talanta* 64 (2004) 1109.

[160] A.K. Das, M. de la Guardia, M.L. Cervera, *Talanta* 55 (2001) 1.

[161] A.R. Walsh, J. O'Halloran, *Water Res*. 30 (1996) 2393.

[162] A.R. Walsh, J. O'Halloran, *Water Res*. 30 (1996) 2401.

[163] M.J. Marques, A. Salvador, A.E. Morales-Rubio, M. de la Guardia, *Fresenius J. Anal. Chem.* 362 (1998) 239.

[164] J. Kotas, Z. Stasicka, *Environ. Pollut*. 107 (2000) 263.

[165] J.L. Parks, L. McNeill, M. Frey, A.D. Eaton, A. Haghani, L. Ramirez, M. Edwards, *Water Res.* 38 (2004) 2827.

[166] M. Pettine, S. Capri, *Anal. Chim. Acta* 540 (2005) 231.

[167] R. Chomchoei, M. Miró, E.H. Hansen, J. Shiowatana, *Anal. Chem*. 77 (2005) 2720.

[168] V.A. Sasyuk, *Zh. Anal. Khim*. 46 (1991) 741.

[169] J.L. Gardea-Torresdey, K. Dokken, K.J. Tiemann, J.G. Parsons, J. Ramos, N.E. Pingitore, G. Gamez, *Microchem. J*. 71 (2002) 157.

[170] K. Chandrasekhar, N.S. Chary, C.T. Kamala, K.R. Supriya, T.R Rao, *Int. J. Environ. Stud*. 5 (2002) 1.

[171] J.B. Sirven, B. Bousquet, L. Canioni, L. Sarger, *Anal. Chem*. 78 (2006) 1462.

[172] J. Posta, H. Berndt, S. Luo, G. Schaldach, *Anal. Chem*. 65 (1993) 2590.

3.6. CONCLUSIONS

Two methods for determining total chromium and one method for determining two species of chromium (Cr(III) and Cr(VI)) have been established. They are based on sequential injection analysis and second-order calibration with MCR-ALS. With these methods, reduced volumes of samples and reagents, and shorter analysis times can be used to determine chromium in the presence of interferents.

With these methods chromium can be quantified with very simple calibrations. Only four or five samples of known concentration, which could be synthetic standards, are required. The experimental cost is, therefore, considerably reduced in comparison to multivariate calibrations like principal component regression (PCR) or partial least squares (PLS). Although these latter methods can make predictions in the presence of interferents, in this case, they must be modelled.

In the calibration step, we worked with augmented matrices that consisted of the matrix of the sample and the matrix of the reference standard. When samples are complex, augmentation with a matrix that contains some reagents is a good choice, because it helps to solve rank deficiency and breaks the rotational ambiguity.

The first two methods analyse total chromium. The second method, even based on the same, has clear advantages of automatization, analysis time, consumption of reagents and generation of waste.

In the third method, Cr(III) and Cr(VI) can be analysed simultaneously. We checked that Cr(III) determination was not dependent on the quantities of Cr(VI) in the sample and vice versa and we studied the calibrations for each of the species by changing the concentration of the other species. This means that this method has considerable potential to be applied in a wide range of samples with varied concentrations.

This method has many advantages over other speciation methods because it enables chromium to be speciated in a single analysis with low volumes of sample and waste, and in a short analysis time.

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The accuracy of the results was checked by using atomic absorption spectrometry as a reference technique. Bivariate least squares and the joint test of slope and intercept were used to check the absence of bias with a 95% confidence interval.

The fourth paper gives an overview of the requirements for chromium determination and speciation. Chromium is determined in many fields but mainly in water samples. Spectroscopic methods are widely used for total chromium determination, but when the aim is to determine one or both of the chromium species, they must be accompanied by a pre-treatment to provide the selectivity of one species with respect to the other. We concluded that new reagents can be developed for sample-pretreatment processes and that chemometric tools can be used for chromium determination and speciation with no need for any pretreatments.

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4. Study of dyes in wastewater samples from tanning effluents

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4.1. INTRODUCTION

In the process of dyeing leather, two or three dyes are normally used to obtain the desired colour. The dyeing process contains various steps and quality of the process is determined by controlling the final product. Normally, the concentration of dye in solution is not controlled at the end of each step. Also, once the dyeing process is over, the residual baths contain certain amounts of dyes. If they are taken to a purifying plant, the resulting water fees can represent a considerable cost to the tanning industry.

This chapter, then, consists of four papers, with two practical objectives: on one hand, to control the amount of dye remaining in solution throughout the process in order to optimize some of the steps and, on the other hand, to study several strategies for reducing the percentage of dyes in wastewater samples before they are taken to wastewater treatment plants.

In this study, we have worked with three dyes that are commonly used in the tanning industry: Acid Red 97, Acid Brown 425 and Acid Orange 61. These dyes have similar chemical structures that cause spectral overlapping. Therefore, if they are to be determined simultaneously, the methods must incorporate pretreatments or multicomponent analysis.

In the first step, we developed an analytical method based on sequential injection analysis (SIA) and second-order calibration (MCR-ALS) to determine three dyes in wastewater samples from the tanning industry in a single step, *Sequential injection analysis with second-order treatment for the determination of dyes in the exhaustion process of tanning effluents*. The method developed was applied to tanning samples that used chromium salts or vegetal species as tanning agents. The application to this latter type of samples gave rise to a second paper: *Matrix effect in second-order data. Determination of dyes in a tanning process using vegetable tanning agents*, because the vegetable tanning agent is a highly absorbing species and causes what is known as the matrix effect, so strategies for correcting these effects had to be studied and applied.

To achieve the second objective, we first reviewed the bibliography. We considered adsorbing dyes onto activated carbon as an attractive strategy because of the low cost the

adsorption process referring to the instrumentation and reagents, and because the analytes required little handling. The third paper, *Kinetic and adsorption study of acid dye removal using activated carbon*, studied the adsorption and kinetic parameters, as well as individual dyes and their mixtures. The objective of the fourth paper, *Experimental designs for optimizing and fitting the adsorption of dyes onto activated carbon*, as the name suggests, is to obtain a response surface that, depending on the concentrations of dyes in wastewater, can establish the experimental conditions to obtain a predetermined adsorption.

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4.2. PAPER

Sequential injection analysis with second-order treatment for the determination of dyes in the exhaustion process of tanning effluents

V. Gómez, J. Font, M.P. Callao
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Sequential injection analysis with second-order treatment for the determination of dyes in the exhaustion process of tanning effluents

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Abstract

A sequential injection (SI)-DAD spectrophotometric method to control the exhaustion of dyes in a mixture of three dyes from a tanning industry process has been developed. It is based on an interdiffusion process of the sample and reagents which leads to a gradual fall in pH through the channel to the detector recording a data matrix. The aim of this paper is to develop a second-order calibration model that is unaffected by interferents by applying multivariate curve resolution with alternating least squares (MCR-ALS). We obtained a linear calibration in the 5–30 mg L^{-1} range with a correlation coefficient of 0.999 for each dye with detection limits of 2.6, 3.9 and 2.1 mg L^{-1} for Acid Red 97, Acid Brown 425 and Acid Orange 61, respectively. The simultaneous determination of the three dyes from tanning samples showed a satisfactory precision for the three analytes. The method has been validated comparing the concentration of some spiked samples with the expected concentration using a *t*-paired test. When we used this method to study the exhaustion of dyes, we found that there were several stages in this process. These data may be the key to optimizing the exhaustion process.

Keywords: Dyes determination; Tanning effluents; Multivariate curve resolution; Sequential injection analysis

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1. Introduction

Large quantities of dyes are used in a wide range of applications. These include textiles, paint pigments, printing inks and food colouring. The textile industry is the largest consumer of dyes, accounting for two-thirds of the dyestuff market. Interest in determining dyes are both technological (to determine the proportions of base dyes needed to achieve a certain tone) and environmental (their synthetic precursors, intermediates and degradation products are potential health hazards [1–5]).

Several analytical procedures, e.g., spectrophotometry [6], solid-phase spectrophotometry [7], voltammetry [8], polarography [9–10], capillary electrophoresis [11–13] and chromatography, have been used to determine dyes [14–16]. Spectrophotometric methods have been used for the routine quantitative determination of synthetic dyes [17]. Binary dye mixtures showing a strong spectral overlap have easily been resolved by derivative and bivariate techniques [18–19]. As the colour normally comprises a mixture of two or more colorant dyes, a method that is able to determine multiple components is needed. Preference is therefore given to chromatographic methods over spectrophotometry since spectrophotometry sometimes suffers spectral interference that cannot be solved by the derivative ratio spectra methods[20].

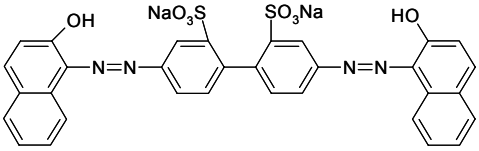
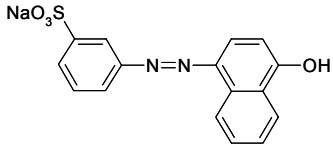
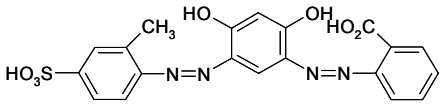
Chemometric techniques such as multiple linear regression (MLR), principal component regression (PCR), partial least squares (PLS) and bilinear least squares (BLLS) have already been applied for analyzing mixtures of several dyes [21–24].

The aim of this paper is to develop an analytical method for studying the exhaustion of dyes in the standard dyeing process of the leather industry. The colour obtained in the process depends on how the dyes enter into the leather, i.e., on factors such as bath volume, the surface area of the leather, and total turning time. In this paper, we hope to obtain information that will help to optimize the dyeing process and to determine the amount of dye that remains in wastewaters once at the end of the process. We studied three dyes. Table 1 shows the structure, name and colour index of these dyes.

Our method is based on combined sequential injection analysis and multivariate curve resolution with alternating least squares (SIA-MCR-ALS)[25]. This SIA system does not use hazardous reagents and the amount of chemical waste generated during the analysis is minimal. Multi-way analysis is becoming popular due to the availability of high-order instrumental data and the proliferation of chemometric algorithms for data processing [26–29]. Unlike other techniques like PARAFAC or Tucker, MCR-ALS is highly flexible when defining the type of data because it enables us to work with trilinear data when two modes are in common (spectra and time) and with non-trilinear data when only one mode (the spectra) is in common. A particularly appealing property of multi-way data is second-order advantage [30], a term which was coined to describe analysis in the presence of unexpected interferents.

We have found no references in the literature that use this method to treat such complex mixtures as those in this study.

Table 1. Structures, name, color index and color of the dyes used in the tanning industry

Structure	C.I.[1] number and name	Color
	Acid Red 97	Bright yellowish red
 * chromium complex	Acid Orange 61	Orange
 * chromium complex	Acid Brown 425	Dull reddish brown

[1]Color index

2. SIA-MCR-ALS

Sequential injection analysis (SIA), known as the second generation of flow systems [31], enables the sample and the reagents to be introduced sequentially into the system. Figure 1 shows a scheme for an SIA system. First, the sample and the reagents are aspirated towards the syringe and stored into the waiting coil (A). They are then pumped to the reaction coil (B), where the reagents mix with the sample via an interdiffusion process.

The analytical process involved sequential aspiration of first: about 4500 μL of the carrier (water), second: reagents (sodium hydroxide and/or sulphuric acid) and third: sample (mixtures containing the three dyes). The interdiffusion process of the sample and reactants lead to a gradual fall in pH through the channel to the detector. The synthetic dyes are polyprotic species, so when the sample reaches the detector, the most acid species appears first, followed by the mixture of this species with its conjugate base. The working conditions (volume and concentrations of reagents and sample volume) for obtaining a suitable response (sequential appearance of the acid and basic species of each dye) were determined using several dyes standards.

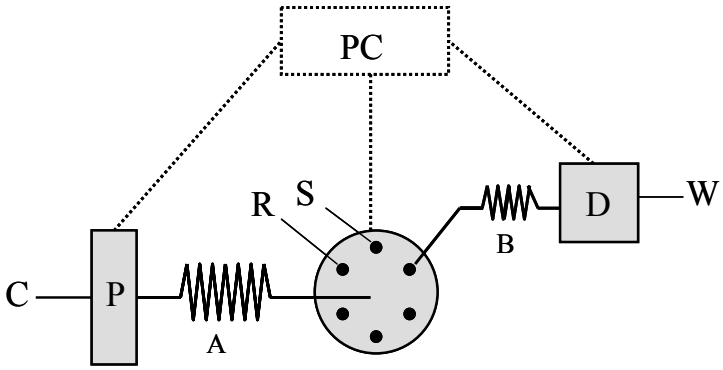


Figure 1. Scheme of the proposed sequential injection system. C: carrier; S: sample; R: reagents; P: pump; V: multi-position valve; D: detector; PC: computer; A: holding coil; B: reaction coil; W: waste.

The obtained signal is a data matrix, **D** ($m \times n$) and corresponds to a set of spectra at different pH. m are the times and n are the wavelengths at which the absorbance is recorded.

As an example, Figure 2 shows the data matrix obtained when the process is applied to one of the dyes studied.

The aim of second-order data treatment techniques such as MCR-ALS is to decompose the raw matrix **D** into the product of two matrices according to Eq. 1:

$$\mathbf{D} = \mathbf{C}\mathbf{S}^{T} + \mathbf{E} \qquad (1)$$

where matrix **C** ($m \times p$) has column vectors corresponding to the profiles of concentration of the p pure components that are present in matrix **D**. The row vectors of matrix $\mathbf{S}^{T}$ ($p \times n$) correspond to the spectra of the p pure components, and **E** is the matrix of the residuals. A scheme of this process is given in Figure 2. The spectral profiles can be used to identify the substances that provide a signal and the concentration profiles can be used to obtain the areas of each species, which are directly related to the concentration. A set of constraints [32] and working with augmented matrices [33–35] is introduced in the resolution process.

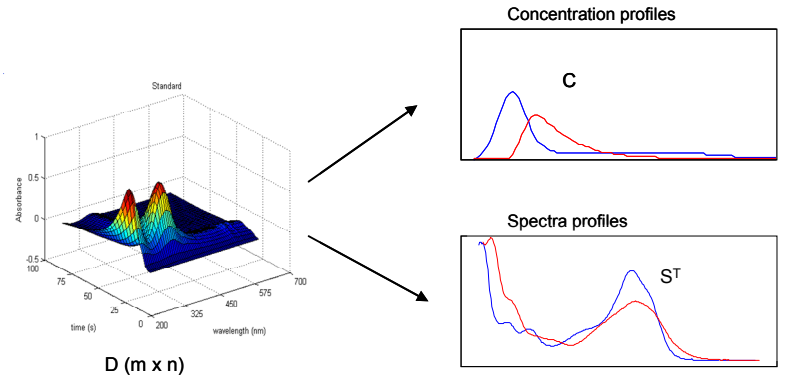


Figure 2. MCR-ALS procedure working with a matrix of a single dye (Acid Red).

To establish a calibration model, augmented matrices are obtained from a matrix corresponding to a calibration standard, a matrix corresponding to a standard solution of constant composition, which we call the reference standard and the vectors corresponding to the spectra of the dyes. From the concentration profiles we obtained the relative area r_p (2).

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$$r_p = \frac{a_p}{a_{rst,p}} \quad (2)$$

where a_p and $a_{rst,p}$ are the areas of the calibration standard and of the reference standard, respectively and we established a univariate linear regression as (3):

$$r_p = b_0 + b_1 \cdot c_p \quad (3)$$

where c_p is $c_{st}/c_{rst,p}$, in which c_{st} is the concentration of the standards and $c_{rst,p}$ is the concentration of the reference standard, and b_1 and b_0 are the parameters of the regression line. The samples were analyzed by the same procedure and the value of the concentration of each analyte in the sample, was obtained from its corresponding r_p value and the calibration parameters.

3. Experimental

3.1. Reagents and samples

In all analyses, we used analytical grade chemicals. These were NaOH and H_2SO_4 from PROLABO (France) and purified water from a Milli-Q water system from MILLIPORE (USA). Standard buffer solutions of pH 4-7 (Hamilton) were used to calibrate the pHmeter. Dyes were obtained from Trumpler Española, S.A. (Spain).

Standard solutions of Acid Red 97, of Acid Brown 425 and of Acid Orange 61 were prepared in Ultrapure Milli-Q water and diluted as required. Acid Orange 61 and Acid Brown 425 correspond to a chromium complex whose ligand has the structure shown in Table 1.

The carrier stream is Ultrapure Milli-Q water. In Table 2 we can see the steps of the whole tanning process. The seven samples in the study belong to the fourth step, *Greasing and dyeing*. We can divide these samples into two groups: samples 1–6, which are taken every 10 min during the first turning process after the addition of dyes, and samples 7 and 8, which are taken after 15 min in the second turning process when formic acid is added.

3.2. Instrumental and software

This system comprised a Cavro XL 3000 stepper motordriven syringe pump of 5 mL equipped with a six-port multiposition automatic selection valve (Eurosas EPS 1306 BPB), and a Hewlett-Packard 8452A spectrophotometer with a Hellma 178.713QS flow-through cell. All tubes to connect the various components of the flow system were made of Omnifit PTFE with 0.8mm (i.d.). The lengths of the holding and reaction coils were 2 and 0.7 m, respectively. The syringe pump, the automatic valve and the data acquisition provided by the spectrophotometer were controlled by a personal computer (PC) via an RS-232 interface, a PCL-711S PC- Lab-Card and an HP-IB IEEE488 interface for communications, respectively.

Table 2. Tanning process

First step: remoisturizing
Add acetic acid and moisturizing tensoactive agent
Turn for 10 min
Empty bath
Second step: retanning
Add acrylic lubricant resin
Turn for 20 min
Add organic chromium
Turn for 90 min
Empty bath
Add formic acid
Turn for 15 min
Empty bath
Third step: neutralizing
Add sodium formiate
Turn for 15 min
Add sodium hydrogen carbonate
Turn for 90-100 min
Empty bath
Add water
Turn for 10 min
Empty bath
Fourth step: greasing and dyeing
Add sulphated triolein and phosphoric ester
Turn for 40 min
Add dyes (6.96 g of each)
Turn for 60 min *SAMPLES 1-6*
Add formic acid
Turn for 30 min *SAMPLES 7-8*
Empty bath
Add water
Turn for 5 min
Empty bath and wring out

The spectra were recorded from 220 to 720 nm in 2 nm steps. As each sample passed through the detector, 100 measurements, one every 0.7 s, were taken. The data were acquired and monitored by the spectrophotometer using the HP89531A software. The instrumentation was controlled by customized software. A Crison pHmeter was used to measure the pH of the samples.

All calculations for multivariate curve resolution with alternating least squares (MCR-ALS) were performed with laboratory-written software under a MATLAB 5.3 computer environment [36] available from http://www.ub.es/gesq/mcr/mcr.htm [37].

4. Results and discussion

First we studied the spectral characteristics of each dye at different pHs in static mode. We measured the absorbance in solutions ranging from pH 1 to 14 in increments of 1 unit of pH. The spectra obtained for each dye are shown in Figure 3.

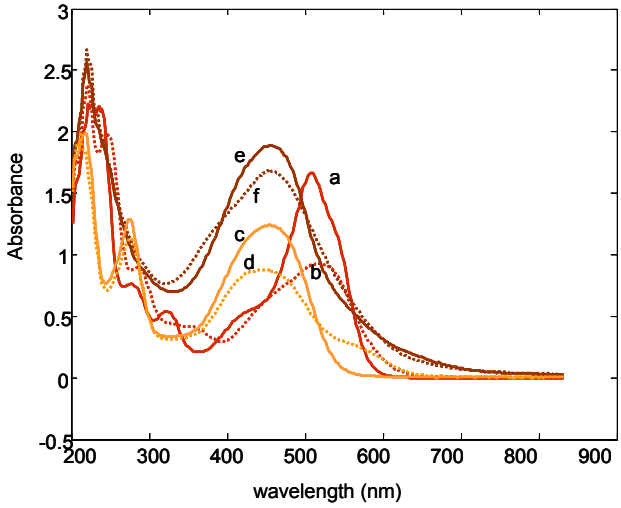

Figure 3. Spectra of each dye obtained using a stopped-flow mode. Acid Red was measured at 50 mg L^{-1} while Acid Brown and Acid Orange were measured at 100 mg L^{-1}. Continuous lines are the acid forms and the dotted lines are the alkaline forms. *a* and *b* correspond to Acid Red, *c* and *d* correspond to Acid Orange and *f* and *e* correspond to Acid Brown.

For Acid Red, we obtained spectrum *a* up to pH 11, between pH 11 and 12 the dye evolved, and at higher pHs we obtained spectrum *b*. For Acid Orange, we obtained spectrum *d* up to pH 2, between pH 2 and 3 the dye evolved, and at higher pHs we obtained spectrum *c*. Finally, for Acid Brown we obtained spectrum *e* up to pH 8, between pH 8 and 9 the dye evolved, and at higher pHs we obtained spectrum *f*.

Acid Red was the most sensitive dye, followed by Acid Brown and Acid Orange, in ratios of approximately 3:1.5:1. By studying each dye individually, we checked that the two species for each dye could be resolved by applying a pH gradient (with NaOH for Acid Red and Acid Brown and with H_2SO_4 for Acid Orange). The linearity of the response of each dye was also studied in these conditions. Acid Red was lineal between 1 and 50 mg L^{-1}, Acid Brown was lineal between 5 and 100 mg L^{-1} and Acid Orange was lineal between 5 and 150 mg L^{-1}.

We then prepared an analytical sequence to resolve the six species in a mixture of three dyes. Because of the pHs at which the three dyes evolve (2–3 for Acid Orange, 10–11 for Acid Brown and 11–12 for Acid Red), we studied an analytical sequence in which we first aspirated NaOH of concentrations between 0.01 and 0.1 M, then the sample and finally H_2SO_4 of the same concentrations. The studied NaOH and H_2SO_4 volumes ranged between 0.042 and 0.083 mL, and the sample volume was 0.25 mL. Flow rate was 1.5 mL min^{-1}. We did not consider this variable, because lower values lengthens the residence time too much and leads to concentration profiles that are too wide and the areas are less reproducible.

The species reached the detector in the following order: first the three acid species, then the basic species of Acid Orange, followed by the basic species of Acid Brown and the basic species of Acid Red. Although we modified the concentrations and volumes of the reagents, this sequence did not produce satisfactory results because we were not able to resolve all the species.

Although the concentration and spectra profiles of Acid Orange could not be obtained after the resolution, we decided to work with an alkaline analytical sequence rather than a complete gradient. In this way, the system contained five components: Acid Red, Basic Red, Acid Brown, Basic Brown and Basic Orange (*a–c*, *e*, *f* following the notation in Figure

3). The experimental conditions were selected by changing the NaOH concentration in the 0.01–0.1M range and the NaOH volume in the 0.042 to 0.25 mL range. NaOH concentrations were those used in the batch mode. NaOH and sample volumes were selected taking into account the dimensions of the reactors of the system and the carrier volume necessary to avoid that the reagents and the sample enter into the syringe. The optimal conditions for analyzing mixtures containing the three dyes were: a NaOH concentration of 0.025 M, a NaOH volume of 0.083 mL, a sample volume of 0.25 mL.

Figure 4 shows the concentration profiles and the spectra of the five species of interest obtained using MCR-ALS of an augmented matrix from a mixture of the three dyes and the individual spectra of each species. Spectra obtained in the resolution show similar sensitivities because of the normalization of the ALS algorithm.

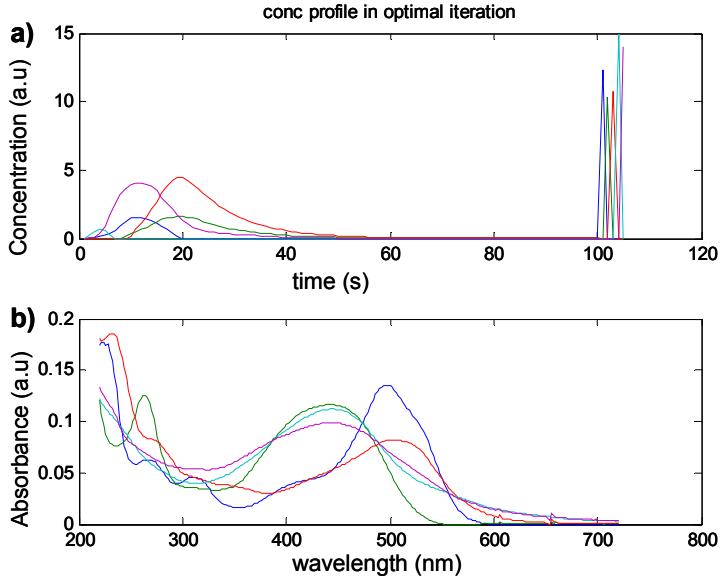

Figure 4. Normalized concentration profiles (a) and pure spectra (b) obtained by multivariate curve resolution of the analysis of an augmented data matrix formed with Sample 1 (sample taken after one minute of turning in the tanning process) and the standard vectors of each dye considered (species *a*–*c*, *e* and *f*). *a* is in blue, *b* in red, *c* in green, e in turquoise and *f* in purple.

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To establish calibration models for each dye, we prepared a set of calibration standards containing the three studied dyes of known concentrations in the 5–30 mg L^{-1} range (5–10–15–20–30). Each of these standards contained the same concentration of dye at each level. To avoid working with solutions that were too absorbent, the working interval was lower than the maximum for each dye.

To determine the value of r_p in Eq. 2, the sum of the areas of both the acid and the basic species of Acid Red and Acid Brown had to be taken into account because this increases the reproducibility of the results. This is because a change in the pH gradient in the reactor increases the area of one of the species and decreases the area of the other.

Figures of merit for each calibration line are shown in Table 3. As calibration was modelled with relative values of both the signal (quotient of areas) and the concentration (quotient of concentrations), ideally the regression line should have a slope of 1 and an ordinate at the origin of 0.

Table 3. Figures of merit for calibrations of the three dyes: Acid Red, Acid Brown and Acid Yellow.

	Dye		
	Acid Red	Acid Brown	Acid Orange
b_1	0.9744	1.0451	0.9675
b_0	0.0197	-0.0291	0.0474
R	0.9995	0.9980	0.9996
s_{b1}	0.0169	0.0387	0.0166
s_{b0}	0.0102	0.0234	0.0101
s_e	0.0108	0.0248	0.0107
n	5	5	5
x_d (mg L^{-1})	2.6	3.9	2.12
F cal	1.29	1.03	8.22
Fcritic (0.05,2,3)		16.04	

b_1 and b_0 are the slope and the intercept of the calibration curve
R is the correlation coefficient
s_{b1} and s_{b0} are the standard deviation of the slope and the intercept
s_e is the standard error
n is the number of points
x_d is the limit of detection
F cal is the calculated value
F critic is the stadistic value

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To check whether the regressions lines satisfy this condition, we compared the slopes and ordinates at the origin of each line with those of the ideal line. This was done using a joint test of ordinate and slope [38]. The *F* values obtained for 95% of confidence are shown in the table. Limits of detection (x_d), calculated taking into account the uncertainty in the regression line [38] with 95% confidence, are also shown in this table.

The concentrations of each dye in the samples were calculated from Eq. 3, and applying the corresponding dilution factors. Samples had to be diluted to be analyzed in the concentration range of the regression line. The standard deviation of the results was calculated from the calibration line parameters [39]. These results are shown in Table 4. Although the standard deviation of the analyzed samples was mostly the same, when we applied the corresponding dilution factors it can be seen that this value increases as the concentration of the sample is bigger.

The accuracy of the method is checked calculating the dyes concentration in spiked samples where known concentrations of standards were added. The difference between each pair of results (concentrations of the spiked samples and concentrations of the samples without spiking) should ideally be the concentration of the added standard. With these data (differences of concentration/concentration of standards) we applied a paired *t*-test [39], adopting the null hypothesis in which the differences between both paired series of data correspond to a population with $\mu_d = 0$. After applying the corresponding *t*-test, we have concluded that there is no significant difference at 90% confidence.

Table 4. Concentrations of the dyes

Sample nº	Time of turning (min)	Concentration ±s(5)[a] (mg L^{-1})		
		Acid Red	Acid Brown	Acid Orange
1	1	4100 ± 140	4200 ± 300	4220 ± 140
2	10	2180 ± 40	2630 ± 90	3230 ± 40
3	20	1160 ± 24	2170 ± 60	1773 ± 24
4	30	1020 ± 24	1690 ± 50	1665 ± 24
5	45	808 ± 18	1060 ± 40	1437 ± 19
6	60	687 ± 18	880 ± 40	873 ± 18
7	75	316 ± 7	50 ± 20	235 ± 7
8	90	191 ± 8	30 ± 20	0 ± 10

[a] s evaluated from the regression line parameters

The evolution of the dyes concentration in front of the time of turning is shown in Figure 5. Error bars show the uncertainty of each value calculated at 95% confidence. We can see that there were four stages in the exhaustion of the dyes. In the first stage (up to 20 min), there was a sharp drop in the concentration. Between minute 20 and 60, the decline was more gradual. Between minute 60 and 75, there was another sharp drop in concentration due to the addition of formic acid. After minute 75 there were practically no more changes in concentration. In sample 7 (75 min of turning), the concentrations were 300, 40 and 240 mg L^{-1} for Acid Red, Acid Brown and Acid Orange, respectively. This means that the percentage dye fixation in all cases was over 93% and over 99% in the case of Acid Brown.

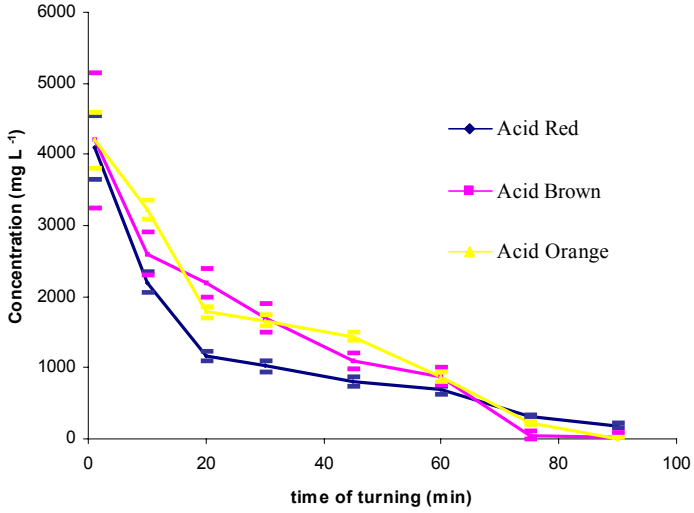

Figure 5. Exhaustion of the dyes expressed in mg L^{-1}. Error bars have been calculated from the linear calibration parameters.

5. Conclusions

SIA-DAD with MCR-ALS is a good method for studying the evolution of a mixture of three dyes in the dyeing process of the tanning process. This system is simple and fast, uses common reagents and has a high frequency of analysis (12 samples h^{-1} including all steps in a SIA process, i.e., cleaning, preparing and analyzing). The main advantage of this system is

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that three analytes can be quantified satisfactorily in a single analysis. Also, it may be possible to optimize the dye exhaustion process in order to reduce the exhaustion time by adding formic acid earlier.

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References

[1] R.D. Combes, R.B. Haveland-Smith, *Mutat. Res*. 98 (1982) 101.

[2] C.C. Sigman, P.A. Papa, M.K. Doeltz, L.R. Perry, A.M. Twhigg, C.T. Helmes, *Environ. Sci. Health* 20 (1985) 427.

[3] J.R. Dipalma, *Am. Farm. Physician* 42 (1990) 1347.

[4] M.S. Bathia, *Indian J. Med. Sci*. 50 (1996) 285.

[5] L.Koutsogeorgopoulou, C. Maravelias, G. Methenitou, A.Koutselinis,*Vet. Hum. Toxicol*. 40 (1998) 1.

[6] J.J.B. Nevado, J.R. Flores, C.G. Cabanillas, M.J.V. Llerena, A.C. Salcedo, *Talanta* 46 (1998) 933.

[7] L.F. Capitán-Vallvey, M.D. Fernández, I. de Orbe, R. Avidad, *Talanta* 47 (1998) 861.

[8] A.G. Fogg, A.M. Summan, *Analyst* (1983) 891.

[9] P.L. López de Alba, L. López-Martínez, L.M. De León-Rodríguez, *Electroanalysis* 14 (2002) 197.

[10] S. Combeau, M. Chatelut, O. Vittori, *Talanta* 56 (2002) 115.

[11] Y. Masukawa, *J. Chromaogr. B* 1108 (2006) 140.

[12] H. Borwitzky, W.E. Haefeli, J. Bruhenne, *J. Chromatogr. B* 826 (2005) 244.

[13] H.Y. Huang, C.W. Chiu, S.L. Sue, C.F. Cheng, *J. Chromatogr.A* 995 (2003) 29.

[14] M.C. Genaro, E. Gioannini, S. Angelino, R. Aigotti, D. Giacosa, *J. Chromatogr. A* 767 (1997) 87.

[15] C.S. Eskilsson, R. Davidsson, L. Mathiasson, *J. Chromatogr. A* 955 (2002) 215.

[16] M.C. Garrigós, F. Reche, M.L. Marín, K. Pernas, A. Jiménez, *J. Chromatogr. A* 963 (2002) 427.

[17] J.J. Berzas, J. Rodríguez Flores, M.J. Villaseñor Llerena, N. Rodríguez Fariñas, *Anal. Chim. Acta* 391 (1999) 353.

[18] P.L. López-de-Alba, L. López-Martínez, K. Wróbel-Kaczmarczyk, K. Wróbel, J. Amador-Hernández, *Anal. Lett*. 29 (1996) 487.

[19] P.L. López-de-Alba, L. López-Martínez, M. González-Leal, Y. Estrada-Hernández, *Quim. Anal.* 18 (1999) 291.

[20] E.C. Vidotti, W.F. Costa, C.C. Oliveira, *Talanta* 68 (2006) 516.

[21] M. Blanco, T. Canals, J. Coello, J. Gené, H. Iturriaga, S. Maspoch, *Anal. Chim. Acta* 419 (2000) 209.

[22] J.J. Berzas-Nevado, C. Guiberteau-Cabanillas, A.M. Contento-Salcedo, *Anal. Lett*. 32 (1999) 1879.

[23] J.J. Berzas-Nevado, J.R. Flores, M.J.V. Llerena, *Talanta* 48 (1999) 895.

[24] N.R. Marsili, A. Lista, B.S. Fernández-Band, H.C. Goicoechea, A.C. Olivieri, *Analyst* 130 (2005) 1291.

[25] A. Pasamanontes, M.P. Callao, *Trends Anal Chem*. 25 (2006) 77.

[26] N.M. Faber, R. Bro, P.K. Hopke, *Chemom. Intell. Lab. Syst*. 65 (2003) 119.

[27] R. Bro, *Chemom. Intell. Lab. Syst.* 38 (1997) 149.

[28] M. Linder, R. Sundberg, *Chemom. Intell. Lab. Syst*. 42 (1998) 159.

[29] R. Tauler, A. Smilde, R. Kowalsky, *J. Chemometrics* 9 (1995) 31.

[30] K.S. Booksh, B.R. Kowalski, *Anal. Chem*. 66 (1994) 782A.

[31] J. Ruzicka, G.D. Marshall, G.D. Christian, *Anal. Chem*. 62 (1990) 1861.

[32] R. Tauler, A. Izquierdo-Ridorsa, E. Cassasas, *Chemom. Intell. Lab. Syst*. 18 (1993) 293.

[33] A. de Juan, E. Casassas, R. Tauler, in: R.A. Meyers (Ed.), *Encyclopedia of Analytical Chemistry*, John Wiley and Sons Ltd, Chichester, 2000, pp. 9800–9837.

[34] A. Izquierdo-Ridorsa, J. Saurina, S. Hern´andez-Cassou, R. Tauler, *Chemom. Intell. Lab. Syst.* 38 (1997) 183.

[35] C. Ruckebusch, L. Duponchel, J.P. Huvenne, J. Saurina, *Anal. Chim. Acta* 515 (2004) 183.

[36] Matlab, The Mathworks, South Natick, MA, USA.

[37] R. Tauler, A. de Juan, Multivariate Curve Resolution homepage, http://www.ub.es/gesq/mcr/mcr.htm.

ISBN: 978-84-691-0990-8/D.L: T.2293-2007

[38] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. de Jong, P.J. Lewi, J. Smeyers-Verbeke, *Handbook of Chemometrics and Qualimetrics Part A*, Elsevier, Amsterdam, 1997.

[39] J.N. Miller, J.C. Miller, *Statistics and Chemometrics for Analytical Chemistry*, 4th ed., Prentice-Hall, Madrid, 2002.

4.3. PAPER

Matrix effect in second-order data

Determination of dyes in a tanning process using vegetable tanning agents

V. Gómez, R. Cuadros, I. Ruisánchez, M.P. Callao

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Matrix effect in second-order data
Determination of dyes in a tanning process using vegetable tanning agents

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Abstract

The aim of this paper is to determine the concentration of three dyes throughout the tanning process of leather using vegetable tanning agents with a sequential injection analyzer with second-order data treatment. As the vegetable tanning agents used are highly absorbent species, we focus on three aspects: (i) difficulties with the resolution (ii) the reduction in the working concentration range; and (iii) matrix effects. Ideally, second-order instruments provide "second-order advantage"; i.e. calibration is possible in the presence of unknown and uncalibrated interfering species. However, if the interfering species change the instrumental response of the analyte (in scale or shape), standard additions must be used to ensure the accuracy of the estimated analyte concentration. Here we study the presence of matrix effects for three dyes in several samples in order to significantly improve the accuracy of predictions in the presence of such effects. We found that there were matrix effects in at least 80% of the samples with an alpha risk of 5%. We used this method to study the exhaustion of dyes in the dyeing process.

Keywords: Dye determination; Vegetable tanning agent; Tanning of leather; Matrix effect; Sequential injection analysis; Multivariate curve resolution

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1. Introduction

Tanning operations include several chemical and mechanical treatments, such as remoisturizing, retanning, neutralizing, fatting and dyeing, to convert a putrescible organic material into a biochemically and mechanically stable product. Two main tanning agents – chrome tanning and vegetable tanning – are used. Chrome tanning is used in most cases (70%) and vegetal tanning is used for specific preparations, such as the manufacture of shoe soles [1]. Vegetable tannins are polyphenolic compounds of two types: hydrolysable tannins (e.g. chestnut and myrobalan), which are derivatives of pyrogallols and condensed tannins (e.g. hemlock and wattle), which are derivatives of catechol [2].

In this study we examine the exhaustion process for three dyes by determining them throughout the dyeing stage. The analytical method is based on a pH-gradient sequential injection system with diode array detection and further data analysis using second-order data treatment with multivariate curve resolution with alternating least squares (MCR-ALS) [3]. In this process a vegetable tanning agent was used. Recent studies have demonstrated the usefulness of this type of systems for determining analytes in complex samples [4,5]. These systems are fast and simple and have a high frequency of analysis. Also, various analytes can be quantified satisfactorily in a single analysis.

Second-order data-treatment techniques provide information about concentration profiles and about the spectra profiles of the species of interest. Second-order calibration methods can recover the pure analyte response from the analysis of a gross signal containing other interfering contributions [6,7]. This is known as “second-order advantage”. Pure analyte standards are commonly used to quantify unknown samples even in the presence of unknown and uncalibrated interferents.

However, the presence of species other than the analytes can cause several problems in the resolution stage. If there is an absorbent species: (i) the system gets complicated because of the greater number of species to resolve, which could make the resolution more ambiguous; (ii) the sample presents high absorbances, which may reduce the concentration range applicable for determining each analyte; and (iii) the sensitivity of the analytes may

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change in the presence of other species and the calibration curve may suffer from the so-called matrix effect [8].

The possible change in the sensitivity of the analyte response as a result of the matrix effect cannot be modelled with second-order calibration models [9], since pure standards do not provide information about the chemical matrix of the sample. Therefore, if the sensitivity of the calibration depends on the chemical composition of the matrix, predictions may be poorer when calibration curves obtained from pure standards are used.

Many studies have been based on second-order techniques but few of these have considered matrix effects in complex samples or provided solutions for solving them [5,10–13]. In our study, the vegetable tanning agent used in the tanning process is a highly absorbent species. We therefore examined the three hypothetically problematical aspects mentioned above in the analysis of the three dyes: (i) difficulties with the resolution; (ii) the possible reduction in the working concentration range; and (iii) matrix effects.

2. Experimental

2.1. Reagents and samples

In all analyses we used analytical grade chemicals. These were NaOH from PROLABO (Fontenay sous Bois, France) and purified water from a Milli-Q water system from MILLIPORE (USA).

Dyes were obtained from Trumpler Española, S.A. (Barberà del Vallès, Barcelona, Spain). Standard solutions of Acid Red 97, Acid Brown 425 and Acid Orange 61 were prepared in Ultrapure Milli-Q water and diluted as required. Colour index for the acid dyes (C.I.) are 97 for the red dye, 425 for the brown dye and 61 for the orange dye. Table 1 shows the chemical structures of these dyes.

The eight samples in the study correspond to tanning solutions with different turning times in reactor for producing the colour of the dyes on the leather. Turning times ranged from 5 to 150 min. These are named M1 to M8.

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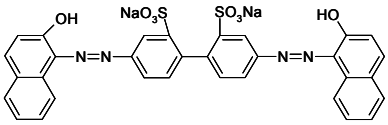
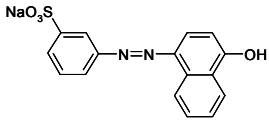
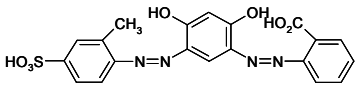
Table 1. Structures of dyes present in the tanning samples

Dye	Structure
Acid Red 97	
Acid Orange 61	 * chromium complex
Acid Brown 425	 * chromium complex

2.2. Apparatus

The sequential injection analyzer comprised: a CAVRO XL 3000 stepper motor-driven syringe pump connected to the PC with an RS-232 interface; a 6-position Eurosas EPS 1306 BPB automatic selection valve connected to the computer through a PCL-711S PC-Lab-Card; a 70 cm×0.8mm Omnifit PTFE tubing reaction coil; a 200cm×0.8mm holding coil; an HP8452A diode-array spectrophotometer controlled by an HP Vectra 5/75 computer equipped with an HP-IB IEEE 488 interface for communications; and a Hellma 178.713QS flow-through cell. Figure 1 shows a scheme of the instrumental set up.

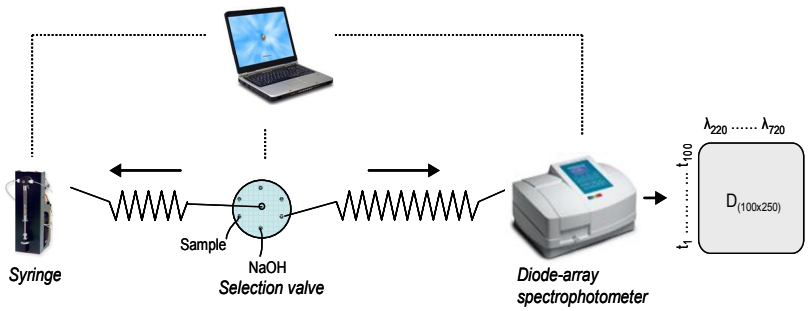


Figure 1. Sequential injection analyzer and data collection

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2.3. Software

HP89531A software was used to record and store the spectra. Customized software was used to control the SIA. All calculations relating to multivariate curve resolution with alternating least squares (MCR-ALS) were performed with laboratory written software under a MATLAB 5.3 computer environment [14]. This software is available from http://www.ub.es/gesq/mcr/mcr.htm.

3. Data treatment

3.1. Data collection

The MCR-ALS method has been described elsewhere [15–17]. With this resolution method, the data matrices are modelled using Eq. 1:

$$\mathbf{D} = \mathbf{C}\mathbf{S}^{T} + \mathbf{E} \quad (1)$$

where matrix **C** ($m \times p$) has column vectors corresponding to the profiles of concentration of the p pure components that are present in matrix **D**. The row vectors of matrix $\mathbf{S}^{T}$ ($p \times n$) correspond to the spectra of the p pure components, and ***E*** is the matrix of the residuals. m are the times and n are the wavelengths at which the absorbance is recorded. The aim of the MCR method is to estimate matrices **C** and $\mathbf{S}^{T}$ by only analyzing **D** using an alternating least squares (ALS) optimization.

The resolution was performed using augmented data matrices. In this case, the spectral domain has been chosen for performing column-wise (i.e. wavelength-wise) matrix augmentation. After the curve resolution of the pure analyte contributions, a calibration curve of pseudo-zero order was built in which the analyte responses (quotient of areas obtained from the concentration profiles) were plotted as a function of the quotient of the analyte concentration in the standards, as in the univariate calibration approach [18]:

$$r_i = b_1 \cdot c_i + b_0 \quad (2)$$

where r_i is the relative area (the quotient between the area of the calibration standard and the reference standard) and c_i is the relative concentration (the quotient between the concentration of the calibration standard and the reference standard). *Calibration standards* are each different standards used to build the calibration line and the *reference standard* is a standard of known concentration that we maintain constant during the resolution process.

3.2. Standard addition method and hypothesis tests[19]

The chemical matrix effect can be detected by comparing the slope of the calibration curves corresponding to the pure standards (b_1) with the slope of the calibration curves corresponding to the samples in which pure standards have been added (b'_1).

The following hypotheses are postulated:

$$H_0: b_1 = b'_1 \quad (3)$$

$$H_1: b_1 \neq b'_1 \quad (4)$$

The resolution of these hypotheses involves known errors such as type I (or α) and type II (or β). A type I error means accepting H_1 when H_0 is true. This means affirming that there are matrix effects when in fact there are not. A type II error means accepting H_0 when H_1 is true. This means affirming that there are no matrix effects when in fact there are.

The values of the slopes are compared using a Student's *t*-test [20]. The value of *t* calculated from the experimental results is compared with the tabulated value of *t* for the selected type I error. If H_0 is rejected, the risk of error, i.e. the probability that H_0 is true, is α.

The probability α of accepting hypothesis $H_0(b_1 = b'_1)$ when in fact b_1 and b_1' are different can be calculated using the corresponding abacus [19] as a function of the number of degrees of freedom (ν) and the parameter λ (Eq. 5) when there is no independent value of σ[19]:

$$\nu = n_1 + n_2 - 4$$

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$$\lambda = \frac{b_1 - b_1^*}{\sigma\sqrt{\left(\frac{1}{n_1} + \frac{1}{n_2}\right)(n_1 + n_2 - 1)}} \qquad (5)$$

b_1^* is a critical value set by the experimenter and considered different from b_1. The unknown value of σ is replaced by its estimate, s (pooled standard deviation), n_1 and n_2 are the measurements taken to evaluate b_1 and b_1', respectively.

4. Procedure

The analytical process involved sequential aspiration of 4.67 mL of the carrier (water), 0.083 mL of reagent (sodium hydroxide 0.025M) and 0.25 mL of sample (mixtures containing the three dyes) using a flow rate of 1.5mL min^{-1}. This solution is then pumped to the detector. The interdiffusion process of the sample and reactants leads to a gradual fall in pH through the channel to the detector. The synthetic dyes are polyprotic species so, with this pH gradient, when the sample reaches the detector, the most acid species appear first, i.e. Brown dye and Red dye, followed by a mixture of these species and the conjugate base of Orange dye, Brown dye and Red dye. The acid form of the Orange dye was not obtained after the resolution because we worked with an alkaline analytical sequence [4]. We studied the spectral characteristics of each dye at different pHs in static mode in solutions ranging from pH 1 to 14 in increments of 1 unit of pH, and we detected two species for each dye.

The spectra were recorded every 2nm in the 220–720nm range, with an integration time of 0.1 s. As each sample passed through the detector, 100 measurements (one every 0.7 s) were taken. Figure 1 (right side) shows a scheme of the data obtained.

If we observed the evolution of spectra against the time, these were changing continuously because were the response of a dynamic system, i.e. were the result of the contribution of each species, that had concentrations evolving. The data treatment with MCR-ALS allowed obtaining individual information of these data matrices.

5. Results and discussion

Figure 2 shows the procedure for applying MCR-ALS. The resolution method was applied to an augmented matrix with the same structure both for mixtures of standards (a) and samples (b). This matrix is made up of matrix $\mathbf{D_1}$, obtained either from a calibration standard with the mixture of the three dyes or from a sample, matrix $\mathbf{D_2}$, obtained from a reference standard with a mixture of the three dyes of known concentrations that is constant for all cases in the study, and the vectors (pure spectra) $\mathbf{v_1}$–$\mathbf{v_5}$ of each species.

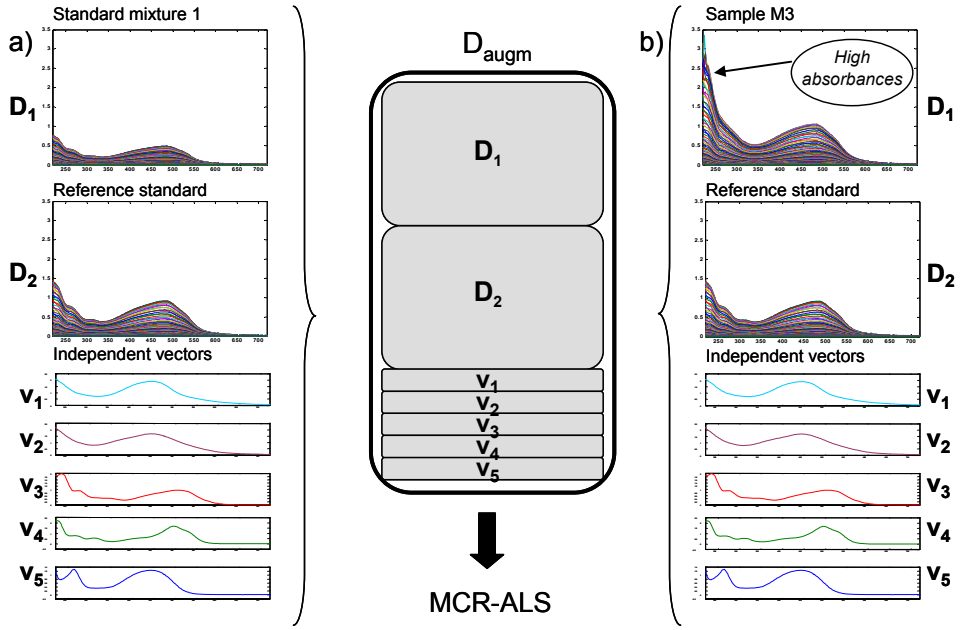

Figure 2. Arrangements of matrices for analyzing synthetic samples (a) or vegetable tanning samples (b). $\mathbf{v_1}$ belongs to the acid form of Acid Brown 425, $\mathbf{v_2}$ belongs to the basic form of Acid Brown 425, $\mathbf{v_3}$ belongs to the acid form of Acid Red 97, $\mathbf{v_4}$ belongs to the basic form of Acid Red 97 and $\mathbf{v_5}$ belongs to the basic form of Acid Orange 61.

The results are shown in Figures 3 and 4. These figures correspond to resolutions in which $\mathbf{D_1}$ is obtained from a calibration standard or from a sample, respectively. These figures show the concentration profiles (a) on one side and the spectra of the dyes (b) on the other. By comparing these figures, we can see that the behaviour of the samples is different from

that of the standards. At least one new component, corresponding to a majority species appears in the resolution of the sample (Figure 4). As a result of this, neither the order nor the areas of the dyes are suitable or as expected. The new component, which is assigned to the tanning agent, presents an intermediate spectrum with respect to the dyes, which may be due to rank deficiency. Also, absorbance in general, and especially in the first wavelengths, is much higher than in the standards of the same order of concentrations (see Figure 2).

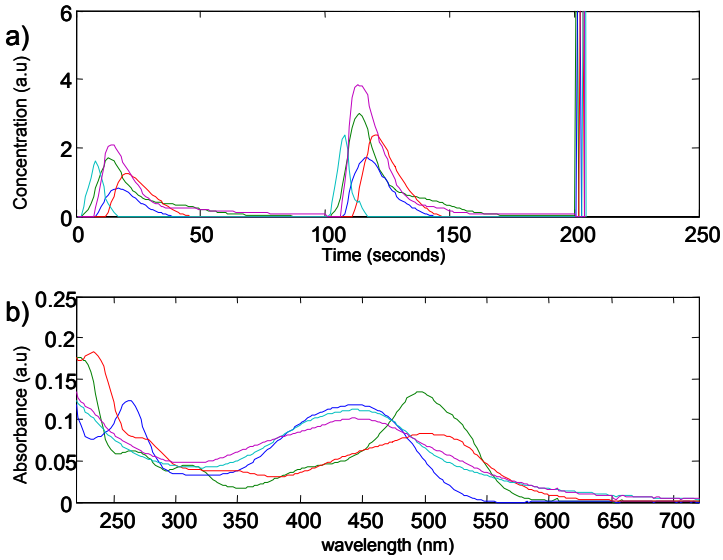

Figure 3. Normalized concentration profiles (a) and pure spectra (b) obtained by multivariate curve resolution of the analysis of an augmented data matrix formed with a standard mixture ($\mathbf{D_1}$), a reference standard ($\mathbf{D_2}$) and the standard vectors of each dye considered ($\mathbf{v_1}$, $\mathbf{v_2}$, $\mathbf{v_3}$, $\mathbf{v_4}$, $\mathbf{v_5}$). $\mathbf{v_1}$ is in turquoise, $\mathbf{v_2}$ is in purple, $\mathbf{v_3}$ is in red, $\mathbf{v_4}$ is in green and $\mathbf{v_5}$ is in blue (a.u. are arbitrary units).

To solve these two problems, which negatively affect the resolution and, therefore, the values of the areas obtained for each dye, we conducted several studies. First we tried to adapt the analytical sequence (volumes and concentrations of the sample and reagent) in order to change the pH gradient, but this did not improve the resolution. We managed to solve the problem of rank deficiency by adding more information to the augmented matrix: this was a new matrix that contained information of the background of the sample with the minimum

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concentration of analyte and was obtained from analysis of the final solution of the study, when the presence of the dyes was minimal. In this way we obtained consistent resolutions.

With regard to the increase in absorbance, we studied whether it was possible to shorten the working range of wavelengths (220–720 nm). The maximum absorbances correspond to the first wavelengths of the studied range, so we removed the first rows of the data matrix and we worked in the 248–720nm range. The reduction of rows of the matrix (wavelengths) does not affect the resolution process but allows reducing the values of absorbances.

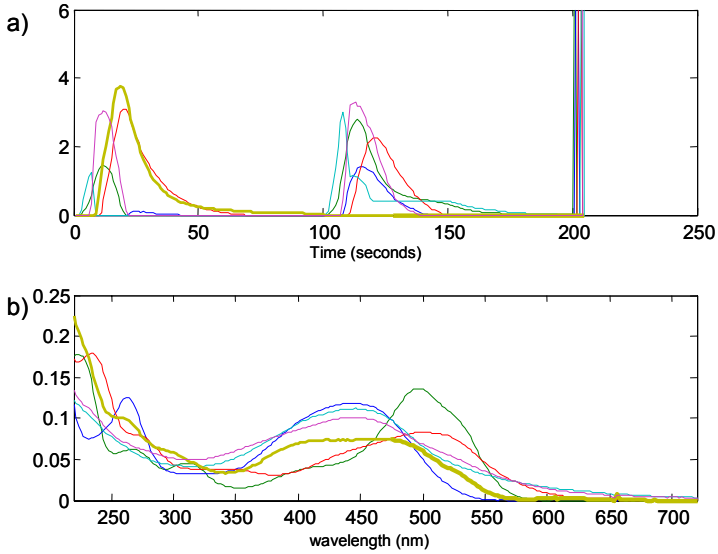

Figure 4. Normalized concentration profiles (a) and pure spectra (b) obtained by multivariate curve resolution of the analysis of an augmented data matrix formed with sample 3 ($\mathbf{D_1}$), a reference standard ($\mathbf{D_2}$) and the standard vectors of each dye considered ($\mathbf{v_1}$, $\mathbf{v_2}$, $\mathbf{v_3}$, $\mathbf{v_4}$, $\mathbf{v_5}$). $\mathbf{v_1}$ is in turquoise, $\mathbf{v_2}$ is in purple, $\mathbf{v_3}$ is in red, $\mathbf{v_4}$ is in green and $\mathbf{v_5}$ is in blue (a.u. are arbitrary units).

In the rest of the spectrum, however, the absorbance of the sample was higher than that of the standards. This can negatively affect samples with low concentrations of dyes because, to measure levels of absorbance that are compatible with the detector, the sample would have to be diluted, which would reduce the concentration of the dyes and risk not detecting them. We analyzed the effect of absorbance on the areas obtained in the resolution

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process for the three dyes. To do so, we studied three samples (M1, M3 and M5) with decreasing concentrations of dyes but similar concentrations of vegetable tanning agents.

In general, we observed that, as the ratio between the concentration of dye and the concentration of tanning agent decreased, more absorbent solutions produced greater deviations in the areas obtained. We also found that absorbances above 1.5–2.0 can lead to inconsistent results. This is a limitation of using these methods in complex samples with absorbent species because, although the analytes of interest can be resolved, the range of concentrations that can be determined is narrower.

To evaluate the matrix effects, several standard additions were performed for each tanning sample (see Table 2 for vegetal sample 1). The six standard additions were denoted M1SA0, M1SA1, M1SA2, M1SA3, M1SA4 and M1SA5. The other samples (M2–M8) were prepared under the same conditions. We also prepared standard solutions with the same concentrations of the three analytes as the concentrations added to each sample in the study.

Table 2. Performed standard addition samples

	Conentration added (mg L^{-1})		
	Acid Red	Acid Orange	Acid Brown
M1SA0	0	0	0
M1SA1	5	5	5
M1SA2	10	10	10
M1SA3	12	12	12
M1SA4	15	15	15
M1SA5	20	20	20

Table 3 shows the results obtained for each dye. The first column shows the sample, the second column shows the slope of the calibration curve. The next two columns show the statistical parameters to compare each standard addition regression line with the regression line of standards. Column 3 shows the *F* values for the variance comparison test and column 4 shows the *t*-calculated values for the slope comparison test. With the exception of Acid Red 97 in sample M5, the variances in the slopes are comparable for an α value of 0.05.

The results of comparing the calculated value of *t* with the tabulated value for α = 0.05 and the degrees of freedom corresponding to the two compared slopes are shown in the

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final column of Table 3. Generally, there are matrix effects except for Acid Red 97 in sample M1, Acid Orange 61 in samples M3 and M5 and Acid Brown 425 in samples M5 and M6.

Table 3. Results for each dye in the tanning samples

	b_1'	Fcal	tcal	Matrix effect
Acid Red 97				
M1	0.9667	0.59	1.18	No (α=0.05 β= 0.09)
M2	1.0024	0.21	3.44	Yes (α=0.05)
M3	1.1462	3.14	6.07	Yes (α=0.05)
M4	1.0510	0.58	5.31	Yes (α=0.05)
M5	1.1786	7.61	4.80	Yes (α=0.05)
M6	1.0320	1.04	3.80	Yes (α=0.05)
M7	1.0474	0.49	5.31	Yes (α=0.05)
M8	1.0938	0.87	6.73	Yes (α=0.05)
b_1 (standards)	0.9424			
Acid Orange 61				
M1	0.9513	0.94	3.82	Yes (α=0.05)
M2	0.9516	2.69	2.69	Yes (α=0.05)
M3	1.1048	1.71	1.55	No (α= 0.05 β= 0.26)
				No (α= 0.1 β= 0.17)
M4	0.9135	1.77	4.30	Yes (α=0.05)
M5	1.0824	1.58	0.91	No (α= 0.05 β= 0.13)
				No (α= 0.1 β= 0.06)
M6	1.1873	1.14	4.70	Yes (α=0.05)
M7	1.1463	1.80	2.81	Yes (α=0.05)
M8	1.1677	2.14	3.26	Yes (α=0.05)
b_1 (standards)	1.0538			
Acid Brown 425				
M1	0.9554	0.32	3.36	Yes (α=0.05)
M2	1.0463	0.80	6.22	Yes (α=0.05)
M3	1.0020	1.48	3.73	Yes (α=0.05)
M4	0.9818	0.25	4.68	Yes (α=0.05)
M5	0.9283	0.41	1.94	No (α=0.05 β=0.11)
				Yes (α=0.1)
M6	0.9392	1.46	1.85	No (α=0.05 β=0.4)
				Yes (α=0.1)
M7	1.0061	0.89	4.58	Yes (α=0.05)
M8	1.0583	0.56	7.24	Yes (α=0.05)
b_1 (standards)	0.8804			

In these cases we studied the probability of making a type II or β error, i.e. the probability of affirming that there were no matrix effects when in fact there was. To do so, we set a critical b_1^* value for each dye as the value of b_1 in the regression line of the standards

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plus 5%. This would affect the concentration by approximately 5%. Clearly, the more permissive one is with these differences, the lower is the type II error for a preset value of α.

If we apply Eq. 5 we obtain the values of λ. Using these values, and those of α and the corresponding degrees of freedom, we obtain the values of β[19], as we can see in the final column of Table 3. For Acid Red 97 in sample M1, this β error is 9%, which means that the probability of committing an error is low. Given the experimental cost involved in considering matrix effects, we can conclude that there is in fact no risk. At the other extreme, for Acid Brown 425 in sample M6, the probability of accepting the null hypothesis when in fact it is false is 40%, which is very high. The other cases provide intermediate conclusions. If we wish to offset these two risks of error, the value of α can be increased in order to obtain lower values of β. By doing this we conclude that there are matrix effects for Acid Brown 425 in sample M6. There are therefore 2 out of 24 comparisons for which we can say with low risks of error that there are no matrix effects. Generally, however, we can conclude that in complex samples, we must consider the possibility of matrix effects.

Figure 5 (dotted line) shows the exhaustion curves. Fixation of dyes after 150 min running time is 97.9%, 97.5% and 97.2% for Acid Red 97, Acid Orange 61 and Acid Brown 425, respectively and after 100 min running time it is 97.5%, 92.2% and 93.1% for Acid Red 97, Acid Orange 61 and Acid Brown 425, respectively.

The technique can therefore be optimized by reducing the time, since after 100 min the increase in dye fixation is not very significant. For the purpose of comparison, we have also calculated the concentration of dyes for the exhaustion curve without considering matrix effects (continuous line). We can see from this figure that Acid Brown 425 was the one that presented the greatest differences in this analysis. We can also see the concentrations are different but the way in which the dyes are fixed on the leather is practically the same.

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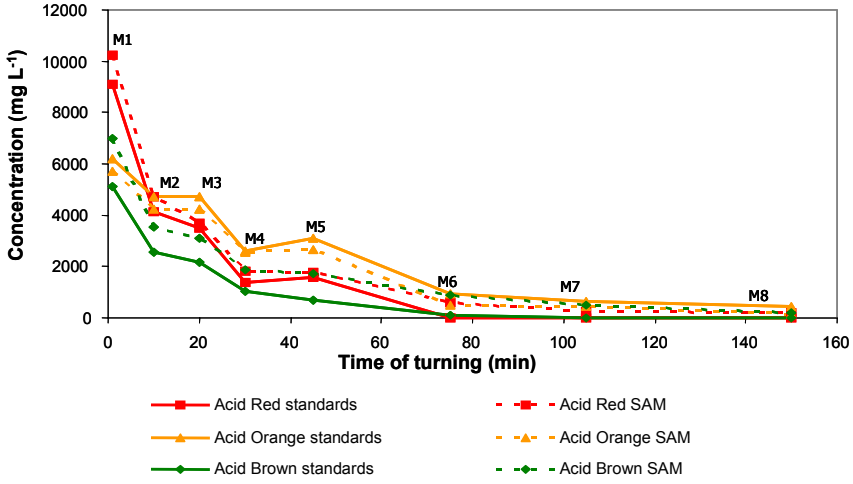

Figure 5. Exhaustion of dyes expressed in mg L^{-1}

6. Conclusions

A flow system such as SIA-DAD and using MCR-ALS to treat the data is a good method for studying the evolution of a mixture of three dyes in the dyeing stage of the tanning process. The main advantage of this method, thanks to the great potential of MCR-ALS, consists on the possibility of the simultaneous quantification of three analytes in complex samples in a single analysis. This system is fast and simple, uses common reagents, and has a high frequency of analysis.

Interferents can be modelled with these second-order chemometric techniques and some strategies can be used for solving matrix effects. We have extended the standard addition method to three-way analysis using multivariate curve resolution to prove the existence of such effects.

With this approach, no real samples were needed in the calibration since only standard additions from pure analyte solutions were performed.

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References

[1] Prospects of EM Technology In Controlling Leather Industry Pollution, http://www.embiotech.org.

[2] Treatment of Tannery Wastewater Naturgerechte Technologien, Bauund Wirtschaftsberatung (TBW) GmbH, Frankfurt (Germany), April 2002, http://www.gtz.de/gate/gateid.afp.

[3] A. Pasamontes, M.P. Callao, *Trends Anal. Chem*. 25 (2006) 77.

[4] V. Gómez, J. Font, M.P. Callao, *Talanta* 71 (2007) 1393.

[5] A. Checa, R. Oliver, J. Saurina, S. Hernández-Cassou, *Anal. Chim. Acta* 572 (2006) 155.

[6] E. Sánchez, B.R. Kowalski, *J. Chemom*. 2 (1990) 247.

[7] K.S. Booksh, B.R. Kowalski, *Anal. Chem*. 66 (1994) 782.

[8] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. De Jong, P.J. Lewi, J. Smeyers-Verbeke, *Handbook of Chemometrics and Qualimetrics*, Elsevier, Amsterdam, 1997.

[9] K. Booksh, J.M. Henshaw, L.W. Burgués, B.R. Kowalski, *J. Chemom*. 9 (1995) 263.

[10] J. Saurina, R. Tauler, *Analyst* 125 (2000) 2038.

[11] A. Herrero, S. Zamponi, R. Marassi, P. Conti, M.C. Ortiz, L.A. Sarabia, *Chemom. Intell. Lab. Syst.* 61 (2002) 63.

[12] M.J. Culzoni, H.C. Goicoechea, A.P. Pagani, M.A. Cabezón, A.C. Olivieri, *Analyst* 131 (2006) 718.

[13] M.M. Sena, M.G. Trevisan, R.J. Poppi, *Talanta* 68 (2006) 1707.

[14] Matlab, The Mathworks, South Natick, MA, USA.

[15] R. Tauler, *Chemom. Intell. Lab. Syst*. 30 (1995) 133.

[16] R. Tauler, A. Smilde, B.R. Kowalski, *J. Chemom*. 9 (1995) 31.

[17] J. Jaumot, R. Gargallo, A. de Juan, R. Tauler, *Chemom. Intell. Lab. Syst*. 76 (2005) 101.

[18] N.M. Faber, *Chemom. Intell. Lab. Syst*. 50 (2000) 107.

[19] Statistique appliqueé à l'exploitation des mesures, Cetama, 2é edition, MASSON.

[20] J.N. Miller, J.C. Miller, *Statistics and Chemometrics for Analytical Chemistry*, fifth ed., Pearson Education Ltd., Harlow, 2005.

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4.4. PAPER

Kinetic and adsorption study of acid dye removal using activated carbon

V. Gómez, M.S. Larrechi, M.P. Callao

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SEQUENTIAL INJECTION ANALYSIS USING SECOND-ORDER CALIBRATION FOR THE DEVELOPMENT OF ANALYTICAL METHODS
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Kinetic and adsorption study of acid dye removal using activated carbon

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Abstract

The adsorption of three acid dyes, Acid Red 97, Acid Orange 61 and Acid Brown 425 onto activated carbon was studied for the removal of acid dyes from aqueous solutions at room temperature (25 ºC). The adsorption of each dye with respect to contact time was then measured to provide information about the adsorption characteristics of activated carbon. The rates of adsorption were found to conform to the pseudo-second-order kinetics with a good correlation. The experimental isotherms obtained, except for Acid Orange 61 studied in mixture, were of the S-type in terms of the classification of Giles and co-workers. The best fit of the adsorption isotherm data was obtained using the Freundlich model. When a comparative study was made of the results obtained with single and mixed dyes, it can be seen that some of them affect others and modify their behaviour in the adsorption process. The results indicate that activated carbon could be employed for the removal of dyes from wastewater.

Keywords: Activated carbon; Adsorption; Dye removal; Kinetics; Isotherms; Wastewater

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1. Introduction

Wastewater generated by the dye production industry and many other industries which use dyes and pigments is high in both colour and organic content. About 10,000 different commercial dyes and pigments exist, and over 7×10^5 tons are produced annually world-wide. It has been estimated that about 10–15% of these dyes are released in effluents during dyeing processes[1]. The discharge of highly coloured waste is not only aesthetically displeasing, but it also impedes light penetration, thus upsetting biological processes within a stream. In addition, many dyes are toxic to some organisms and may cause direct destruction of aquatic communities. Hence, removal of dyes from such wastewaters is a major environmental problem and it is necessary because dyes are visible even at low concentrations.

There are many processes available for wastewater treatment of dyes: chemical oxidation, foam flotation, electrolysis, biodegradation, adsorption, chemical coagulation and photocatalysis[2-13]. Adsorption onto activated carbon has proven to be one of the most effective and reliable physicochemical treatment methodology[14-17].

The objective of the present study was to assess the ability of activated carbon to remove three acid dyes, Acid Red 97, Acid Orange 61 and Acid Brown 425 from aqueous effluents. These dyes are commonly used in the tanning industry.

Most of the studies reported in the literature deal with the adsorption of individual adsorbates from solution onto solids. Few adsorption studies have been made on binary or ternary mixtures on solid surfaces, although in technological processes several dyes and other substances are often present in wastewater. To quantify binary or ternary mixtures of substances, we need selective procedures. When there are possible interferences that affect the signal or if the species present have overlapping signals, samples need to be treated in order to obtain selectivity. Nevertheless, studies on how several species behave in the presence of an adsorbent must surely be interesting since competitive interaction may take place with the solid surface as well as the mutual interaction between them. We have developed an analytical method for determining the concentration of dyes in mixtures[18]. This method does not use hazardous reagents, the amount of chemical waste generated during

the analysis is minimal (333 μL per sample analyzed), and the analysis frequency is 12 samples per hour. By applying this method we can determine the concentration of single dyes and their mixtures.

In this study, we investigated the kinetics and the equilibrium of adsorption of acid dyes onto activated carbon. Similar studies have been done for adsorption of other organic compounds such as Rhodamine-B and Congo Red[15,16,19]. The results will give a better insight into those aspects of the system that will enable us to study the optimization of the experimental factors and lead to an efficient process of removing these dyes from dye production wastewater at minimum cost. We put particular emphasis on the behaviour of these dyes when they are used singly or mixed in solution.

2. Materials and methods

2.1. Materials

Adsorbent: The commercial material Activated Carbon (CAC) was supplied by J.T. Baker (Holland).

Adsorbates (Analytes): Acid Red 97, Acid Orange 61 and Acid Brown 425 were selected as the adsorbates to be used to investigate the adsorption selectivity of acid dyes. Dyes were of analytical grade from Trumpler, S.A (Spain) and were used without further purification. The structures of dyes are shown in Table 1.

Reagents: The sodium hydroxide used was of analytical/ laboratory grade, procured from Prolabo (France). We used purified water from a Milli-Q water system from Millipore (USA).

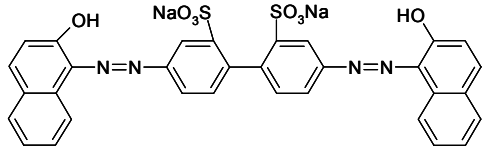
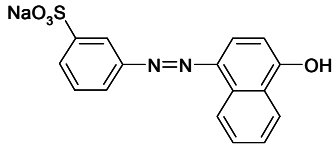
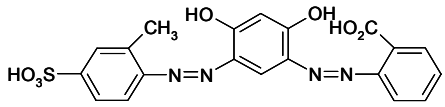
2.2. Analytical method

Instrument

The instrumentation used to determine the concentration of dyes consists of a sequential injection (SI) system. This system has the following components: a CAVRO XL 3000 stepper motordriven syringe pump connected to the PC with an RS-232 interface; a 6-

position Eurosas EPS 1306 BPB automatic selection valve connected to the computer through a PCL-711S PC-Lab-Card; a 70 cm × 0.8 mm Omnifit PTFE tubing reaction coil; a 200 cm × 0.8 mm holding coil; an HP8452A diode-array spectrophotometer controlled by an HP Vectra 5/75 computer equipped with an HP-IB IEEE 488 interface for communications; and a Hellma 178.713QS flow-through cell.

Table 1. Dye structures

Dye	Structure
Acid Red 97	
Acid Orange 61	 * chromium complex
Acid Brown 425	 * chromium complex

Analytical procedure

In SI systems the sample and the reagents are aspirated towards the syringe and stored into the holding coil. They are then pumped to the reaction coil, where the reagents mix with the sample via an interdiffusion process, and directed towards the detector.

The analytical process[18] involved sequential aspiration of the carrier (water), reagent (sodium hydroxide) and sample (solutions containing the dyes). The interdiffusion process of the sample and reactants led to a gradual fall in pH through the channel to the detector. The synthetic dyes are polyprotic species so, with this pH gradient, when the sample

reaches the detector the most acid species appear first, followed sequentially by their basic forms. The signal obtained is a data matrix, and consists of absorbances recorded at different wavelengths and at different pHs. This matrix is treated with the second-order algorithm, Multivariate Curve Resolution with Alternating Least Squares (MCR-ALS), developed by Tauler et al. [20], and available on their webpage [21] which enables the concentration of the analytes to be recovered. The analytical method that we propose is adequate for multicomponent determinations. Mathematically, imposing chemical constraints and incorporating the spectra of all the components, we can distinguish between each dye and predicted concentration of one dye is not affected by the presence of others.

Adsorption measurements

The dye adsorption data from water solutions were obtained by the immersion method. All of the dye solution was prepared with distilled water. For adsorption experiments, 0.2 g of activated carbon was added to 500 mL of dye water solutions at a concentration of 30 mg L^{-1} for each dye.

The solution and solid phase were separated by filtration with nylon syringe filters from Sharlau. A 5 mL aliquot of the supernatant was removed and analyzed for dyes. The adsorption capacity of dyes was then calculated using the relation $q = V\Delta C/m$, where V was the volume of the liquid phase, m was the mass of the adsorbent, and ΔC was the difference between the initial and final concentration of dye in solution. For the experiments of adsorption kinetics, the amounts of dye adsorption were determined by analyzing the solution at appropriate time intervals at room temperature (25 ºC).

The standard deviations of the results, i.e. the measured dyes concentration at equilibrium and the adsorption capacity, were calculated from the calibration line parameters [22]. Figures of merit of the calibration lines are described in [18]. The standard deviation of the adsorption capacity is evaluated from the transmission error expression, considering the corresponding errors of the parameters used for its calculation.

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2.3. Theory

Kinetic models

The equation corresponding to the pseudo-first-order kinetic model is the following:

$$\log \frac{q_e}{q_e - q_t} = \frac{k_1}{2.303} t \qquad (1)$$

where q_e and q_t refer to the amount of dye adsorbed (mg g^{-1}) at equilibrium and at any time, t (min), respectively, and k_1 is the equilibrium rate constant of the pseudo-first-order sorption (min $^{-1}$). Eq. 1 can be rearranged to obtain a linear form:

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t \qquad (2)$$

The equation corresponding to the pseudo-second-order kinetic model is the following:

$$\frac{1}{q_e - q_t} = \frac{1}{q_e} + k_2 t \qquad (3)$$

where k_2 is the equilibrium rate constant of the pseudo second-order adsorption(g mg^{-1} min^{-1}). Eq. 3 can be arranged to obtain a linear form:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \qquad (4)$$

Isotherm models

The theoretical Langmuir isotherm equation can be represented as:

$$q_e = \frac{q_{mon} K_L C_e}{1 + K_L C_e} \qquad (5)$$

where K_L is the Langmuir constant related to the energy of adsorption (L mg^{-1}) and q_{mon} is the maximum amount of adsorption corresponding to complete monolayer coverage on the

surface (mg g^{-1}). The constants K_L and q_{mon} can be determined from the following linearized form of:

$$\frac{1}{q_e} = \frac{1}{q_{mon}} + \frac{1}{K_L q_{mon}} \frac{1}{C_e} \quad (6)$$

The essential features of Langmuir adsorption isotherm can be expressed in terms of a dimensionless constant called the separation factor or equilibrium parameter (R_L). Conformation of the experimental data into Langmuir isotherm model indicates the homogeneous nature of the activated carbon surface.

The Freundlich isotherm can be used for non-ideal sorption that involves heterogeneous surface energy systems and is expressed by the following equation:

$$q_e = K_F C_e^{1/n} \quad (7)$$

where K_F is a rough indicator of the adsorption capacity and *1/n* is the adsorption intensity. In general, as the K_F value increases the adsorption capacity of an adsorbent for a given adsorbate increases. Eq. 7 may be linearized by taking logarithms:

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \quad (8)$$

Multicomponent equilibrium studies have been developed attaining different perspectives. The ideal adsorbed solute theory (IAST) has been used to predict isotherm data for binary systems[23] and ternary systems [24].

On the basis of the Langmuir and Freundlich isotherms (Eqs. 5 and 7), the relationships for predicting multicomponent adsorption has been derived and are known as the extended Langmuir equation 9 [25] and the extended Freundlich equation 10 [26]:

$$q_{e,i} = \frac{q_{mon} K_{L,i}^0 C_{e,i}}{1 + \sum K_{L,i}^0 C_{e,i}} \quad (9)$$

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$$C_{e,i} = \frac{q_i}{\sum_{j=1}^{N} q_j} \left(\frac{\sum_{j=1}^{N} n_j q_j}{n_i K_i} \right)^{n_i} \quad \text{(i= 1 to N)} \qquad (10)$$

MCR-ALS algorithm

The aim of second-order data treatment techniques such as MCR-ALS [20,27-28] is to decompose the raw matrix **D** into the product of two matrices according to:

$$\mathbf{D} = \mathbf{C}\mathbf{S}^{T} + \mathbf{E} \qquad (11)$$

where matrix **C** ($m \times p$) has column vectors corresponding to the profiles of concentration of the p pure components that are present in matrix **D**. The row vectors of matrix $\mathbf{S}^{T}$ ($p \times n$) correspond to the spectra of the p pure components, and **E** is the matrix of the residuals.

The resolution of the profiles of species consisting of two steps: a preliminary spectral estimation of species ($\mathbf{S}^{T}$) and a estimation of concentration profiles (**C**) from Eq. 11. Subsequently, the spectral profiles are updated using the **C** matrix. These steps are repeated until reaching the optimal **C** and $\mathbf{S}^{T}$ according to various optimization criteria. A set of chemical constraints and working with augmented matrices is introduced in the resolution process.

The spectral profiles can be used to identify the substances that provide a signal and the concentration profiles can be used to obtain the areas of each species, which are directly related to the concentration[18]. Regression lines were built for each dye and regression errors where below 5% in all cases.

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3. Results and discussion

3.1. Kinetic study

The influence of contact time on dye colour removal by activated carbon in individual dyes is presented in the Figure 1 (dotted lines). We also studied the influence of contact time on dye removal by activated carbon in mixtures containing all three dyes (continuous line).

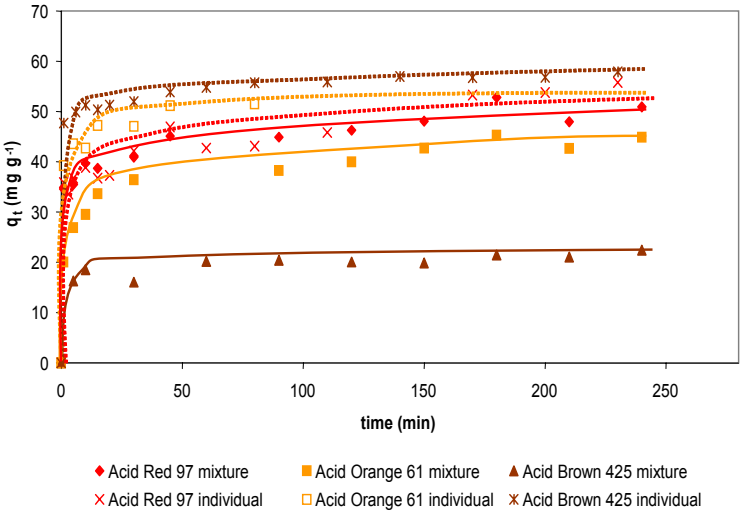

Figure 1. Kinetics of dye removal by activated carbon (0.4 g L^{-1} for individual dyes and for the mixture of the three dyes) at concentration of residual dye in wastewater (30 mg L^{-1} each dye).

It is evident that the carbon is efficient at absorbing acid dyes from wastewater, and the process gradually attains equilibrium. It can be seen that the maximum amount adsorbed of each dye when they reach the equilibrium, decreases when dyes are in a mixture, in a first stage because the total amount of dye in solution is higher. Anyway, this reduction is not the same for all dyes, for the Acid Red 97 the behaviour is mostly the same when it is alone, for the Acid Orange 61 the behaviour is more different and for the Acid Brown 425 it is drastically different. This behaviour shows that there is competition between dyes and that there are some priorities in the occupation of the adsorption sites of the activated carbon.

The kinetic adsorption data were processed to study the dynamics of the adsorption process in terms of the order of rate constant. Firstly, kinetic data were treated with the pseudo-first-order kinetic model. Values of the rate constant (k_1), equilibrium adsorption capacity (q_e), the correlation coefficient (r), and the residual error (s_e) were calculated from the plots of $\log(q_e - q_t)$ versus t for each individual dye and are presented in Table 2. s_e corresponds to the estimated error from the residuals obtained from the regression lines built to obtain the kinetic parameters. The calculated equilibrium adsorption capacities do not agree with experimental values. This indicated that the adsorption of these acid dyes onto activated carbon is not an ideal pseudo-first-order reaction.

Kinetic data were further treated with the pseudo-second-order kinetic model[29,30]. If the pseudo-second-order kinetics is applicable, the plot of t/q_t versus t should show a linear relationship. Table 2 shows the second-order rate constant (k_2), the equilibrium adsorption capacity (q_e), the correlation coefficients (r), and the residual errors (s_e) obtained from the regression line. The linear plots of t/q_t versus t show that the experimental data agree with the pseudo-second-order-kinetic model for the three dyes. The calculated q_e values agree very well with the experimental data and the correlation coefficients for the second-order kinetic model are higher than 0.99 in all cases. These indicate that the adsorption of acid dyes from wastewater onto activated carbon obeys the pseudo-second-order kinetic model. Namasivayam and Kavitha reported similar findings for the adsorption of Congo Red onto activated carbon prepared from agricultural waste[31]. Mall et al. found higher values for the same adsorbate, but with different type of adsorbents[15]. Malik reported similar findings for the adsorption of direct dyes onto activated carbon developed from sawdust[32].

Table 2. The pseudo-first and pseudo-second order adsorption rate constants and the calculated and experimental q_e values for adsorption of each acid dye individually and in a mixture onto activated carbon

Sample	First-order kinetic model					Second-order kinetic model			
	q_e (exp) (mg g^{-1}) (s)	k_1 (min^{-1}) (s)	q_e (cal) (mg g^{-1}) (s)	r	s_e	k_2 (g mg^{-1} min^{-1}) (s)	q_e (cal) (mg g^{-1}) (s)	r	s_e
Acid Red 97	55.25 (0.03)	0.0111 (0.0016)	21.58 (2.33)	0.9530	0.22	0.0025 (0.0004)	54.35 (0.98)	0.9940	0.20
Acid Orange 61	51.33 (0.06)	0.0348 (0.0131)	10.38 (2.14)	0.8391	0.13	0.0162 (0.0051)	51.28 (0.82)	0.9995	0.02
Acid Brown 425	56.82 (0.03)	0.0154 (0.0012)	8.82 (0.56)	0.9747	0.11	0.0094 (0.0009)	57.14 (0.51)	0.9999	0.07
Acid Red 97 (mixture)	51.54 (0.03)					0.0026 (0.0012)	51.81 (0.86)	0.9971	0.18
Acid Orange 61 (mixture)	45.75 (0.06)					0.0018 (0.0004)	46.30 (0.84)	0.9945	0.23
Acid Brown 425 (mixture)	21.84 (0.03)					0.0092 (0.0029)	21.98 (0.51)	0.9982	0.39

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We obtained similar results when studying mixtures that contain all three dyes. The second-order rate constant (k_2) and the equilibrium adsorption capacity (q_e) were calculated from the intercept and slope of the plots of t/q_t versus t for each dye in a mixture solution. Results are shown in Table 2. We can see that the values of the rate constants are similar to those obtained when each dye is studied individually, but the individual dyes and the mixtures have different adsorption capacities at equilibrium: they are slightly lower for Acid Red 97 and Acid Orange 61. The highest differences are for Acid Brown 425, which in mixtures has the lowest adsorption.

3.2. Isotherm study

Figure 2 shows adsorption isotherms that express the adsorbed amounts as a function of equilibrium concentration for Acid Red 97, Acid Orange 61 and Acid Brown 425 in individual solutions. The standard deviations of measured dyes concentration at equilibrium (C_e) and adsorption capacity (q_e) are at the most 0.7 and 1.8, respectively, in all cases.

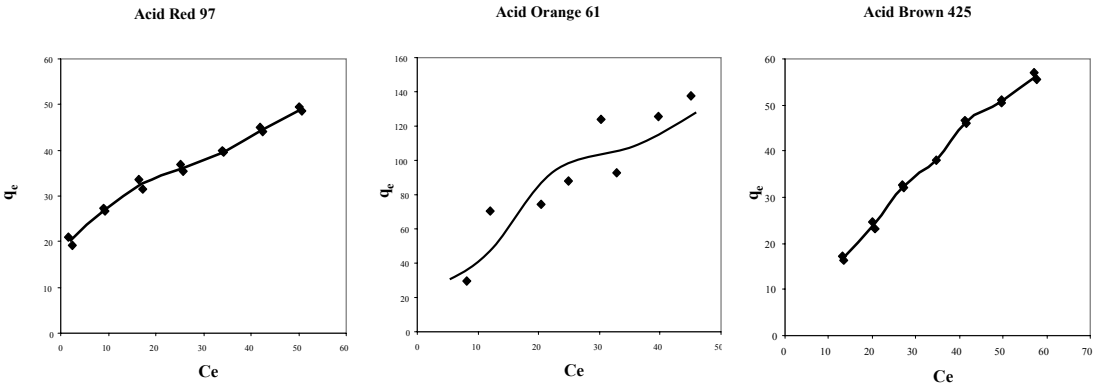

Figure 2. Isotherms for adsorption of Acid Red 97, Acid Orange 61 and Acid Brown 425 onto activated carbon. Contact time = 80, 30 and 50 min, respectively, temperature = 25 ºC.

Using the Giles classification[33], the experimental isotherms obtained in the present study were of type S. The initial part of the S curve indicates little interaction between acid dyes and the solid at low concentrations. However, as the concentration in the liquid phase increased, adsorption occurred more readily. This behaviour is due to a synergistic effect, with the adsorbed molecules facilitating the adsorption of additional molecules as a result of

attractive adsorbate–adsorbate interactions. This was particularly the case when the solute molecule had a fairly large hydrophobic component.

Figure 3 shows the isotherms obtained when mixtures of the three dyes were studied. For Acid Red 97 and Acid Brown 425 type S isotherms are observed. The Acid Orange 61 isotherm is different and has been observed by several authors [34]. The presence of the other dyes changes the concentration ratio in equilibrium compared to the quantity adsorbed. There are three stages: at first the amount adsorbed increases as the equilibrium concentration increases, then it stabilizes and finally there is a decrease when the equilibrium concentration increase. If we compare the obtained isotherms for Acid Red 97 and for Acid Brown 425 (these are the dyes that maintain similar behaviours when they are alone or in mixtures) we could appreciate that when they are alone, the isotherms are comparable. Meanwhile when they are in a mixture, the red dye has similar behaviour being alone or in a mixture, but the brown dye has considerably lower adsorption capacity onto activated carbon.

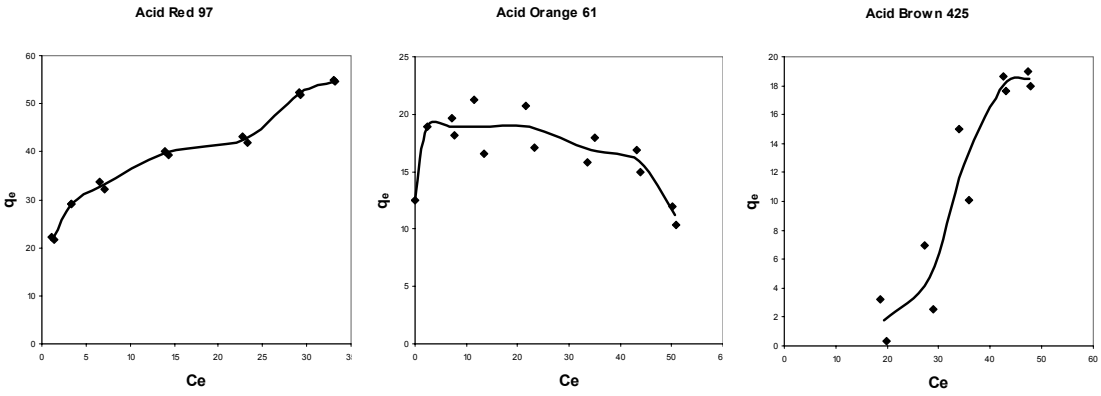

Figure 3. Isotherms for adsorption of Acid Red 97, Acid Orange 61 and Acid Brown 425 of a mixture onto activated carbon. Contact time = 210 min, Temperature = 25 ºC.

To optimize the design of an adsorption system, it is important to establish the most appropriate correlation for the equilibrium curves. Pikaar et al. reported an evaluation of 6 models of isotherms with adsorption data published by other authors and concluded that, in general, the model that best fitted with the data was the dual-Langmuir model, an extension of the Langmuir model considering two terms[19]. Mall et al. studied four isotherm models for the adsorption of Congo Red, Freundlich, Langmuir, R-P and Temkin, and concluded that

their results fitted well to the R-P and Freundlich model[15]. Kadirvelu et al. considered that Langmuir and Freundlich models were the most useful to describe adsorption isotherms at a constant temperature for water and wastewater treatment applications[16].

According to [16], the adsorption isotherm data were measured and fitted into the Langmuir and Freundlich model. The Langmuir equation is applicable to homogeneous adsorption systems when there is no interaction between the sorbate molecules, while the Freundlich equation is an empirical equation used to describe heterogeneous systems and is not restricted to the formation of the monolayer.

Table 3 shows the results found when these two models were applied to the isotherms obtained when the dyes were studied individually. The inability of the Langmuir model to represent the experimental data could have been due to the fact that this isotherm does not take into account adsorbate–adsorbate interactions. The Freundlich equation provided a more satisfactory description.

The values of R_L confirm that activated carbon favours the adsorption of acid dyes from wastewater under the conditions used in this study. The data of maximum adsorption (q_{mon}) were in the following decreasing order: Acid Brown 425, Acid Orange 61 and Acid Red 97 and the binding strength values (K_L) were in the reverse order.

Table 3 shows the values of q_{mon}, K_L, R_L for the Langmuir isotherms and K_F, n for the Freundlich isotherms for the acid dyes in mixtures. Correlation coefficient (r) and the residual error (s_e) of the regression lines are also shown. s_e corresponds to the estimated error from the residuals obtained from the regression lines built to obtain the isotherm parameters. For Acid Orange 61, the experimental data do not fit either of the two models, so the other values in the table have no meaning. All the data on Acid Brown 425 indicate practically null adsorption. The binding strength values were only positive for the Acid Red 97. It was observed that in the mixture the dye adsorption is competitive and that the orange and the brown dye are hardly adsorbed.

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Table 3. Langmuir and Freundlich constants for adsorption of each acid dye individually and in a mixture onto activated carbon

Dye	Langmuir isotherm					Freundlich isotherm			
	q_{mon} (mg g^{-1}) (s)	K_L (L mg^{-1}) (s)	R_L	r	$s_e \cdot 10^{-3}$	K_F (s)	n (s)	r	$s_e \cdot 10^{-2}$
Acid Red 97	52.08 (2.44)	0.112 (0.012)	0.23	0.9585	1.8	12.65 (0.81)	2.99 (0.18)	0.9834	1.7
Acid Orange 61	169.49 (40.01)	0.053 (0.019)	0.39	0.8542	1.6	16.94 (6.91)	1.84 (0.43)	0.8894	5.9
Acid Brown 425	222.22 (48.47)	0.006 (0.001)	0.85	0.9959	1.4	1.95 (1.15)	1.19 (0.03)	0.9958	1.9
Acid Red 97 (mixture)	40.82 (1.74)	0.935 (0.101)	0.034	0.9627	2.4	21.50 (0.51)	4.45 (0.23)	0.9899	1.6
Acid Orange 61 (mixture)	17.89 (0.78)	-4.437 (2.906)	-0.008	0.5018	5.6	21.41 (1.74)	-16.98 (9.63)	0.5544	4.4
Acid Brown 425 (mixture)	7.83 (1.36)	-0.016 (0.003)	1.923	0.9764	20.7	0.01 (0.001)	0.52 (0.06)	0.9669	7.6

Some models for multi-constituent adsorption equilibrium relationships (Eqs. 9 and 10) have been applied to the data, but these models do not explain properly the behaviour of these dyes. It could be due because the experimental data do not correspond to the modelled data or to the necessity of having values of high quality obtained from the models of a single component. Some papers agree with these considerations[23,24].

4. Conclusions

The present study shows that activated carbon is efficient at removing three acid dyes from wastewater, both in individual solutions and in mixtures. The kinetics of these acid dye adsorptions on activated carbon follows the pseudo-second-order model.

The adsorption isotherms of three acid dyes from aqueous solutions onto activated carbon were also determined. The experimental isotherms obtained when the dyes were studied individually were of the S type [33] and were appropriately described by the Freundlich model. In a mixture of the three dyes, adsorption is mainly favourable for Acid Red 97. The adsorption is highly dependent on the dye concentration, and is reduced for mixtures.

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References

[1] A.A. Vaidya, K.V. Datye, *Colourage* 14 (1982) 3.

[2] S.S. McClung, A.T. Lemley, *Text. Chem. Color* 26 (1994) 17.

[3] S.H. Lin, C.C. Lo, *Environ. Technol*. 17 (1996) 841.

[4] A. Bousher, X. Shen, R.G.J. Edyvean, *Water Res.* 31 (1997) 2084.

[5] J. Oakes, P. Gratton, *J. Chem. Soc*., Perkin Trans. 2 (1998) 2201.

[6] I. Poulios, I. Aetopoulou, *Environ. Technol*. 20 (1999) 479.

[7] S. Papic, N. Koprivanac, A. Loncaric Bozic, *J. Soc. Dyers Color*. 116 (2000) 352.

[8] V. Lopez-Grimau, M.C. Gutierrez, *Chemosphere* 62 (2006) 106.

[9] S.S. Tahir, N. Rauf, *Chemosphere* 63 (2006) 1842.

[10] M. Mohorcic, S. Teodorovic, V. Golob, J. Friedrich, *Chemosphere* 63 (2006) 1709.

[11] J.X. Chen, L.H. Zhu, *Chemosphere* 65 (2006) 1249.

[12] M. Arami, N.Y. Limaee, N.M. Mahmoodi, *Chemosphere* 65 (2006) 1999.

[13] F.L. Fu, Y. Xiong, B.P. Xie, R.M. Chen, *Chemosphere* 66 (2007) 1.

[14] A. Pala, E. Tokat, *Water Res*. 36 (2002) 2920.

[15] I.D. Mall, V.C. Srivastava, N.K. Agarwal, I.M. Mishra, *Chemosphere* 61 (2005) 492.

[16] K. Kadirvelu, C. Karthika, N. Vennilamani, S. Pattabhi, *Chemosphere* 60 (2005) 1009.

[17] H. Metivier-Pignon, C. Faur, P. Le Cloirec, *Chemosphere* 66 (2007) 887.

[18] V. Gómez, J. Font, M.P. Callao, *Talanta* 71 (2007) 1393.

[19] I. Pikaar, A.A. Koelmans, P.C.M. van Noort, *Chemosphere* 65 (2006) 2343.

[20] R. Tauler, A. Smilde, B.R. Kowalsky, *J. Chemom*. 9 (1995) 31.

[21] R. Tauler, A. de Juan, 2006. Multivariate Curve Resolution homepage, http://www.ub.es/gesq/mcr/mcr.htm.

[22] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. de Jong, P.J. Lewi, J. Smeyers-Verbeke,. *Handbook of Chemometrics and Qualimetrics Part A*. 1997, Elsevier, Amsterdam.

[23] J.F. Porter, G. McKay, K.K.H. Choy, *Chem. Eng. Sci*. 54 (1999) 5863.

[24] K.K.H. Choy, J.F. Porter, G. McKay, *Langmuir* 20 (2004) 9646.

[25] K.K.H. Choy, G. McKay, J.F. Porter, *Resour. Conserv. Recy*. 27 (1999) 57.

[26] A.L. Ahmad, M.F. Chong, S. Bhatia, *Ind. Eng. Chem. Res*. 45 (2006) 6793.

[27] R. Tauler, *Chemom. Intell. Lab. Syst*. 30 (1995) 133.

[28] J. Jaumot, R. Gargallo, A. de Juan, R. Tauler, *Chemom. Intell. Lab. Syst*. 76 (2005) 101.

[29] Y.S. Ho, G. McKay, *Process Biochem*. 34 (1999) 451.

[30] Y.S. Ho, G. McKay, *Water Res.* 34 (2000) 735.

[31] C. Namasivayam, D. Kavitha, *Dyes Pigments* 54 (2002) 47.

[32] P.K. Malik, *J. Hazard. Mater. B* 113 (2004) 81.

[33] C.H. Giles, T.H. MacEwan, S.N. Makhwa, D.J. Smith, *J. Colloid Interf. Sci.* 3 (1960) 3973.

[34] D. Palit, S.P. Moulik, *Colloid J.* 65 (2003) 350.

4.5. PAPER

Experimental designs for optimizing and fitting the adsorption of dyes onto activated carbon

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Experimental designs for optimizing and fitting the adsorption of dyes onto activated carbon

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Abstract

We used experimental design methodologies to obtain the response surface of the adsorption process for three acid dyes from aqueous effluents used in the dyeing step of a tanning process. The dyes were Acid Red 97, Acid Orange 61 and Acid Brown 425. They were determined simultaneously in a single step using sequential injection analysis with multivariate curve resolution and alternating least squares (SIA-MCR-ALS).

This method involves a screening design and a response surface-modelling step to optimize the response. Individual responses for each dye and a total response are considered.

Keywords: Experimental design; Response surface; Adsorption; SIA-MCR-ALS

1. Introduction

The tanning industry uses a wide spectrum of dyes to impart a particular colour to the leather matrix. About 60-80% of the dyes available are adsorbed on to the leather matrix and the unspent dyes are discharged in the wastewater, causing an environmental and economic problem.

There is considerable environmental interest in determining dyes because their biodegradability is poor and conventional wastewater treatment plants are not very efficient at removing them [1-2]. The discharge of dye-laden wastewater by the tanning industries has been severely criticized by the public for aesthetic reasons. Attempts have been made to remove unspent dyes by coagulation-flocculation [3], chemical oxidation [4-6], foam flotation [7], electrolysis [8-9], adsorption [10-12] and by the use of membranes [13]. Adsorption onto activated carbon has proven to be one of the most effective and reliable physicochemical treatment methodologies [14-18].

In a previous paper, we described a method for simultaneously determining three acid dyes in wastewater samples from the tanning industry [18] (Acid Red 97, Acid Orange 61 and Acid Brown 425). The ability of activated carbon to remove these acid dyes was also assessed [19] and we concluded that the adsorption process is competitive. The adsorption of the brown dye was clearly influenced by the presence of the other two dyes and the adsorption of the orange dye by the red dye. The adsorption of the red dye was similar whether it was by itself in solution or in a mixture.

The objective of the present study was to assay an experimental strategy to find the experimental conditions in which the adsorption process was efficient, and build a mathematical model for the percentage of dyes remaining in solution as a function of variables involved (in this case, the initial concentration of each dye, the mass of the adsorbent and the contact time).

There are many strategies for finding the optimal conditions in chemical experimentation. The simplest one is to change one variable while the others are held constant. When the best response is found, this variable is held constant and another variable

is studied, and so on. Another strategy is to use design of experiments (DOE) [20-21]. DOE is very important because it takes less time, effort and resources than univariate procedures, and it provides large quantities of information in a minimum number of experiments [22-26].

Our method uses fractional factorial designs and response surface methodologies to optimize and fit the relevant adsorption factors for the percentage of individual and total dyes remaining in solution. Dye concentrations were evaluated with a sequential injection analysis system and data treatment with a second-order calibration method, multivariate curve resolution with alternating least squares (MCR-ALS).

2. Experimental

2.1 Materials

- Adsorbent: The commercial material Activated Carbon (CAC) was supplied by J. T. Baker (Holland).
- Adsorbates (analytes): Acid Red 97, Acid Orange 61 and Acid Brown 425 were selected as the adsorbates that would be used to investigate the adsorption selectivity of acid dyes. Dyes were of analytical grade from Trumpler, S. A (Spain) and were used without further purification.
- Reagents: The sodium hydroxide used was of analytical/laboratory grade, provided by Prolabo (France). We used purified water from a Milli-Q water system from Millipore (USA).

2.2. Adsorption process

The adsorption of dyes onto activated carbon was studied by the immersion method. The desired amount of carbon was immersed in the solution of dyes for a certain amount of time, and the solution was agitated at ambient temperature (25ºC). All of the dye solution was prepared with purified Milli-Q water.

The solution and solid phase were separated by filtration with nylon syringe filters from Sharlau (Spain). A 5 mL aliquot of the supernatant was removed and analyzed for dyes.

2.3. Analytical method

Instrument

The instrumentation used to determine the dyes consists of a sequential injection (SI) system. This system has the following components: a syringe pump (CAVRO XL 3000 stepper motor-driven) connected to a HP Vectra 5/75 computer with an RS-232 interface; a 6-position automatic selection valve (Eurosas EPS 1306 BPB) connected to the computer through a PCL-711S PC-Lab-Card; a 70 cm × 0.8 mm Omnifit PTFE tubing reaction coil; a 200 cm × 0.8 mm holding coil; a diode-array spectrophotometer (HP8452A) with a Hellma 178.713QS flow-through cell controlled by the computer equipped with an HP-IB IEEE 488 interface for communications.

Analytical procedure

The analytical process is outlined in Figure 1. It consisted of obtaining a data matrix of absorbances recorded at different wavelengths and at different times when a reaction took place between sodium hydroxide and the sample. The synthetic dyes are polyprotic species so, with a pH gradient, the interdiffusion process of the sample and reactants led to a gradual fall in pH, and acid/basic species arrived sequentially at the detector[18]. The matrix obtained was treated with the second-order algorithm, Multivariate Curve Resolution with Alternating Least Squares (MCR-ALS) [27], available in [28] which enables quantitative and qualitative information of the analytes to be recovered.

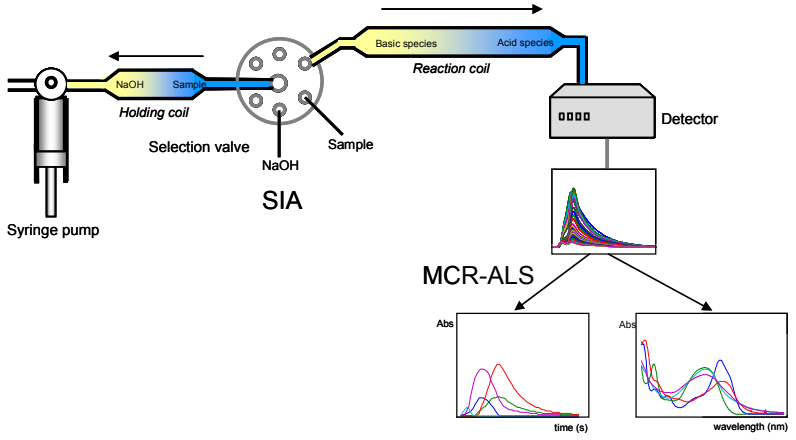

Figure 1. Schematic illustration of the SIA-MCR-ALS method for determining three acid dyes used in tanning samples

2.4. Optimization and modeling process

Figure 2 shows a scheme of the procedure followed in this paper.

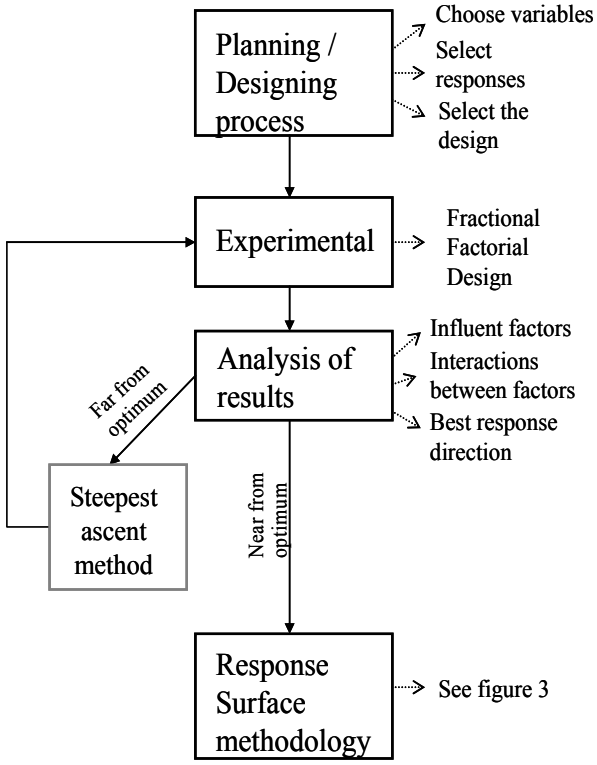

Figure 2. Scheme of the procedure used in the optimization process

Definition of factors and responses

The following variables involved in the adsorption were studied: each initial dye concentration (i.e. of Acid Red 97, Acid Brown 425 and Acid Orange 61), mass of carbon, and contact time.

Because the main objective of this paper was to establish the conditions in which dyes can be efficiently adsorbed onto activated carbon, the studied responses were the percentage of the relative concentration of each dye at the end of the process (r_i), individual response, and the percentage of the relative concentration of the sum of dyes (r_t), total response, calculated as in Eq. 1 and Eq. 2, respectively:

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$$r_i = \frac{c_{i,f}}{c_{i,0}} \cdot 100 \qquad (1)$$

$$r_t = \frac{\sum c_{i,f}}{\sum c_{i,o}} \cdot 100 \qquad (2)$$

where $c_{i,f}$ is the concentration of dye after the adsorption process and $c_{i,0}$ is the concentration of dye before the adsorption process.

Screening process with a fractional factorial design

Fractional factorial designs are good alternatives to full factorial designs for reducing the number of experiments, especially in the initial stage of a project [29].

In this paper, we worked with a fractional factorial design 2^{5-1}. With this design and with the four-factor interaction as the generator of the fifth factor, the main effects are confounded with four-factor interactions, and two-factor interactions are confounded with three-factor interactions. Assuming that the interactions between three or more factors are negligible, the main effects and the two-factor interactions can be determined.

The significance of estimated effects was studied using the ANOVA test [30]. The importance of the effects can be visually studied with a Pareto chart, which shows important factors in the response in the form of a graph.

Application of the steepest ascent method [20]

When the initial estimate of the optimum operating conditions is a long way from the actual optimum, the steepest ascent method is a simple and economically efficient experimental procedure for getting into the general vicinity of the optimum.

This method moves sequentially along the path of steepest ascent; that is, in the direction of the maximum increase in the response. We assume that a first-order model is an adequate approximation to the true surface in the experimental domain.

The fitted first-order model is:

$$\hat{y} = \hat{\beta}_0 + \sum_{i=1}^{k} \hat{\beta}_i x_i \qquad (3)$$

where $\hat{\beta}_i$ are the estimations of the effects of each factor, evaluated in the screening step.

For each factor (x_j), the step size (Δx_j) is affixed by the experimenter depending on the experiment and the step size for the rest of the factors (Δx_i) is evaluated as in Eq. 4:

$$\Delta x_i = \frac{\hat{\beta}_i}{\hat{\beta}_j / \Delta x_j} \quad i = 1, 2, \ldots, k; \qquad i \neq j \qquad (4)$$

Finally, coded variables have to be converted into natural variables.

Experiments are conducted until no further increase in response is observed. Then a new first-order model can be fitted and a new steepest-ascent path determined.

Establishing and validating the response surface

Once we know the experimental domain in which the response values are considered suitable, the experimental methodology for obtaining the response surface is established, following the scheme in Figure 3. The eventual objective of response surface methods is to determine the optimum operating conditions for the system or to determine a region in the factor space in which operating requirements are satisfied.

When a fractional factorial design is used we can fit a first-order model but we should be alert to the possibility that a second-order model may be more appropriate. Replicating certain points (e.g. center points) protects against curvature from second-order effects and also enables an independent estimate of error to be obtained. Validation consists of evaluating the pure quadratic curvature sum of squares ($SS_{Pure\ quadratic}$) and applying an F-test to compare $SS_{Pure\ quadratic}$ and $SS_{Residual.}$ If both values are comparable, there is no quadratic

curvature. On the other hand if they are not comparable, then quadratic curvature is present and we will have to assume a second-order model.

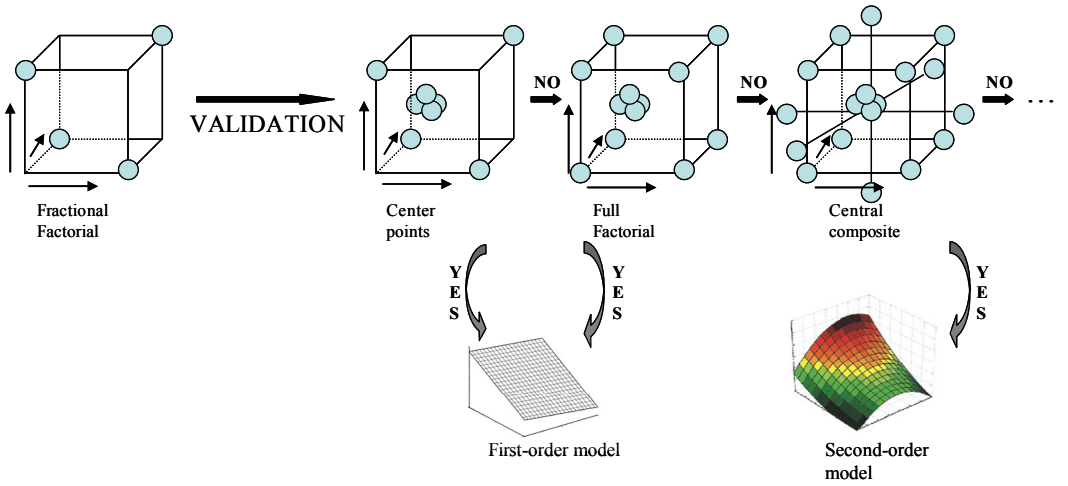

Figure 3. Scheme used to establish and validate the response surfaces

The pure quadratic curvature sum of squares in the analysis of variance is computed as follows:

$$SS_{\text{Pure quadratic}} = \frac{n_F n_C (\bar{y}_F - \bar{y}_C)^2}{n_F + n_C} \qquad (5)$$

where $\bar{y}_F$ is the average of the runs at the factorial points of the design and $\bar{y}_C$ is the average of the runs at the center point. n_F and n_c are the number of runs at the factorial design and at the center point.

To estimate the parameters of the quadratic terms, a central composite design is one option. Central composite designs contain imbedded full factorial designs with center points that are augmented with a group of axial (star) points that enable curvature to be estimated[31]. In a central composite design, the experimental domain can be carried out in a sequential way. In the first step, the full factorial design is completed and validation for a first-order model is checked again, even though the initial hypothesis should be maintained

and the obtained conclusions might be the same as with a fractional factorial design. If the first-order model is not validated, axial points are added.

3. Results and discussion

The experimental domain of the first design is based on previous knowledge [19]. The experimental domain and the results obtained are shown in Table 1. The notation of the variables will be maintained throughout the paper. The results revealed that approximately 70% of dyes remain in the solution. Acid Red 97 is the most adsorbent dye, with 54% of the dye being in solution, followed by Acid Orange 61 with 76% of the dye in solution and, finally, Acid Brown 425 with 80% of the dye in solution. These results agree with those obtained in previous papers [19].

Table 1. Experimental domain and responses of the first fractional factorial design

Experimental domain					Dyes in solution (%)			
$A^{(1)}$	$B^{(2)}$	$C^{(3)}$	$D^{(4)}$	$E^{(5)}$	Acid Red	Acid Brown	Acid Orange	Total
30	30	30	0.1	120	47.43	90.26	60.56	66.08
50	30	30	0.1	60	62.10	90.16	70.11	71.94
30	50	30	0.1	60	60.19	85.28	73.16	75.13
50	50	30	0.1	120	53.22	79.31	76.36	68.60
30	30	50	0.1	60	65.47	71.72	83.38	75.32
50	30	50	0.1	120	51.54	86.84	69.61	66.64
30	50	50	0.1	120	47.80	84.02	72.15	71.10
50	50	50	0.1	60	68.34	81.07	86.88	78.76
30	30	30	0.3	60	56.76	58.02	90.99	68.59
50	30	30	0.3	120	49.49	73.41	69.03	61.34
30	50	30	0.3	120	37.01	86.60	56.16	64.77
50	50	30	0.3	60	65.82	68.01	82.13	72.73
30	30	50	0.3	120	38.08	77.07	67.64	62.15
50	30	50	0.3	60	57.96	59.10	79.25	69.94
30	50	50	0.3	60	54.89	66.61	81.62	69.68
50	50	50	0.3	120	52.32	74.57	79.24	68.71

[1] Acid Red concentration (mg L^{-1})

[2] Acid Brown concentration (mg L^{-1})

[3] Acid Orange concentration (mg L^{-1})

[4] Mass of carbon (g)

[5] Time of contact (min)

From this first design, the individual and total responses show that all factors and interactions are significant. For the total response, the most significant factor was the contact time, followed by the mass of carbon.

Because the response of the initial design was a long way from the real optimum, we used the steepest ascent method. The new experimental conditions (see Table 2, step 1) are determined by taking into account that the effect of the mass of carbon on the total response is 2/3 of the effect of time. The initial dye concentrations are maintained constant because it is the most common range in wastewater tanning samples. We increased the highest level of time by 60 minutes and the highest level of mass of carbon by 0.13 grams. The response improved and a second experimental point was reconsidered following the same criterion as in the first optimization (step 2 Table 2). The response was again better.

A new fractional factorial design (step 3 Table 2) was proposed to determine whether the influence of the factors changed given that adsorption is not linear over time [19], and in this case, approach a suitable experimentation. The experimental domain of this second design was established by considering that the experimental points of the second optimization step were the central points of the domain. We worked with a fractional factorial design that was analogous to the previous one.

Table 2. Experimental points or experimental domain used in the optimization step

Step	Method	Factor			Response (%)[1]			
		A/B/C	D	E	Red	Brown	Orange	Global
1	STM	40	0,43	180	31	80	58	56
2	STM	40	0,56	240	10	67	50	42
3	FFD	30-50[2]	0.46-0.66[2]	210-270[2]	14	64	49	42
4	STM	40	1	180	9.5	48	28	28.5
5	STM	40	1.4	180	8	40	25	24
6	FFD	30-50[2]	1.3-1.5[2]	150-210[2]	11	38	23	24

STM: Steepest ascent method

FFD: Factorial fractional design

[1] Average response in designs (percentage of dyes in solution)

[2] Experimental domain

When we studied the effect of the factors, we noticed that the time factor was not important in most of the responses, so the domain of this factor belonged to a flat zone in the kinetic adsorption. The most influential factor was the mass of carbon, followed by the initial

concentration of the red dye. It seemed that the mass of carbon we were working with prevented the brown dye from competing with the orange dye and the red dye from being adsorbed onto the activated carbon.

Since our aim was to find a model that fitted the response to the factors considered, we used again the steepest ascent method and modified its direction. The new experimental point is shown in step 4 of Table 2. Contact time was fixed at the initial conditions of the optimization process (step 1) and the mass of carbon was increased so that response was better. In this way, response improved noticeably and the new experimental conditions were moved in this direction (step 5, see Table 2). At this last experimental point, response improved slightly, indicating that we were in a stable zone where the percentage of dyes in solution was 24%.

These values were considered to be suitable because a high percentage of dyes could be eliminated at reasonable cost (time and mass of carbon). This is why the modeling strategy was initiated around experiment 5 of Table 2, following the scheme in Figure 3 (step 6 Table 2).

Figure 4 shows the Pareto chart obtained with the total number of dyes in solution as response. The vertical line corresponds to the *p value* above which the effects are important.

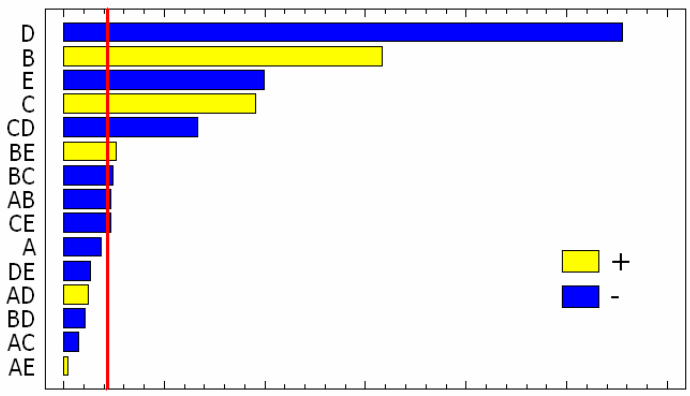

Figure 4. Pareto chart from the fractional factorial design considering the % total dyes in solution as response

It can be seen that, with the exception of factor A (initial concentration of red dye), all the factors were relevant. Some interactions were significant, but the only important one was the initial concentration of the orange dye with the mass of carbon. The initial concentration of the red dye was not important, which indicated that we worked in experimental conditions in which it was mainly adsorbed.

We established a first-order model with the fractional factorial design for the total response (a) and for the individual responses, Acid Red 97 (b), Acid Brown 425 (c) and Acid Orange 61 (d):

(a) $y = 23.4 + 1.8\ X_B + 1.1\ X_C - 3.2\ X_D - 1.1\ X_E - 0.3\ X_A X_B - 0.3\ X_B X_C + 0.3\ X_B X_E - 0.8\ X_C X_D - 0.3\ X_C X_E$

(b) $y= 10.5 - 2.3\ X_A + 1.2\ X_B + 0.7\ X_C - 0.4\ X_{AC} + 0.4\ X_{BE} - 0.5\ X_{CD}$

(c) $y= 37.7 + 3.6\ X_A - 0.7\ X_B + 2.1\ X_C - 3.4\ X_D - 1.4\ X_E$

(d) $y= 23.1 + 1.4\ X_A + 1.4\ X_B - 6.3\ X_D - 1.9\ X_E - 1.2\ X_{CD}$

with responses expressed as a percentage of dyes remaining in solution.

We validated this surface with the central points of the design evaluating $SS_{Pure\ quadratic}$ and $SS_{Residual}$, and calculated the corresponding F-value. Table 3 shows the results of this validation process. All the surfaces obtained are validated for a significance level of 5%.

Table 3. Analysis of the quadratic effects of the first-order model

Exp. Nº	Factor					
	A	B	C	D	E	Response (%)
1	40	40	40	0,43	180	56
2	40	40	40	0,56	240	42
3	40	40	40	1	180	28
4	40	40	40	1.4	180	24

We also selected four other points in the domain to do an external validation of the model. The experimental factors and responses of each point are shown in Table 4. We

applied a joint test of slope and intercept. We determined that they were statistically comparable with the predicted values from the model with 5% significance and concluded that the linear model fits the process studied.

Table 4. External validation of the first-order model

Exp. Nº	A	B	C	D	E	Experimental/Predicted response			
						Total	Acid Red	Acid Brown	Acid Orange
1	35	50	50	1.45	165	25.7/24.7	13.4/13.3	36.2/36.3	21.9/21.0
2	45	35	35	1.35	195	21.7/22.7	9.1/8.3	38.1/39.8	21.5/25
3	35	45	45	1.35	165	25.6/27.1	13.6/12.7	34.9/39.0	22.6/27.5
4	35	45	35	1.5	195	20.6/20.6	11.8/12.1	28.2/30.4	15.2/16.4

Figure 5 shows the fitted surface for the total response for 40 mg L^{-1} of each dye at different contact times and mass of carbon. The contour plot is also presented, which shows the different combinations of contact time and mass of carbon for achieving the same response.

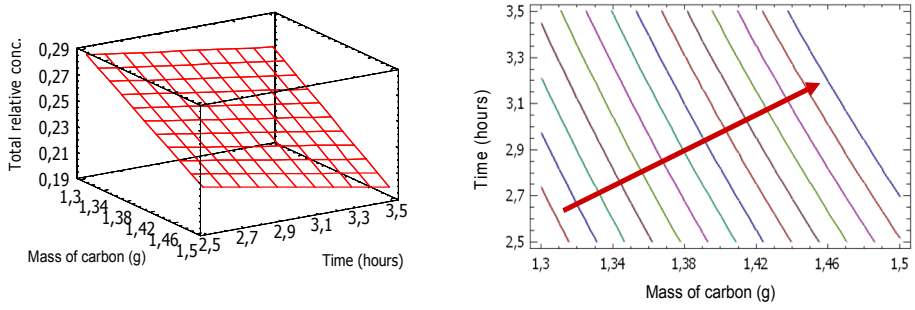

Figure 5. Response surface and contour plot of the total percentage of dyes in solution after the dyes have been adsorbed onto activated carbon. Experimental conditions: 40 mg L^{-1} each dye.

4. Conclusions

Our study of the adsorption of dyes onto activated carbon has shown that all the factors studied (initial dye concentration, mass of carbon and contact time) were important, having a positive effect for the dyes concentration and a negative effect for the mass of carbon and time of contact for the relative concentrations of the dyes. In the domain studied, 24% of the dyes were in solution, after the fractional factorial designs and the steepest ascent method had been applied.

We established a first-order response surface for the adsorption of dyes onto activated carbon using the relative concentration of dyes at the end of the process. When the responses were individual concentrations, the first-order model was also fitted. In the experimental domain where the response surfaces were fitted between 14.5 and 32.3 % of total dyes were in solution, between 5 and 16 % of Acid Red 97, between 26.5 and 48.9% of Acid Brown 425 and between 10.9 and 35.3 % of Acid Orange 61, with the extremes of the domain being applied to all factors.

Although the dyes studied compete with each other in the adsorption process, conditions to obtain the best response were not opposite. Experimental conditions can depend on which dye we are interested in removing, for visual or toxic reasons, by obtaining response surfaces for the total and the individual responses.

This methodology can be used as a strategy to eliminate the percentage of dyes in wastewater samples. However, the presence of other species in the sample can affect the final function obtained, especially if the affinity of these species is greater than those of interest. In this case, the method can be applied in the same way.

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References

[1] L.H. Ahlström, A. Sabine, L. Mathiasson, *J. Sep. Sci*. 28 (2005) 2407.

[2] G.A. Umbuzeiro, H.S. Freeman, S.H. Warren, D. Palma de Oliveira, Y. Terao, T. Watanabe, L.D. Claxton, *Chemosphere* 60 (2005) 55.

[3] S. Papic, N. Koprivanac, A. Loncaric Bozic, *J. Soc. Dyers Color*. 116 (2000) 352.

[4] J. Oakes, P. Gratton, *J. Chem. Soc*. 2 (1998) 2201.

[5] I. Poulios, I. Aetopoulou, *Environ. Technol*. 20 (1999) 479.

[6] J.X. Chen, L.H. Zhu, *Chemosphere* 65 (2006) 1249.

[7] S.H. Lin, C.C Lo, *Environ. Technol*. 17 (1996) 841.

[8] S.M. McClung, A.T. Lemley, *Text. Chem. Color* 26 (1994) 17.

[9] V. Lopez-Grimau, M.C. Gutierrez, *Chemosphere* 62 (2006) 106.

[10] A. Bousher, X. Shen, R.G.J. Edyvean, *Water Res.* 31 (1997) 2084.

[11] S.S. Tahir, N. Rauf, *Chemosphere* 63 (2006) 1842.

[12] F.L. Fu, Y. Xiong, B.P. Xie, R.M. Chen, *Chemosphere* 66 (2007) 1.

[13] M. Arami, N.Y. Limaee, N.M. Mahmoodi, *Chemosphere* 65 (2006) 1999.

[14] A. Pala, E. Tokat, *Water Res*. 36 (2002) 2920.

[15] I.D. Mall, V.C. Srivastava, N.K. Agarwal, I.M. Mishra, I.M. *Chemosphere* 61 (2005) 492.

[16] K. Kadirvelu, C. Karthika, N. Vennilamani, S. Pattabhi, *Chemosphere* 60 (2005) 1009.

[17] H. Metivier-Pignon, C. Faur, P. Le Cloirec, *Chemosphere* 66 (2007) 887.

[18] V. Gómez, J. Font, M.P. Callao, *Talanta* 71 (2007) 1393.

[19] V. Gómez, M.S. Larrechi, M.P. Callao, *Chemosphere* 69 (2007) 1151.

[20] D.C. Montgomery, *Design and Analysis of Experiments*, John Wiley & Sons, New York, 1997.

[21] T. Lundstedt, E. Seifert, L. Abramo, B. Thelin, A. Nystrom , J. Pettersen , R. Bergman, *Chemometr. Intell. Lab. Syst*. 42 (1998) 3.

[22] R.H. Myers, D.C. Montgomery, *Response Surface Methodology, Process and Product Optimization Using Designed Experiments*, John Wiley & Sons, New York, 1995.

[23] J. Brandvik, *Chemometr. Intell. Lab. Syst*. 42 (1998) 51.

[24] N. Ortega, S.M. Albillos, M.D. Busto, *Food Control* 14 (2003) 307.

[25] C. Severini, A. Baiano, T. De Pilli, R. Romaniello, A. Derossi, *Lebensm.-Wiss. Technol*. 36 (2003) 657.

[26] J.V. Nardi, W. Acchar, D. Hotza, *J. Eur. Ceram. Soc*. 24 (2004) 375.

[27] R. Tauler, *Chemometr. Intell. Lab. Syst*. 30 (1995) 133.

[28] R. Tauler, A. de Juan, 2006. Multivariate Curve Resolution homepage http://www.ub.es/gesq/mcr/mcr.htm.

[29] G. Hanrahan, K. Lu, *Crit. Reviews in Anal. Chem*., 36 (2006) 141.

[30] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. de Jong, P.J. Lewi, J. Smeyers-Verbeke, *Handbook of Chemometrics and Qualimetrics Part A*. Elsevier, Amsterdam, 1997.

[31] G. Hanrahan, J. Zhu, S. Gibani, D.G. Patil, Chemometrics: experimental design, in *Encyclopedia of Analytical Science*, Elsevier, Oxford, 2005.

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4.6. CONCLUSIONS

Three dyes were simultaneously determined in a single analysis by coupling SIA and MCR-ALS. These species do not present selectivity, which is why they cannot be determined with univariate calibrations. This technique has considerable advantages because of its simplicity and rapidity. It could be applied as a routine method to control dye fixation in leather samples.

Applying strategies for matrix arrangements (augmented matrices) and the second-order standard addition method, matrix effects—that is, changes in sensitivity due to the sample matrix—were evaluated and corrected, and dye concentrations were determined in these cases. In this way, MCR-ALS makes quantification possible in the presence of unknown and uncalibrated interferents and the standard addition method can be applied to a correct data matrix to determine dyes in samples that presented matrix effects.

Studying the behaviour of these dyes in the process of absorption onto activated carbon has allowed us to determine both their kinetic and thermodynamic behaviour, and the competition between the species. The results obtained enabled us to initiate an optimization process and then model the removal of the dyes. We obtained four response surfaces that fitted the remaining concentration of dyes (total and of each individual dye) as a function of the initial concentration of each dye, contact time and mass of carbon.

By fixing the factor conditions (initial concentration of each dye), the most appropriate experimental values for mass of carbon and contact time can be obtained in order to get a desirable response. This strategy can be easily extrapolated to the study of other dyes or when the sample contains other adsorbent species.

The results have made it possible to optimize the dyeing process in tanning samples. The process has been shortened and the wastewater samples at the end of the process are cleaner.

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5. Hyphenated techniques involving flow systems

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5.1. INTRODUCTION

The experimental work presented in the previous chapters describes analytical methods based on a basic SIA system for obtaining highly dimensional data, which can be treated with MCR-ALS to simultaneously determine more than one analyte.

The aim of the last step of the thesis was to explore the possibilities of increasing the capacity of simultaneous analysis by means of flow systems and second-order calibration methods.

In this chapter we present two papers that were the result of our investigations into this issue. The first paper, *Multicomponent analysis in flow systems*, is an overview concerning this item. Multianalyte determinations in flow analysis are, for the time being, just a small percentage of the total number of analytical determinations. This paper has been structured in different sections, each one of which describes a different strategy for carrying out multicomponent analysis. Finally, the future possibilities of these determinations are discussed.

The second paper, *Coupling of sequential injection chromatography with multivariate curve resolution-alternating least squares for enhancement of peak capacity*, is the result of an experiment that combines two of the strategies proposed to carry out multicomponent analysis. This study has been made in collaboration with the group of Analytical Chemistry, Automation and Environment of the University of the Balearic Islands (UIB), where the experimental work was carried out.

In 2003, a new separation approach in flow methods coupled monolithic columns with a sequential injection technique, named Sequential Injection Chromatography (SIC). SIC consists on placing a monolithic separation column between the multiposition valve of the conventional SIA system and the flow cell of the detector. It is built on a classical SIA manifold (syringe pump, selection valve and detector). The chromatographic part consists of a short (usually 25 or 50mm of length) commercially available monolithic column (with or without a monolithic precolumn 5 or 10mm long).

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The resolution capacity of SIC is lower than that of HPLC, but greater than that of other strategies used with flow systems. That is why coupling SIC with diode array detection and Multivariate Curve Resolution with alternating least squares (SIC-MCR-ALS) was proposed as a new complementary tool for enhancing SIC resolution capabilities.

To assay the resolution capacity of this method, we developed a method for determining phenol derivatives in samples of disinfectant. The phenols confer microbicide, fungicide and deodorizing properties. The most common derivatives in disinfectants are: phenol, dimethylphenol, 4-chloro-3-methylphenol, 2-benzyl-4-chlorophenol, 2-phenylphenol and methylphenol. These species have similar UV spectra and close retention times in short reversed-phase silica-based monolithic columns using a SIC system. Mixtures have low chromatographic resolution values between species (0.05-0.57). The totally overlapped chromatographic separation of these mixtures can be solved by coupling SIC and MCR-ALS.

5.2. PAPER

Multicomponent analysis using flow systems

Verónica Gómez, María Pilar Callao

Trends in Analytical Chemistry 26 (2007) 767-774

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Multicomponent analysis using flow systems

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Abstract

Flow systems are very common in analysis, with low consumption of sample and reagents and great versatility, whether referring to detectors or to the flexibility of modules for carrying out pretreatment before the analytical signal is recorded. Despite the great potential of these systems, they are mostly used for single determinations and do not exploit the possibility of carrying out multicomponent analysis in a single step.

In this article, we present an overview of the various strategies used to make multicomponent determinations in flow systems, with particular focus on recently published papers.

Keywords: Chemometrics; Flow system; Multichannel manifold; Multicomponent determination; Selective detector; Sequential injection chromatography; Solid-phase extraction

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1. Introduction

Since its introduction in 1974, flow injection analysis (FIA) has been widely accepted by the analytical chemical community, as reflected by the considerable number of scientific publications that it has generated. Up to now, FIA has undergone certain changes and modifications, which define three separate generations:

(1) FIA;
(2) sequential-injection analysis (SIA); and,
(3) bead-injection (BI)-lab-on-valve (LOV) [1].

Other configurations [2], based on those above, have also appeared (e.g., multicommutated FIA (MCFIA), multisyringe FIA (MSFIA), and multipumping flow systems (MPFSs). Figure 1 shows a typical FIA, SIA and LOV manifold, in which we can see the general features of flow systems on the left, and the specific characteristics of each configuration on the right.

One of the most outstanding features of flow systems is their inherent ability to accommodate a plethora of unit operations (e.g., on-line liquid–liquid extraction, precipitation or coprecipitation in knotted reactors, or solid-phase extractions in column reactors with either hydrophilic or hydrophobic packing materials), which are facilitated by incorporating additional reactors and modules, either in the flow lines or at the external multiposition valve ports. These configurations make possible sample-pretreatment processes and multicomponent determinations in a single analysis.

It is rather unfortunate that such a powerful technique is used mostly for single-component analysis. A literature survey showed that more than 72% of all publications on SIA concerns single component analysis [3].

This article presents an overview of the various strategies used in multicomponent determinations in flow systems, which we think have the greatest potential in this field. Because of the numerous papers published in this area, there is some overlap between some aspects of our paper and other overviews or reviews (e.g., speciation with SIA systems [4],

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metal analysis with FIA systems [5] or analysis of pharmaceutical products with FIA [6] or SIA [7].

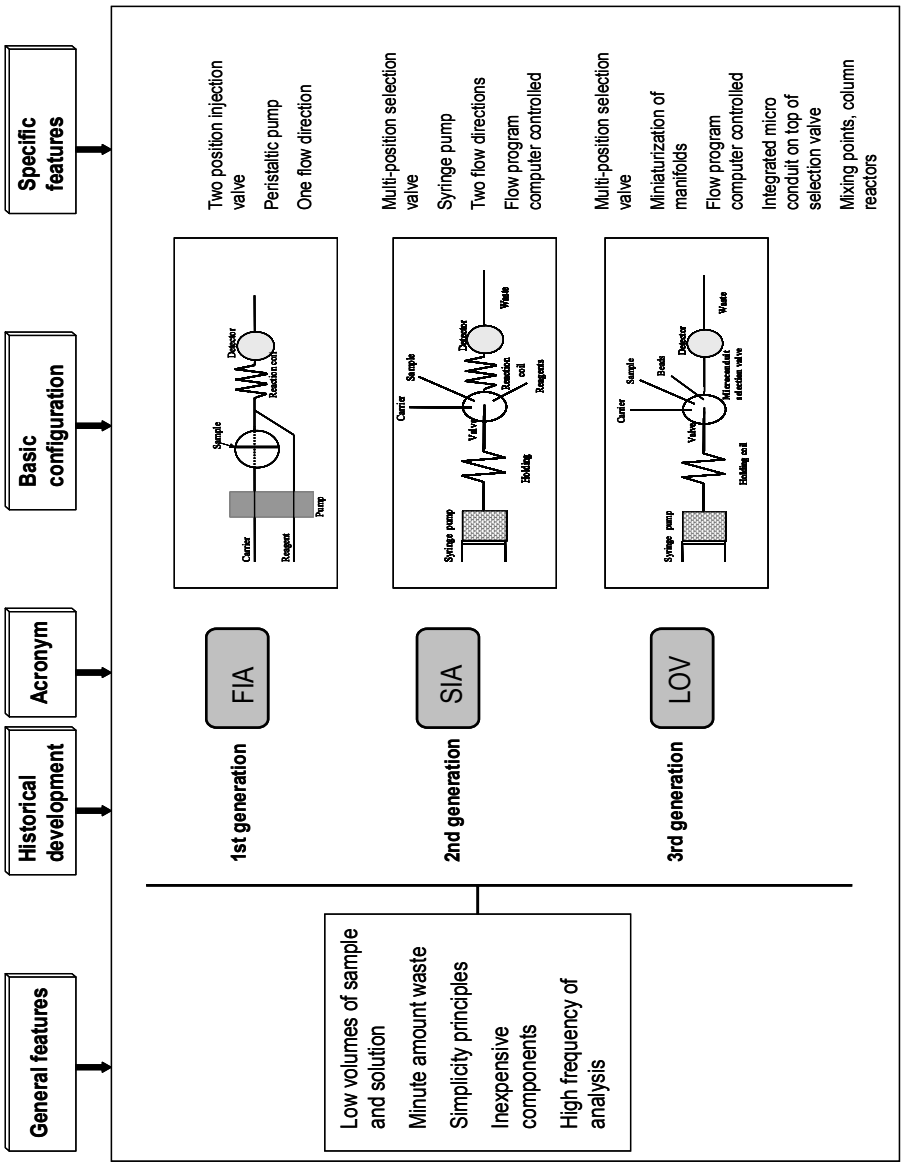

Figure 1. Basic configuration of flow systems. General and specific features.

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We have structured the article in sections (Figure 2), each covering one of the strategies considered:

(1) solid-phase extraction (SPE);
(2) sequential injection chromatography (SIC) and other approaches;
(3) multichannel manifolds;
(4) selective detectors and hyphenations of flow systems; and,
(5) chemometric treatments of multivariate data.

Due to the great versatility of flow systems (e.g., instrumental configuration (continuous-flow or stopped-flow), application fields, measurement strategies (kinetic or equilibrium methods), and detectors), it is possible that some relevant items might not be included in any one specific section of the paper, while being covered in some of the other sections.

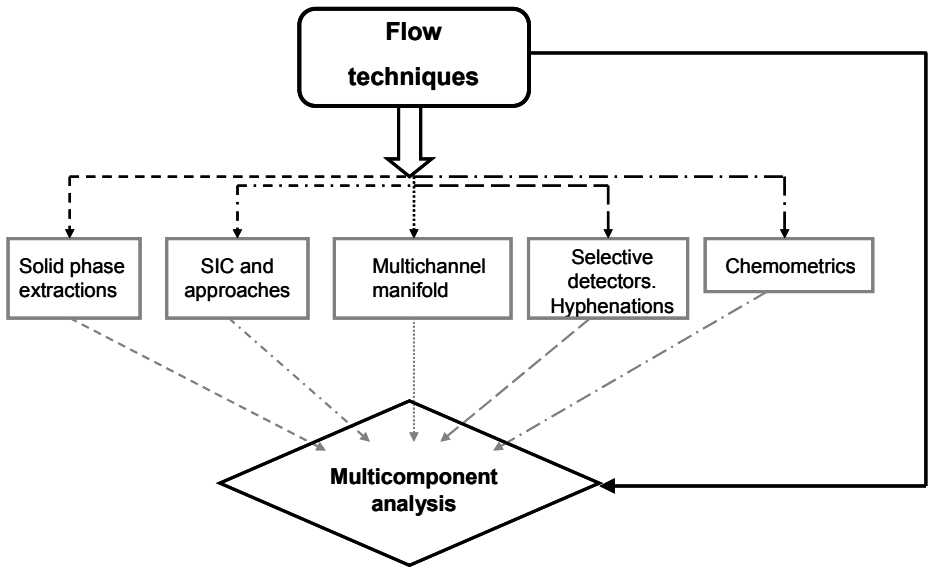

Figure 2. Strategies used in flow systems to achieve multicomponent analysis.

The studies that we reference or mention comprise a representative selection that deals with some of the strategies considered, since the vast range of these scientific techniques means that our study cannot be exhaustive. The flow systems used in the articles

referenced could be more complex than the text suggests because they may include other steps (e.g., elimination of interferences, or preconcentration). In this article, we focus only those on processes involved in multicomponent determinations. Table 1 gives details of applications discussed in the text.

Finally, we discuss some future possibilities for multicomponent analysis using flow systems.

Table 1. Multicomponent applications in flow systems according to the different strategies discussed in the text.

Analytes	Sample type	System	Strategy	Detection	Ref.
ascorbic acid and rutin trihydrate	pharmaceuticals	SI	Solid phase extraction	spectrophotometry	[8]
furosemide and triamterene	pharmaceuticals, urine and serum	MCFI	Solid phase extraction	fluorescense optosensor	[9]
sacarine and aspartame	sweets and drinks	FI	Solid phase extraction	spectrophotometry	[10]
vitamin B2 and B6	pharmaceuticals	MCFI	Solid phase extraction	fluorescense optosensor	[11]
Hg and MetilHg	certified reference materials (natural water, rice flour and pork)	FI	Solid phase extraction	fluorescense	[12]
Sb(III), Sb(V)	bottled drinking water	segmentedFI	Solid phase extraction	HGAAS	[13]
methylparaben, propylparaben and sodium diclofenac	pharmaceuticals	SI	SIC and approaches	spectrophotometry	[14]
methylparaben, propylparaben and triamcinolone acetonide	pharmaceuticals	SI	SIC and approaches	spectrophotometry	[15]
ambroxol hydrochloride and doxycycline	pharmaceuticals	SI	SIC and approaches	spectrophotometry	[17]
ambroxol, methylparaben and benzoic acid	pharmaceuticals	SI	SIC and approaches	spectrophotometry	[18]
naphazoline nitrate and methylparaben	pharmaceuticals	SI	SIC and approaches	spectrophotometry	[19]
salicylic acid and triamcinolone acetonide	pharmaceuticals	SI	SIC and approaches	spectrophotometry	[20]
amoxicillin, ampicillin and cephalexin	pharmaceuticals	MSFI	SIC and approaches	spectrophotometry	[22]
nitrites and nitrates	water, soil and biological samples	FI	Multichannel manifold	spectrophotometry	[23]
Cu, Zn	pharmaceuticals	FI	Multichannel manifold	spectrophotometry	[24]
Mo, W	steel alloys	FI	Multichannel manifold	spectrophotometry	[25]
citrate and piruvate	pharmaceuticals, urine and serum	stopped-flow	Multichannel manifold	chemiluminescense	[26]
Cr(III), Cr(VI)	surface waters and standard reference materials	BI-LOV	Multichannel manifold	ETAAS	[27]
malic and lactic acid	wine	FI	Multichannel manifold	photometry or fluorimetrically	[28]
ammonia and phosphate	surface waters	SI	Multichannel manifold	fluorescense	[29]
Fe(II) Fe(III)+nitrite nitrate	river and marine water	MCFI	Multichannel manifold	spectrophotometry	[30]
Cu, Fe, Zn	serum	FI	Multichannel manifold	spectrophotometry	[31]
Cu, Ni	plant digests	FI	Multichannel manifold	spectrophotometry	[32]
pH, Cl, Ni	electrolytic bath samples	SI	Selective detectors	potenciometry- two ion selective electrodes	[33]
Cd(II), Pb(II) /Ni(II), Co(II)	fertiliser and ore	FI/SI	Selective detectors	anodic and adsorptive stripping voltammetry	[34]
cysteine, glutathione	plasma and blood	FI	Selective detectors	biosensor	[35]
phenolic compounds	industrial wastewater and surface water	FI	Selective detectors	biosensor	[36]
Co, Ni, Cu, Zn, Cd, Pb	ocean seawater standard reference	FI	Selective detectors	ICP-MS	[37]
Ge, As, Se	materials	FI	Selective detectors	ICP-MS	[38]
V, Cr, Mn, Co, Ni, Cu, Zn, Cd, Pb	certified reference materials and natural water	FI	Selective detectors	ICP-MS	[39]
Ag, Pd, Au, Ga, In, Nb	certified reference materials and natural water	FI	Selective detectors	ICP-OES	[40]
Cr(III), Cr(VI)	river, tap and wastewater samples	FI	Selective detectors	ICP-AES	[41]

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Table 1. Continued.

Analytes	Sample type	System	Strategy	Detection	Ref.
Cr(III), Cr(VI)	water samples	FI	Selective detectors	ICP-OES	[42]
seven anions	water, juice and milk	FI	Selective detectors	capillary electrophoresis	[43]
eigth amines	synthetic water samples	SI	Selective detectors	capillary electrophoresis-mass spectrometry	[45]
honokiol and magnolol	pharmaceuticals	FI	Selective detectors	capillary electrophoresis	[46]
four anthraquinones	pharmaceuticals	FI	Selective detectors	capillary electrophoresis	[47]
Sn, Ge, Mo	food samples	FI	Chemometrics (PLS)	spectrophotometry	[48]
Co(II), Cu(II)	water samples	FI	Chemometrics (PLS)	chemiluminescence	[49]
Fe, V	alloys	MPFI	Chemometrics (PLS)	spectrophotometry	[50]
amoxicillin and clavulanic acid	pharmaceuticals	stopped-flow	Chemometrics (PLS)	fluorescense	[51]
rifampicin, isoniazid	pharmaceuticals	FI	Chemometrics(ANN)	chemiluminescence	[52]
Cu(II) Ni(II)	electrolytic bath samples	FI-stopped-flow	Chemometrics(ANN)	spectrophotometry	[53]
two inhibitors	pharmaceuticals	FI	Chemometrics(MCR-ALS)	spectrophotometry	[55]
Cr(III), Cr(VI)	tanning wastewater	SI	Chemometrics(MCR-ALS)	spectrophotometry	[56]
three acid dyes	tanning wastewater	SI	Chemometrics(MCR-ALS)	spectrophotometry	[57]
xanthine and hypoxanthine	urine	stopped-flow	Chemometrics(N-PLS)	spectrophotometry	[58]
four dyes	fruit drink powder	FI	Chemometrics(BLLS)	spectrophotometry	[59]

2. Solid-phase extraction (SPE)

Although flow techniques are a so-called “non-separation method”, the direct incorporation of an SPE microcolumn as a separation element into the manifold can greatly increase their specificity and analytical performance. Columns packed with materials that can selectively adsorb or absorb are probably the most widely used. Separation is achieved because of differential retention or desorption when two or more analytes interact with the solid phase [8–13]. The most generalized strategy comprises an analyte that passes through the solid support in the precolumn and develops its analytical signal when it reaches the flow-through cell. It is eluted by the carrier itself, whereas the other is retained on the solid phase, and is eluted later by changing the composition of the carrier into another more suitable.

The main characteristic of this approach is that the instrumental configuration is simple. To exploit this strategy, it is necessary to find selective solid phases or selective elution conditions. Furthermore, in many cases, a preconcentration step can also be carried out. The most important drawback is that usually only two components can be determined in

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a single step. It is possible to find other applications with more components, but their separation capacity is limited.

3. Sequential injection chromatography (SIC) and other approaches

In 2003, a new separation approach using flow methods coupled monolithic columns with SIA. Known as sequential injection chromatography (SIC) [14,15], it is based on using a monolithic separation column that is placed between the multiposition valve of the conventional SIA system and the flow cell. Recently, an overview on this topic has been published [16].

SIC can be operated under isocratic conditions (using the mobile phase as the carrier) or gradient elution (capitalizing on dispersion of the eluent into the carrier solution). In addition, automatic programming of the flow of the mobile phase by the SI instrumentation makes it possible to use flow gradients that can enhance peak resolution while considerably decreasing the total chromatographic run-times.

Although SIC has so far been used to resolve only organic molecules in pharmaceutical preparations [7,17–20], it has potential in other applications (for not only organic analytes but also anion and cation separations).

In [21], a miniaturized application that automatically renews the stationary phase is presented, based on SIC. The separation process is affinity chromatography in the biomolecular field.

In 2006, a high-performance, low-pressure chromatographic system using a multisyringe burette coupled to a chromatographic monolithic column was developed [22], and applied to the separation of pharmaceutical products.

These techniques retain some of the advantages of classical flow systems: automation; flexibility; low waste generation; and versatility. They also ensure automatic fluidic control of the whole chromatographic process. The unique features of SIC are the

economy and the speed of gradient elution, which is achieved without any gradient device. At present, the disadvantages of the system are:

(1) limited possibility of separation due to restriction of the column length and the flow rate;
(2) limited flow rates for the syringe pump (due to back pressure of about 2.5 MPa);
(3) limited volume for the syringe pump (at present, the commercially-available maximum is 10 mL); and,
(4) lack of sufficient software for evaluating the separation analysis (compared with HPLC software, which is generally available).

4. Multichannel manifolds

An approach often used in simultaneous quantification is ingenuity in the design of flow manifolds, thus providing selectivity for the components. The simplest possibility comprises several runs or injections of the sample into various conduits (reactors), where each component is determined individually by modifying the experimental conditions [23]. The most usual strategy uses splitting flow pieces and delay coils. The split streams follow lines of different lengths and residence times in which the analytes can react with specific reagents. Finally, all channels join at a common point prior to detection. Various successive peaks for each channel or analyte are obtained from a single sample injection.

The specificity of the signal in each reactor is based on many principles. Differential kinetic analysis is used in some applications [24], which exploit the different reaction rate in substituting one ligand that forms an absorbent complex for another one that forms a non-absorbent complex. Two residence times are used in each channel; the longer residence time provides the signal of only the analyte that reacts more slowly, while the shorter residence time provides both signals. Other applications are based on selective behaviours to reagents (e.g., enhancing the selective capacity for catalyzing a reaction with two metals (in one reactor) and with only one (in the other reactor) [25]).

A kinetic method for the determination of citrate and piruvate [26] was based on the stopped-flow mixing approach that enables the progress of the reaction to be followed, due to

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the different kinetic behaviour of the analytes being studied. These methods enable measurement to be made instantly after mixing, and, after several seconds, the kinetic data are obtained.

Other applications have been described for determining species with different oxidation states. In these applications, specific modules (e.g., reduction columns) are filled with a reducing agent and injected into the lines. The first measure determines one of the species and the second measure changes the oxidation state of the other species and measures the joint signal of both of them. These two signals make it possible to determine both species simultaneously [23,27].

Dialysis units with enzymatic derivatization have also been used to separate analytes from complex matrices and simultaneous determination of organic acids [28].

Due to the instrumental versatility of flow systems, the approaches mentioned above can use one detector or more (in series or in parallel) [29,30] or several flow cells in the same detector [31]. These configurations can be applied with differential kinetic analysis [32]. Each reactor is connected to a detector or a flow cell. The main advantage of using two detectors is the considerable reduction in analysis time because signals can be acquired at the same time, although the instrumental configuration is more complex.

Although many of the approaches found in the bibliography describe simultaneous determination of two components, more components can be analyzed in a single step if more reactors are used.

5. Selective detectors and hyphenations of flow systems

A strategy for performing multicomponent determinations is to use various specific detectors in series. In this way, each detector records the signal of each species of interest [33]. Another possibility is the use of detectors that provide specific signals to the various species of interest.

Selective systems that can carry out multicomponent determinations (e.g., voltammetry [34], amperometry [35,36], ICP-MS [37–39], ICP-OAS [40], ICP-AES [41,42], or capillary electrophoresis (CE) [43–47]) can be hyphenated with flow systems because of their capacity to carry out pretreatments, mainly preconcentrations and separation of the species of interest from their matrix or from interferences [37]. These hyphenations are also interesting because small volumes of sample can be automatically injected and because their frequency of analysis is high.

Voltammetry has largely been applied to the determination of metals. Usually, when dealing with samples with extremely low concentrations of analytes, flow hyphenation is introduced as a tool for performing and automating preconcentration before detection in the electrode by electrolysis (anodic stripping voltammetry) or by adsorption (adsorptive stripping voltammetry), minimizing the risks of sample contamination and reducing significantly the analysis time [34].

Amperometry as a biosensor system has been applied to the determination of organic compounds. High sensibilities are achieved by immobilizing enzymes on selective electrodes [35,36].

Hyphenation of flow systems with mass spectrometry (ICP-MS) is a powerful technique for trace multi-elemental and isotopic analysis being applied to a wide range of samples [37].

In [43], hyphenations of flow systems with CE are presented as the sum of three blocks:

(1) the FIA section, which uses the facilities of these systems for any type of sample pretreatment;
(2) the electrophoresis section, which gives the system its separation ability; and, finally,
(3) the interface or union between them.

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In references [44,45], the possibilities of interfaces or unions are discussed. This separation technique has sometimes been applied with flow systems in multideterminations [46,47].

These techniques have considerable potential in multicomponent analysis, and they complement each other; atomic spectroscopic and voltammetric techniques focus on metal analysis, while electrophoresis and amperometric biosensor techniques, which have a wider field of application, can be used for organic compounds.

6. Chemometric treatment

It is well known that processing multivariate data with multivariate chemometric tools makes it possible to determine several species simultaneously in a single analysis.

Multivariate signals can be divided into two big blocks: first-order data; and, second-order data, generally known as n-way data. Figure 3 shows the algorithms that are most used for processing both types of data and their most outstanding characteristics.

The most widely used multivariate linear regression method is Partial Least Squares (PLS) [48–51]. Artificial Neural Networks (ANN) [52,53] are powerful non-linear optimization systems that have several advantages over linear techniques; they allow models to be built without knowing the modeling functions.

To obtain second-order data, multichannel detectors must be used, and another order of data must be generated simultaneously. Flow systems play an important role; they make it possible for the sample to change over time while it passes through the detector. In this way, second-order data is generated and a data matrix can be acquired. The first-order corresponds to the multivariate signal (different wavelengths) and the second-order to the property that allows the sample to change its composition (e.g., pH gradient) [54].

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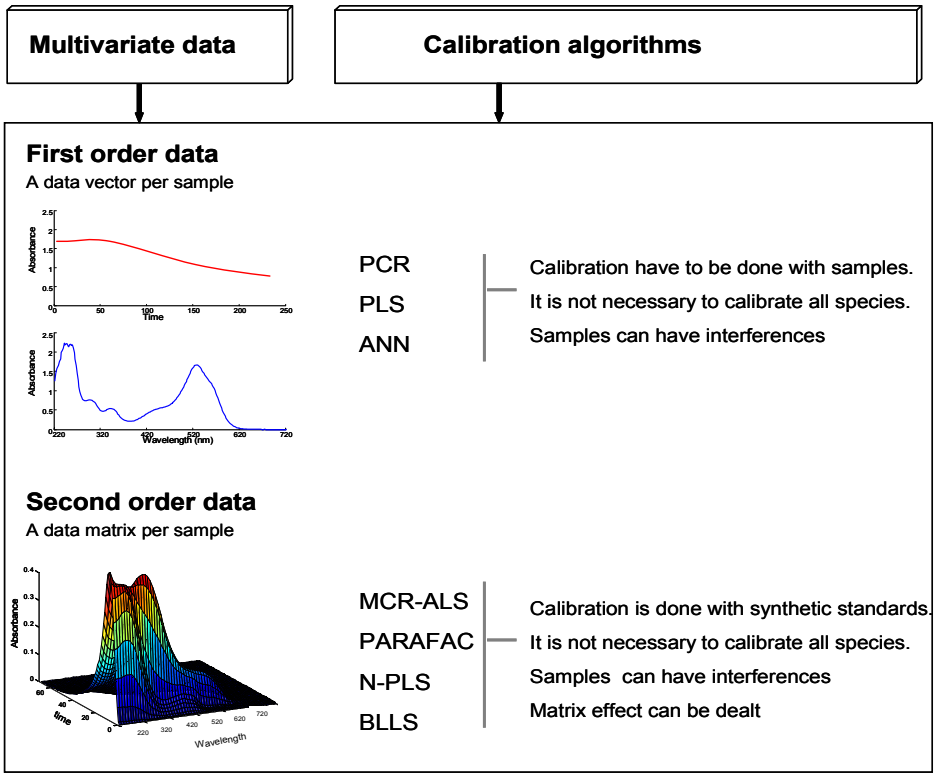

Figure 3. Multivariate data: type of data, most used algorithms and some characteristics.

Recent applications of multicomponent analysis using flow systems can be found in second-order data treatments (e.g., Multivariate Curve Resolution with Alternating Least Squares (MCR-ALS) [55–57], n-PLS [58] and Bilinear Least Squares (BLLS) [59]).

Differential kinetic analysis and stopped-flow techniques can also be coupled with first-order and second-order calibration methods [50,51,53,58].

7. Conclusions and future trends

Multicomponent analysis is gaining popularity for the simultaneous determination of mixtures of compounds in several fields. Using flow systems for simultaneous determinations

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has a number of potential advantages derived from the flexibility of flow assemblies and their typically high throughput, and modest sample and reagent consumption.

Table 2 shows the main characteristics of the strategies described for multicomponent determinations. The fitness of one strategy or another obviously depends on the application to be dealt with (e.g., number of analytes to be analyzed simultaneously, concentration of analytes, complexity of sample matrix, and instrument availability), but the most appropriate strategy in each case can be selected in relation to these characteristics.

Table 2. Comparison of strategies used for multicomponent analysis in flow systems

Strategy	Instrumental configuration	Other characteristics	Number of components (approx.)	Mathematical treatment
Solid phase extraction	Simple	Allows preconcentration	2	Simple
SIC and approaches	Simple	Allows preconcentration Problems with back pressure	3-4	Simple
Multichannel manifold	Intermediate	Not preconcentration	2-4	Simple
Selective detectors and hyphenations	Complex	Allows preconcentration Need interfaces between them	4-8	Simple
Chemometrics	Simple	Not preconcentration	2-4	Complex

Nevertheless, in most current applications, the resolution capacity is normally limited to two components. One effective way to overcome this limitation would be to promote the hyphenation of flow systems with more powerful techniques (e.g., CE or ICP-MS).

We think that all strategies proposed will be expanded in a near future, concretely those related to kinetic analysis and chemometric treatments because they are more economical and quicker than other methods. SIC techniques will extend their field of application in determinations with few analytes, currently determined by HPLC.

The future of multicomponent analysis with flow systems could be with the union of the different strategies presented. In particular, coupling chemometric treatments with other strategies could be one of the most attractive choices, because of the low experimental cost

and the simple configurations. Hyphenations of flow systems with highly selective techniques (e.g., ICP-MS and CE) could be applied when preconcentration, separation or small volumes are needed. In addition, coupling SIC techniques with chemometric tools for second-order treatment holds considerable potential. These mathematical treatments could notably improve the separation capacity of SIC because the partially overlapped signals could be quantified.

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References

[1] E.H. Hansen, M. Miró, *Trends Anal. Chem*. 26 (2007) 18.

[2] V. Cerdà, C. Pons, *Trends Anal. Chem*. 25 (2006) 236.

[3] J.F. van Staden, R.E. Taljaard, *Talanta* 64 (2004) 1203.

[4] J.F. van Staden, R.I. Stefan, *Talanta* 64 (2004) 109.

[5] M. Miró, J.M. Estela, V. Cerdà, *Talanta* 63 (2004) 201.

[6] L. Hlabangana, S. Hernández-Cassou, J. Saurina, *Curr. Pharm. Anal*. 2 (2006) 127.

[7] P. Solich, M. Polasek, J. Klimundova, J. Ruzicka, *Trends Anal. Chem*. 23 (2004) 116.

[8] Z. Legnerová, D. Satínsky, P. Solich, *Anal. Chim. Acta* 497 (2003) 165.

[9] E.J. Llorent-Martínez, P. Ortega-Barrales, A. Molina-Díaz, *Anal. Bional. Chem*. 383 (2005) 797.

[10] L.F. Capitán-Vallvey, M.C. Valencia, E. Arana Nicolás, J.F. García- Jiménez, *Anal. Bional. Chem.* 385 (2006) 385.

[11] E.J. Llorent-Martínez, J.F. García-Reyes, P. Ortega-Barrales, A. Molina-Díaz, *Anal. Chim. Acta* 555 (2006) 128.

[12] H. Wu, Y. Jin, W. Han, Q. Miao, S. Bi, *Spectrochim. Acta, Part B* 61 (2006) 831.

[13] A. Erdem, A.E. Eroglu, *Talanta* 68 (2005) 86.

[14] D. Satínsky, P. Solich, P. Chocholous, R. Karlícek, *Anal. Chim. Acta* 499 (2003) 205.

[15] D. Satínsky, J. Huclova, P. Solich, R. Karlícek, *J. Chromatogr. A* 1015 (2003) 239.

[16] P. Chocholousˇ, P. Solich, D. Satínsky, *Anal. Chim. Acta* 600 (2007) 600 (2007) 129.

[17] D. Satínsky, L.M.L. Dos Santos, H. Sklenarova, P. Solich, M.C.B.S.M. Montenegro, A.N. Araújo, *Talanta* 68 (2005) 214.

[18] D. Satínsky, J. Huclova, R.L.C. Ferreira, M.C.B.S.M. Montenegro, P. Solich, *J. Pharm. Biomed.* 40 (2006) 287.

[19] P. Chocholous, D. Satínsky, P. Solich, *Talanta* 70 (2006) 408.

[20] P. Chocholous, P. Holík, D. Satínsky, P. Solich, *Talanta* 72 (2007) 854.

[21] H. Erxleben, J. Ruzicka, *Analyst* 130 (2005) 469.

[22] H.M. González-San Miguel, J.M. Alpízar-Lorenzo, V. Cerdà-Martín, *Anal. Bional. Chem*. 387 (2007) 663.

[23] K. Suvardhan, K.S. Kumar, S.H. Babu, B. Jayaraj, P. Chiranjeevi, *Talanta* 66 (2005) 505.

[24] L.K. Shpigun, Y.V. Shushenachev, P.M. Kamilova, *Anal. Chim. Acta* 573 (2006) 360.

[25] A.P.G. Gervasio, P.R. Fortes, S.R.P. Meneses, C.E.S. Miranda, E.A.G. Zagatto, *Talanta* 69 (2006) 927.

[26] T. Pérez-Ruiz, C. Martínez-Lozano, V. Tomás, J. Fenoll, *Anal. Chim. Acta* 485 (2003) 63.

[27] X. Long, M. Miró, E.H. Hansen, *J. Anal. Atom. Spectrom.* 20 (2005) 203.

[28] E. Mataix, M.D. Luque de Castro, *Anal. Chim. Acta* 428 (2001) 7.

[29] M.A. Feres, B.F. Reis, *Talanta* 68 (2005) 422.

[30] C. Frank, F. Schroeder, R. Ebinghaus, W. Ruck, *Microchim. Acta* 154 (2006) 31.

[31] N. Teshima, S. Gotoh, K. Ida, T. Sakai, *Anal. Chim. Acta* 557 (2006) 387.

[32] D. Vendramini, V. Grassi, E.A.G. Zagatto, *Anal. Chim. Acta* 570 (2006) 124.

[33] J.E. da Silva, M.F. Pimentel, V. Lins da Silva, M.B.S.M. Montenegro, A.N. Araújo, *Anal. Chim. Acta* 506 (2004) 197.

[34] A. Economou, A. Voulgaropoulos, *Talanta* 71 (2007) 758.

[35] J.J.J. Ruiz-Díaz, A.A.J. Torriero, E. Salinas, E.J. Marchevsky, M.I.S.J. Raba, *Talanta* 68 (2006) 1343.

[36] R. Solná, P. Skládal, *Electroanalysis* 17 (2005) 2137.

[37] S. Nakatsuka, K. Okamura, K. Norisuye, Y. Sohrin, *Anal. Chim. Acta* 594 (2007) 52.

[38] Z.C. Chen, S.J. Jiang, *J. Anal. Atom. Spectrom*. 21 (2006) 566.

[39] J. Yin, Z. Jiang, G. Chang, B. Hu, *Anal. Chim. Acta* 540 (2005) 333.

[40] C. Hang, B. Hu, Z. Jiang, N. Zhang, *Talanta* 71 (2007) 239.

[41] T. Sumida, T. Ikenoue, K. Hamada, A. Sabarudin, M. Oshima, S. Motomizu, *Talanta* 68 (2005) 388.

[42] A.A. Menegário, P. Smichowski, G. Polla, *Anal. Chim. Acta* 546 (2005) 244.

[43] P. Kuban, B. Karlberg, *Trends Anal. Chem*. 17 (1998) 34.

[44] B. Santos, B.M. Simonet, A. Rios, M. Valcárcel, *Trends Anal. Chem*. 25 (2006) 968.

[45] B. Santos, B.M. Simonet, A. Rios, M. Valcárcel, *J. Chromatogr. A* 1127 (2006) 278.

[46] L. Liu, X. Wu, L. Fan, X. Chen, Z. Hu, *Anal. Bional. Chem.* 384 (2006) 1533.

[47] L. Liu, L. Fan, H. Chen, X. Chen, Z. Hu, *Electrophoresis* 26 (2005) 2999.

[48] X. Zou, Y. Li, M. Li, B. Zheng, J. Yang, *Talanta* 62 (2004) 719.

[49] B. Li, D. Wang, J. Lv, Z. Zhang, *Talanta* 69 (2006) 160.

[50] P.R. Fortes, S.R.P. Meneses, E.A.G. Zagatto, *Anal. Chim. Acta* 572 (2006) 316.

[51] A. Muñoz de la Peña, A. Espinosa-Mansilla, M.I. Acedo Valenzuela, H.C. Goicoechea, A.C. Olivieri, *Anal. Chim. Acta* 463 (2002) 75.

[52] B. Li, Y. He, J. Lv, Z. Zhang, *Anal. Bional. Chem*. 383 (2005) 817.

[53] D.M. Magni, A.C. Olivieri, A.L. Bonivardi, *Anal. Chim. Acta* 528 (2005) 275.

[54] A. Pasamontes, M.P. Callao, *Trends Anal. Chem*. 25 (2006) 77.

[55] A. Checa, R. Oliver, J. Saurina, S. Hernández-Cassou, *Anal. Chim. Acta* 572 (2006) 155.

[56] V. Gómez, M.S. Larrechi, M.P. Callao, *Anal. Chim. Acta* 571 (2006) 129.

[57] V. Gómez, J. Font, M.P. Callao, *Talanta* 71 (2007) 393.

[58] J.M. Amigo, J. Coello, S. Maspoch, *Anal. Bional. Chem*. 382 (2005) 1380.

[59] N.R. Marsili, A. Lista, B.S. Fernández Band, H.C. Goicoechea, A.C. Olivieri, *Analyst* 130 (2005) 1291.

5.3. PAPER

Coupling of sequential injection chromatography with multivariate curve resolution-alternating least squares for enhancement of peak capacity

Verónica Gómez, Manuel Miró, María Pilar Callao, Víctor Cerdà
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Coupling of sequential injection chromatography with multivariate curve resolution-alternating least squares for enhancement of peak capacity

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Abstract

Flow-through low-pressure chromatographic separations capitalized on the Sequential Injection Chromatographic (SIC) concept are for the first time coupled to second-order multivariate regression models based on Multivariate Curve Resolution-Alternating Least- Squares (MCR-ALS) for outperforming current chromatographic methods in terms of resolution efficiency. The proposed SIC-MCR-ALS method involving Sequential Injection separation on short monolithic columns along with isocratic elution fosters ultrafast reversed-phase separations of complex multicomponent mixtures regardless of peak overlapping and retention parameters. The ruggedness of SIC systems is enhanced by removing the solenoid valves from the flow network, thus diminishing the column back pressure effects. As a consequence, the flow setup admitted mobile-phase flow rates much higher than those traditionally enabled in SIC.

To ascertain the improved peak capacity of the SIC-MCR-ALS procedure, five phenolic species commonly used in disinfectant products and featuring similar UV spectra and close retention times in short reversed-phase silica-based monolithic phases are selected as model compounds and determined in just 1 minute using mobile-phase flow rates of ≥ 2 mL min^{-1}. Notwithstanding the fact that the five phenolic derivatives coelute in a single chromatographic band, thus rendering resolution values ranging from 0.05 to 1.19, the concentration profiles and the pure spectra of each individual phenol species could be concurrently obtained. Quantitative validation of the chromatographic-chemometric method demonstrated both the reliability of the results and the enhanced resolution of mixtures with regard to former SIC systems with no need for thorough optimization of the separation conditions.

1. Introduction

The various generations of flow injection (FI) have already been consolidated as powerful analytical approaches for both automated microsampling and on-line processing of complex samples aimed at either matrix isolation or preconcentration of trace levels of target compounds[1-4]. Yet, FI-based systems have the inherent shortcoming of limited capability for multi-analyte determinations[5]. Though the second generation of FI, that is sequential injection (SI), features improved versatility as to the clustering of unit operations at will at the peripheral ports of the multiposition valve[6-8], the multicomponent analyses have been so far restricted to straightforward speciation procedures where a given oxidation state of the target analyte is derivatized via a selective reagent[9].

The advent of monolithic columns featuring high porosity and fast mass-transfer kinetics during separation and their coupling to SI, rendering the so-called sequential injection chromatography (SIC), expanded the potential of flow-based systems for multicomponent purposes. The SIC concept, launched by Solich and co-workers[10,11], which is also adaptable to related discontinuous-flow approaches, namely, Lab-on-Valve and multisyringe flow injection analysis[12], might be viewed as an appealing alternative to high-performance liquid chromatography (HPLC) for on-line, low-pressure reversed-phase chromatographic separations in the pharmaceutical field[13-15]. As opposed to forward-flow HPLC with particle-packed capillary columns, low-pressure SIC fosters the straightforward microfluidic handling that could be exploited for on-line derivatization reactions along with the miniaturization of the entire analytical setup that ensures ready portability for process analysis. Another asset of SIC is the considerable reduction in consumption of solvents as a result of the use of flow programming, which, in turn, limits the generation of waste and decreases the analysis costs.

Notwithstanding the fact that monolithic phases feature similar chromatographic properties as to retention and selectivity as particle columns of the same specific surface area and pore diameter[16], the ruggedness of SIC is deteriorated as compared to HPLC because of the short-term operational performance of the solenoid valve placed at the head of the liquid driver, that is, the syringe pump, under increased flow impedance. As a consequence,

SIC admits merely mobile phase rates below 1.0 mL min^{-1} whenever short analytical columns (usually 25 mm long) are interfaced in the flow system[17].

Another inherent shortcoming of SIC is the lack of appropriate selectivity (separation factors) as a consequence of the short column size that restricts its current analytical applicability to the resolution of up to three biologically active compounds in relatively simple matrices (e.g., topical creams and pharmaceutical preparations)[17]. To tackle this problem, maximum benefit can be taken from the exploitation of chemometric approaches for resolution of coeluting peaks[18-20], thus enhancing the peak capacity of monolithic columns. Actually, an overview of chemometric methods that can widely and profitably be invested in chromatographic separations has been recently published[21]. In this context, multivariate curve resolution-alternating least-squares (MCR-ALS)[22-24] procedures have much to offer because pure compound information (concentration and spectra profiles) might be unravelled from nonselective experimental outputs and unknown multiplicative matrix interferences can be appropriately handled[25].

In this paper, SIC with diode-array detection is for the first time coupled to MCR-ALS for outperforming the current low-pressure chromatographic methods in terms of peak resolution efficiency. The aim is to devise a universal and straightforward SIC-MCR-ALS method involving isocratic elution for ultrafast reversed-phase separations of complex multicomponent mixtures regardless of peak overlapping and retention parameters. Though MCR-ALS has been proven effective to handle overlapped HPLC chromatograms[26-31], multivariate data analyses have been frequently applied for resolution of sharp elution peaks embedding a mere of two or three species.

In order to evaluate the improved peak capacity and ruggedness of the new SIC-MCR-ALS methodology, five phenolic species commonly used in disinfectant products and featuring similar UV spectra and close retention times in short reversed-phase, silica-based monolithic columns are selected as model compounds at variable concentration levels. The purity of the elution band is thus to be investigated, and the concentration profiles and the pure spectra of each individual phenolic derivative concurrently are to be obtained. The validation of the method via the evaluation of the figures of merit fosters the ascertainment of both the reliability of the chromatographic-chemometric results and the potential

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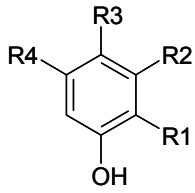
improvement of peak capacity with no need for further optimization of the experimental chromatographic conditions.

2. Experimental

2.1. Reagents and solutions

All chemicals were of analytical-reagent grade, and doubly deionised water (18.2 MΩ cm) obtained from a Millipore system (Milllipore Synthesis A10, France) was used throughout for solution preparation. Phenol, 2,4-dimethylphenol, 4-chloro-3-methylphenol, 2-phenylphenol, and 2-benzyl-4-chlorophenol were purchased from Aldrich and used without further purification. The chemical structures of targeted compounds are shown in Table 1 and their spectral characteristics illustrated in Figure 1.

Table 1. Chemical structure of the targeted phenolic species



Compound	R1	R2	R3	R4
Phenol	H	H	H	H
2,4-dimethylphenol	CH_3	H	CH_3	H
4-chloro-3-methylphenol	H	CH_3	Cl	H
2-phenylphenol	C_6H_5	H	H	H
2-benzyl-4-chlorophenol	$C_6H_5CH_2$	H	Cl	H

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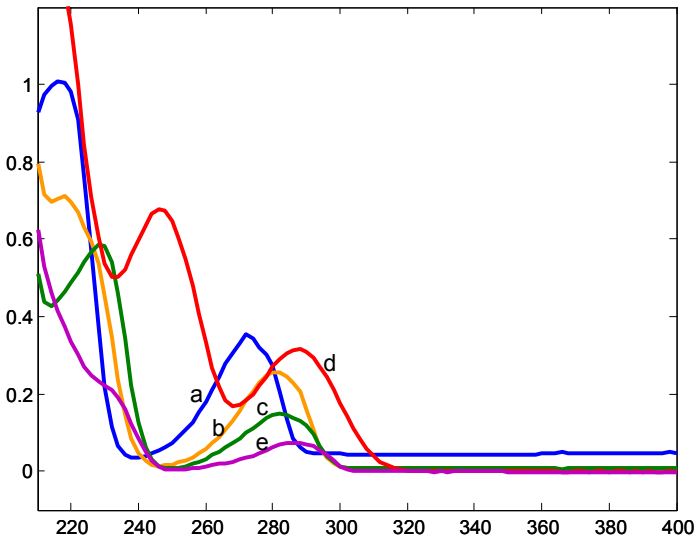

Figure 1. Spectral profiles of the phenolic species. Phenol (a, blue), 2,4-dimethylphenol (b, orange), 4-chloro-3-methylphenol (c,green), 2-phenylphenol (d, red) and 2-benzyl-4-chlorophenol (e, purple). The first two species are prepared at a 200 mg L^{-1} level while the remaining at a 150 mg L^{-1} level.

A stock solution of each analyte (5000 mg L^{-1}) was prepared in a 50:50 (v/v) acetonitrile:water mixture, which contains the minimum amount of organic solvent needed for dissolution of the phenolic derivatives of high molecular weight. Working standard solutions of individual species or mixtures of the various compounds were prepared daily by stepwise dilution of the stock solution with MilliQ water.

Isocratic binary mobile phases were prepared by mixing 50 mmol L^{-1} phosphate buffer (pH 3.0) with acetonitrile or methanol HPLC-grade (Panreac). All solvents, mobile phases and working standard solutions were first filtered under vacuum through a 0.45 µm nylon filter (Albet, Barcelona, Spain) and degassed using an ultrasonic bath for 15 min.

The real sample is a mixture of two constituents of Cresovex-S (SP Veterinaria, Spain), that is, Preventol cmk and Preventol bp (Lanxess Germany GmbH), which are utilized for disinfection of cow sheds and stables. The sample containing 4-chloro-3-methylphenol and 2-benzyl-4-chlorophenol was spiked with phenol, 2,4-dimethylphenol, and 2-phenylphenol and analyzed directly with the SIC system with no further manual processing.

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2.2. Sequential injection chromatographic system

The proposed sequential injection chromatographic (SIC) manifold is composed of a syringe piston pump with programmable speed (MicroBU 2030, Crison, Alella, Spain) furnished with a 5-mL syringe and a 10-port multiposition valve (Crison). The conventional three-way solenoid valve mounted atop of the syringe is eliminated and replaced by a two-way connector that communicates the liquid driver with the selection valve. The flow network was built from poly(tetrafluoroethylene) (PTFE) tubing of 0.5 and 0.8mm i.d., using chemically resistant connectors made of poly(vinylidene difluoride) and poly(oxymethylene). The holding coil was made from 0.8 mm PTFE tubing, the length being 50 cm, which corresponds to a volume of 0.25 mL. On-line sample separation was conducted at room temperature on a reversed-phase monolithic rod Onyx^{TM} Monolithic C18 (25 mm × 4.6 mm i.d. column; Phenomenex) coupled with a monolithic guard column (5 mm × 4.6 mm i.d.; Phenomenex) of the same material. The flow manifold for low-pressure chromatographic separations is schematically illustrated in Figure 2. Initially, the system was conditioned by filling the overall manifold tubing and monolithic column with mobile phase. The analytical procedure involved the consecutive aspiration of 3.0 mL of mobile phase and 25 μL of sample, which via flow reversal, were delivered forward to the monolithic column at 2.0 mL min^{-1}.

A diode-array spectrophotometer (Hewlett-Packard HP8452A) equipped with a quartz flow-cell (Hellma 178.712QS, 18 μL inner volume, 1 cm optical path) was used as detector. Measurements were recorded at a frequency of 1.43 Hz within the wavelength range from 210 to 400 nm with 1 nm scan interval.

The operational procedures for the SIC analyzer were computer controlled by the software package AutoAnalysis 5.04 (Sciware, Spain) based on dynamic link libraries (DLLs). Acquisition and processing of the spectrophotometric data were performed using the same software package. In our particular configuration, the principal protocol of the software was loaded with custom-built DLLs designed for the automatic control of the syringe pump, selection valve and diode-array spectrophotometer.

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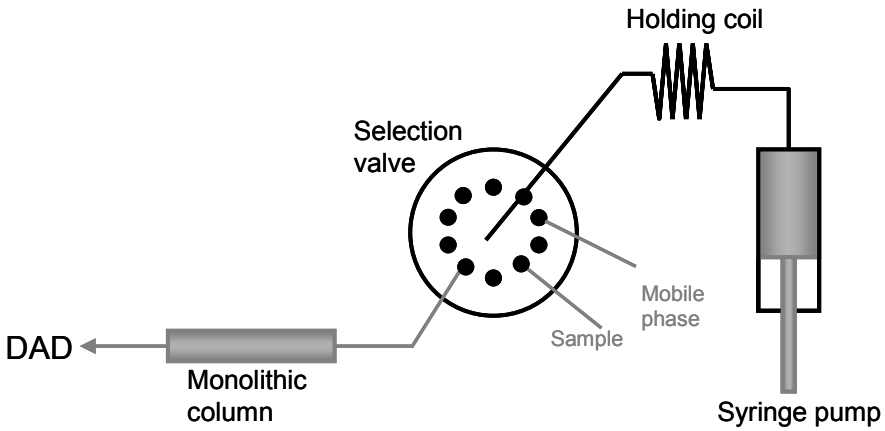

Figure 2. Schematic illustration of the sequential injection chromatographic system for determination of phenolic disinfectant agents

2.3. Chromatographic reference method

Gas chromatography (GC) was selected as a reference method for determination of the phenolic ingredients in disinfectant products. GC analyses were performed on a HP 5890 chromatograph (Hewlett-Packard) equipped with a SPB^{TM}5 fused-silica capillary column (Supelco, 30 m long, 320 µm i.d. and 0.25µm film thickness) and a flame ionization detector. The oven temperature program started from 50 ºC with a holding time of 2 min and increased to 200 ºC at a rate of 10 ºC min^{-1}, followed by heating to 300 ºC at 25 ºC min^{-1} with a final holding time of 5 minutes. Helium was used as carrier gas with an average linear velocity of 1.2 mL min^{-1}.

2.4. Chemometric procedure

The multivariate curve resolution (MCR) chemometric approach is based on a linear model, which assumes the additivity of the response of the overall components in the sample, as described elsewhere[22,23,32].

The analytical data obtained with the aid of a hyphenated chromatographic technique might be presented as a two-way data table, the so-called matrix **D** ($m \times n$). The columns of **D** are the chromatograms of a sample, registered by using a single-wavelength channel of the

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detector, whereas the rows are the spectra at the given elution time, as schematically shown in Figure 3.

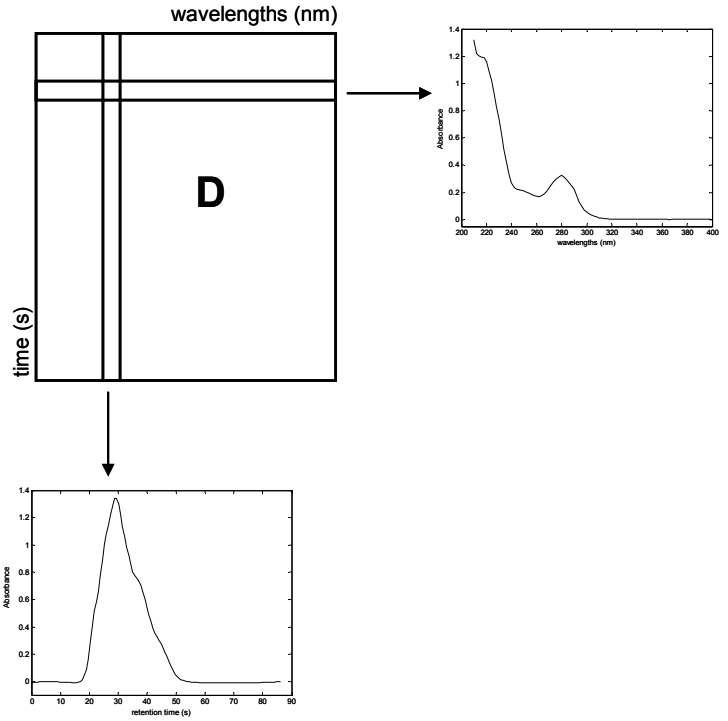

Figure 3. Illustration of the bi-linear chromatographic data. Concentration profiles (chromatograms) and spectral profiles are contained within the columns and rows of matrix D, respectively

These data can be described by assuming a bilinear model based on the multiwavelength extension of Beer's absorption law:

$$\mathbf{D} = \mathbf{C}\mathbf{S}^{T} + \mathbf{E} \qquad (1)$$

where $\mathbf{C}(m \times k)$ is the matrix of the chromatographic profiles for the k analytes, $\mathbf{S}^{T}(k \times n)$ is the transposed matrix of absorption profiles, and $\mathbf{E}$ is the matrix of residuals with the unmodeled absorbance data values. To assess the matrices $\mathbf{C}$ and $\mathbf{S}^{T}$ from the matrix $\mathbf{D}$ of measured data, an alternating least-squares procedure, starting with initial estimates of the pure spectra of the k components, is exploited. During the iterative optimization, a series of

constraints[22,33], was applied in an attempt to improve the optimization and to restrict the number of possible solutions, that is: (a) the pure spectra of each component must be non-negative; (b) the concentration profile of each component must be non-negative; (c) the concentration profile of each component must have only one maximum (unimodality). The optimization ends when a convergence criterion is reached.

A suitable means for resolution improvement involves the implementation of chemical knowledge of the system into the model. It is carried out by incorporating spectral and profile information on the *k* species in the mixture by column-wise matrix augmentation; that is, an augmented matrix is built by setting one matrix in top of the other while maintaining the columns (viz., wavelengths) in common[34,35].

The quality of the recovered profiles is to be assessed via the criterion of similarity, which entails the comparison of the actual spectra with the spectra obtained from the MCR-ALS algorithm, in accordance with the following equation:

$$r = cos\,\gamma = \frac{s_i^T \hat{s}_i}{\|s_i\| \cdot \|\hat{s}_i\|} \qquad (2)$$

where γ is the angle defined by the vectors associated with the recovered spectra ($\hat{s}_i$) and the real spectra (s_i) of the compound studied. Additional parameters commonly utilized for evaluation of the algorithm application are the lack of fit (*lof*), that is, the lack of adjustment of the model proposed in Eq. 1, and R^2, which corresponds to the explained variance reported by the product $\mathbf{C}\cdot\mathbf{S}^T$.

$$lof = \sqrt{\frac{\sum_i \sum_j (d_{i,j} - \hat{d}_{i,j})^2}{\sum_i \sum_j d_{i,j}^2}} \qquad (3)$$

$$R^2 = \frac{\sum_i \sum_j \hat{d}_{i,j}^2}{\sum_i \sum_j d_{i,j}^2} \qquad (4)$$

where d_{ij} represents the elements of the experimental data matrix **D**, and $\hat{d}_{i,j}$ are the values calculated by the model in Eq. 1.

Quantitative information for each particular compound is obtained from the MCR-ALS resolved concentration profiles. Calibration is performed using column-wise augmented matrices, where the matrix above corresponds to the calibration standards and the matrix below corresponds to a reference standard whose concentration is affixed. The most concentrated standard is selected as the reference standard throughout[36]. With a set of standards, a calibration curve is built for each analyte where the area ratios ($a_{std,i}/a_{std,ref}$) are plotted versus the concentration ratios ($c_{std,i}/c_{std,ref}$). $a_{std,i}$ and $a_{std,ref}$ are the areas of the chromatographic peaks of the target analyte in the calibration standards and the reference standard, respectively, and $c_{std,i}$ and $c_{std,ref}$ are the concentrations in the calibration standards and the reference standard, respectively. A regression line with a slope of 1 and an intercept of 0 is thus expected. The prediction step is conducted by calculating the ratio between the area of the chromatographic profile of the particular analyte in the unknown sample and that of a reference calibration standard, followed by the introduction of this value into the regression line as $c_{unk}=((a_{unk}/a_{std,ref})-b_0)\cdot c_{std,ref}/b_1$, where c_{unk} and a_{unk} are the concentrations and the areas of the chromatographic peak of the target analyte in the unknown sample.

To estimate the spread of the measurements around the fitted regression line, the residual error, s_e is calculated for each targeted species as follows:

$$s_e = \sqrt{\frac{\sum (y_i - \hat{y}_i)}{n-2}} \qquad (5)$$

where y_i and $\hat{y}_i$ are the experimental and the predicted absorbance area ratios, and n is the total number of calibration standards.

All calculations related to multivariate curve resolution with alternating least squares (MCR-ALS) were performed via dedicated software under a Matlab 7.0 computer environment[37]. This software is available upon request to the authors[38].

3. Results and discussion

3.1. Analytical performance of the sequential injection chromatographic system

Despite the high permeability of monolithic rods as compared with particulate phases, low-pressure sequential injection chromatographic systems using a syringe pump as a liquid driver are merely reliable at moderate mobile phase flow rates[17]. The instrumental setup reported by Solich et al.[10,14] has been however modified by removing the solenoid valves from the flow network, thus enhancing the system's ruggedness by tolerating pressures up to 8.5 bar while fostering the application of flow rates up to 3.0 mL min^{-1} with no appreciable flow resistance.

Chemical parameters including the type and proportion of organic modifier (viz., acetonitrile or methanol) should be carefully selected to prevent undue back pressure effects. Acetonitrile (ACN) was chosen as an organic modifier for further investigations as a consequence of the low viscosity of the resulting mobile phase. According to the results compiled in Table 2, the larger the organic modifier to water ratio the lower the flow impedance. The effect of the concentration of organic modifier on the separation of the phenolic disinfectant agents was thus investigated over the range 50-70% (v/v) using an isocratic elution mode. The increase of organic modifier in the mobile phase gives rise to the decrease in elution times, thus rendering time and cost-effective chromatographic analyses. The five targeted species were actually embedded in a single elution peak whenever using 70:30 ACN:H_2O (v/v) at pH 3.0 as a mobile phase. The retention times for individual phenolic derivatives were 22.4, 25.1, 25.2, 28.7 and 32.2 s for phenol, 2,4-dimethylphenol, 4-chloro-3-methylphenol, 2-phenylphenol and 2-benzyl-4-chlorophenol, respectively. The application of the second-order data treatment method revealed that in this particular case the matrix presents rank deficiency and therefore cannot be resolved because the elution profiles of 2,4-dimethylphenol and 4-chloro-3-methylphenol are virtually the same. It should be noted

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that the rank of the matrix should be identical to that of the number of components in the sample for appropriate quantification. The composition of the isocratic binary mobile phase was thus finally affixed at 60:40 ACN:H_2O (v/v) and pH 3.0 because of the improvement of the separation for 2,4-dimethylphenol and 4-chloro-3-methylphenol with no need for decrease of flow rate.

Under the foregoing experimental conditions, the retention parameters for the suite of phenolic species are shown in Table 3. Chromatographic resolution is estimated from $R= 2\ \Delta t_{AB}/\ (w_A+w_B)$ where Δt_{AB} is the difference between retention times of resolved peaks and w_A and w_B are the respective peak widths. Acceptable resolution values at ≥ 98% level were merely encountered for phenol and 2-benzyl-4-chlorophenol. Thus, the separation and quantitation of more than two components or other multicomponent mixtures is not feasible by exploiting SIC with univariate calibration.

Table 2. Maximum pressures measured at the inlet of the chromatographic column

Flow	Acetonitrile:water			Methanol:water	
	70:30	50:50	10:90	70:30	50:50
0.5 mL min^{-1}	< 1	< 1	2.5	< 1	< 1
0.8 mL min^{-1}	< 1	< 1	3.5	3	5
1.0 mL min^{-1}	2.5	4	5	5	7
1.2 mL min^{-1}	3	4.5	6	7	8.5
1.5 ml/min	3.5	5	7.5	8.5	
2.0 mL min^{-1}	5	7.5			
2.5 mL min^{-1}	6.5	8.5			
3.0 mL min^{-1}	7.5				

The 3D data for the chromatographic analysis of a mixture of the five phenolic species is depicted in Figure 4. The 2D plots (absorbance versus time in Figure 4a and absorbance versus wavelength in Figure 4b) clearly illustrate a strong coelution of the suite of targeted compounds along with the overlapping of the overall spectral bands. For the poorly resolved analytes (see Table 3), suitable selectivity is not expected to be achieved by merely changing the chromatographic experimental conditions, whereby a universal approach for chemometric resolution of the species is devised as detailed below.

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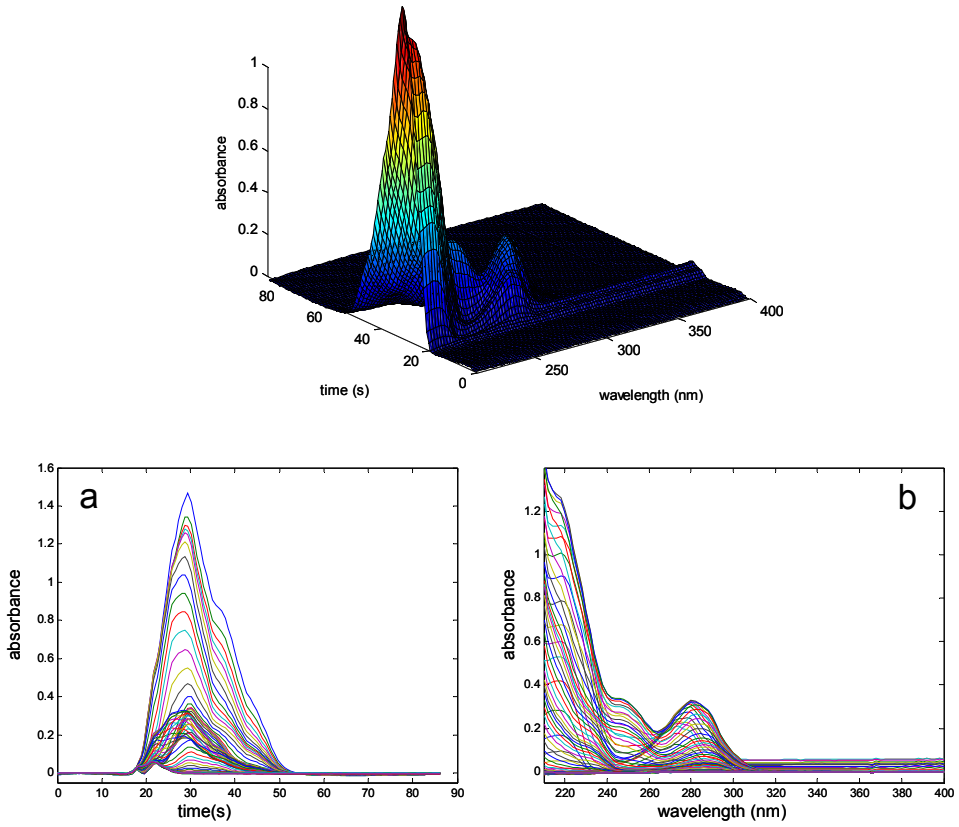

Figure 4. Data matrix resulting from the chromatographic separation and determination of a mixture of five phenolic disinfectant agents. (a) Concentration profiles and (b) spectral profiles. The concentrations of the various species are: 90 mg L^{-1} phenol, 90 mg L^{-1} 2,4-dimethylphenol, 70 mg L^{-1} 2-phenylphenol, 90 mg L^{-1} 4-chloro-3-methylphenol and 70 mg L^{-1} 2-benzyl-4-chlorophenol.

3.2. Figures of merit of the chemometric model

MCR-ALS was applied to an augmented matrix comprising the sample matrix itself along with a reference mixture of known concentrations and five matrices corresponding to each individual phenolic derivative. This information is added to the mathematic algorithm aimed at improving the resolution while precluding the rotational ambiguity[34,35].

The chemometric model yields a resolved chromatogram for the mixture of five phenols (**C**), and their spectra matrix ($\mathbf{S}^T$), as shown in Figure 5. Equation 2 was utilized for

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quantitative evaluation of the spectral profiles. The similarity coefficients (*r*) were 0.9997, 0.9997, 0.9999, 0.9994 and 0.9995 for phenol, 2,4-dimethylphenol, 2-benzyl-4-chlorophenol, 4-chloro-3-methylphenol and 2-phenylphenol, respectively, thus denoting an excellent match between the resolved and pure spectra.

Table 3. Chromatographic parameters obtained for the targeted phenolic agents at 60% ACN:H_2O (v/v) and pH 3.0

			Resolution			
	tr (s)	peak width w_i(s)	phenol	2,4-dimethylphenol	4-chloro-3-methylphenol	2-phenylphenol
phenol	23.1	14.7				
2,4-dimethylphenol	26.6	15.4	0.23			
4-chloro-3-methylphenol	27.3	14.7	0.29	0.05		
2-phenylphenol	31.5	15.4	0.56	0.32	0.28	
2-benzyl-4-chlorophenol	40.6	16.8	1.11	0.87	0.84	0.57
dead time (t_0)	17.5					

According to Eq. 3 the product of the spectral and concentration profiles explained 99.65% of the variance associated to the augmented data matrix. The lack of fit that provides a global measure of the residual was estimated to be 5.93% (Eq. 4), thus indicating that most of the variability of the experimental data is given by the product $\mathbf{C}\cdot\mathbf{S}^{\mathbf{T}}$.

For quantitation purposes, simultaneous analysis of the multiple data matrices corresponding to chromatograms of different standard mixtures was performed at five concentration levels within the 20-90 mg L^{-1} range for the suite of phenolic compounds. Isoabsorbance mixtures were warranted by careful selection of the concentration of individual species. To evaluate discrepancies in retention times and peak areas, the repeatability was evaluated by performing three consecutive injections of each phenol mixture. Regression lines were built using a single mixture of 90 mg L^{-1} phenol, 2,4-dimethylphenol, 4-chloro-3-methylphenol and 70 mg L^{-1} of 2-phenylphenol and 2-benzyl-4-chlorophenol as a reference standard. Analytical figures of merit of the chromatographic-chemometric system including regression parameters (b_0, b_1), its uncertainties (s_{b0}, s_{b1}), residual errors (s_e), correlation coefficients (*r*) and critical and detection limits (X_C, X_D) are compiled in Table 4. The slope ($b_1 \pm s_{b1}$) and intercept ($b_0 \pm s_{b0}$) of the regression lines are not significantly different from one and zero, respectively. The fitting is slightly worsened for those analytes, namely, 4-chloro-3-methylphenol and 2-phenylphenol, embedded completely within the chromatographic peak. Besides, the higher the partition coefficient of the analyte on the reversed-phase monolithic

column the higher is the residual error (s_e) encountered**.** This is attributed to the broadening of the chromatographic peak and the lack of precision in the peak area for the late eluting compounds. Yet, with this novel SIC-MCR-ALS system, multicomponent mixtures of the five phenolic derivatives are analyzed in less than 60 seconds.

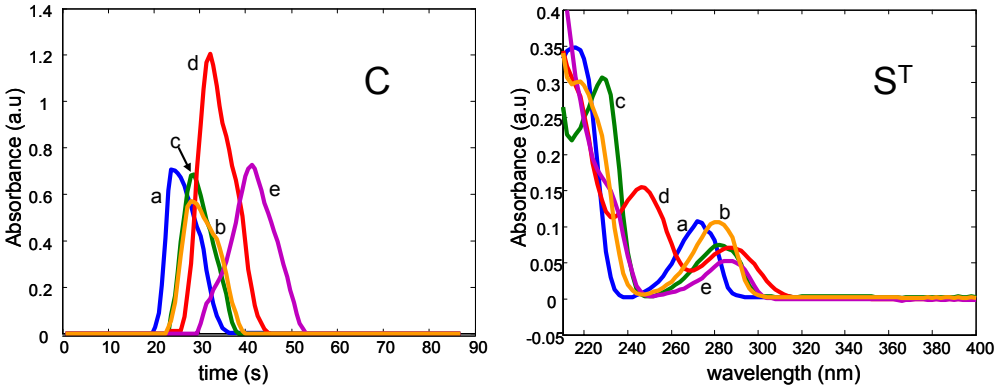

Figure 5. Analytical results from the MCR-ALS resolution of overlapped phenolic derivatives containing 90 mg L^{-1} of phenol, 2,4-dimethylphenol and 4-chloro-3-methylphenol and 70 mg L^{-1} of 2-phenylphenol and 2-benzyl-4-chlorophenol. (C) Concentration profiles. (S^T) Spectral profiles. Phenol (a, blue), 2,4-dimethylphenol (b, orange), 4-chloro-3-methylphenol (c, green), 2-phenylphenol (d, red) and 2-benzyl-4-chlorophenol (e, purple).

Critical limits (X_C) and detection limits (X_D) were calculated taking into account the uncertainty in the regression line at the 95% confidence level[39]. The best values are attained for the analytes with the shortest retention times (see Table 4). The regression lines were validated via the application of an ANOVA test[39]. The statistical F calculated by comparison of the standard error of replicates and the residual error of the regression line revealed the inexistence of significant differences in precision at the 0.05 significance level because the experimental results of F were in all cases lower than the critical F value.

3.3. Application of the SIC-MCR-ALS procedure

The sequential injection chromatography system coupled to multivariate curve resolution system was applied to the simultaneous determination of five phenolic species commonly used as disinfectant agents in different types of environmental waters including

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well water, spring water and seawater aiming at evaluating the reliability of the second-order multivariate regression model and the dependence of the bulk sample matrix on the chemometric resolution.

Table 4. Analytical figures of merit of the SIC-MCR-ALS calibration

	phenol	2,4-dimetylphenol	4-chloro-3-methylphenol	2-phenylphenol	2-benzyl-4-chlorophenol
Interval range	30-90	30-90	30-90	20-70	20-70
b_1	0.99	0.99	1.04	0.95	0.99
b_0	0.01	0.03	-0.07	0.02	0.05
s_{b1}	0.0314	0.0256	0.0342	0.0368	0.0676
s_{b0}	0.0221	0.0180	0.0240	0.0262	0.0481
s_e	0.0270	0.0220	0.0294	0.0338	0.0621
r	0.9935	0.9957	0.9931	0.9905	0.9713
n	15	15	15	15	15
X_C (mg L^{-1})	5.64	4.58	5.83	5.54	9.75
X_D (mg L^{-1})	10.75	8.80	11.09	10.47	17.73
ANOVA, Fcal	3.29	3.33	3.42	3.29	3.26
Ftab (0.05, 3,10)			4.10		

The MCR-ALS data for the spiked water samples are compiled in Table 5. The uncertainty of the results was calculated from the parameters of the regression curve and the deviation error. The model was validated via the application of a joint test of intercept and slope (*F* test) for the investigation of bias[40]. If both the slope and the intercept of the regression curves where the added concentrations are plotted against the predicted values were not significantly different from the ideal values of one and zero, respectively, there would not be bias for a certain level of confidence pre-established. The *F* data listed in Table 5 revealed the inexistence of significant differences for at least a 97.5% confidence level.

The phenolic disinfectant agents were also determined in a commercial formulation composed of Preventol cmk and Preventol bp (Lanxess Germany GmbH) in order to ascertain the accuracy of the SIC-MCR-ALS method. The chemometric results were contrasted with the experimental data obtained by a GC protocol as detailed under the Experimental Section (see Table 5). The significance *t*-test of comparison of two means was reliable for each individual phenol at a 95% confidence level.

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Table 5. Simultaneous determination of 5 phenolic species by coupling of SIC with MCR-ALS

	phenol		2,4-dimethylphenol		4-chloro-3-methylphenol		2-phenylphenol		2-benzyl-4-chlorophenol	
	predicted ($mg\ L^{-1}$)	added/real [1] ($mg\ L^{-1}$)	predicted ($mg\ L^{-1}$)	added/real [1] ($mg\ L^{-1}$)	predicted ($mg\ L^{-1}$)	added/real [1] ($mg\ L^{-1}$)	predicted ($mg\ L^{-1}$)	added/real [1] ($mg\ L^{-1}$)	predicted ($mg\ L^{-1}$)	added/real [1] ($mg\ L^{-1}$)
well	38±3	40	47±3	50	35±2	35	61±4	60	67±5	60
well	75±5	70	35±2	35	84±6	80	55±4	50	38±3	30
well	50±3	50	65±4	70	45±3	50	19±1	20	45±3	40
sea	78±5	70	87±6	80	36±2	30	44±3	55	75±5	65
sea	51±4	55	36±2	40	25±2	20	23±2	25	67±5	70
sea	41±3	40	35±2	30	77±5	75	34±2	30	44±3	50
spring	61±4	60	57±4	60	57±4	60	54±4	50	48±3	50
spring	38±3	40	33±2	35	65±4	60	30±2	30	61±4	70
spring	80±5	80	77±5	80	28±2	30	60±4	60	60±4	60
disinfectant [1]	46±3	50±4	38±3	40±3	49±3	50±4	38±3	40±5	44±3	40±5
Fcal [2]	6.28		1.2		1.55		0.04		6.27	
F(0.025,2,8)					8.022					

[1] Determined by gas chromatography
[2] Joint test of slope and intercept

4. Conclusions

In this paper, a universal protocol for increasing peak capacity of short monolithic columns is presented by coupling SIC with multivariate chemometric approaches. The scope of low-pressure chromatographic separations utilizing sequential injection instrumentation might be thus expanded from the traditional assays of drugs in relatively simple multicomponent matrices (e.g., pharmaceutical preparations) to more complex matrices (e.g., disinfectant products) as demonstrated in this work.

The ruggedness of SIC systems has been improved by removing the solenoid valves from the flow network. As a consequence, the flow setup admitted mobile phase flow rates much higher than those allowed in traditional SIC. This, in turn, resulted in ultrafast chromatographic separations, namely, the targeted five phenolic disinfectant agents could be determined in just 1 min. It should be stressed that similar substituted phenols have been previously separated and quantified via high-performance liquid chromatographic using particulate or monolithic columns[41,42], yet time analysis ranged between 3 and 25 minutes depending upon the peak resolution of the chromatographic column.

The coupling of SIC with the second-order calibration method MCR-ALS reduces not merely the analysis time but solvent expenses and fosters quantitation in case of strong signal overlapping. As opposite to univariate calibration methods, five phenolic species

coeluting in a single chromatographic peak and bearing similar spectral characteristics could be readily resolved. The results obtained from multivariate data analysis have been validated. The absence of bias in the SIC-MCR-ALS method was assessed via the joint confidence interval test and a comparison test of two means using bivariate least-squares. The analysis of disinfectant samples indicates that there are no significant differences between SIC-MCR-ALS and GC-FID, used as a reference method, at the 0.05 significance level. Hence, the accuracy of the proposed method has been demonstrated. SIC-MCR-ALS is therefore a promising evolving method in flow analysis for fast multicomponent analysis by handling unresolved chromatographic peak signals with no constraints as to the complexity of the sample matrix. SIC applications can be actually expanded to further analytical fields, such as environmental and biological samples and foodstuffs, and even to trace level analysis via the hyphenation of monolithic columns with on-line sorptive preconcentration protocols.

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References

[1] E.H. Hansen, M. Miró, *Trends Anal. Chem*. 26 (2007) 18.

[2] M. Miró, J. M. Estela, V. Cerdà, *Curr. Anal. Chem*. 1 (2005) 329.

[3] M. Miró, E.H. Hansen, On-line Processing Methods in Flow Analysis, In *Advances in Flow Methods of Analysis*; Trojanowicz M., Ed.; Wiley-VCH: Weinhem, Chapter 11. In press.

[4] V. Cerdà, Introduction to Flow Analysis Methods (in Spanish), Sciware SL, 2006.

[5] M. Miró, W. Frenzel, *Microchim. Acta* 148 (2004) 1.

[6] C.E. Lenehan, N.W. Barnett, S.W. Lewis, *Analyst* 127 (2002) 997.

[7] A. Economou, *Trends Anal. Chem*. 24 (2005) 416.

[8] M. Miró, E.H. Hansen, *Trends Anal. Chem*. 25 (2006) 267.

[9] J.F. van Staden, R.I. Stefan, *Talanta* 64 (2004) 1109.

[10] D. Satinsky, P. Solich, P. Chocholous, R. Karlıcek, *Anal. Chim. Acta* 499 (2003) 205.

[11] J. Huclova, D. Satinsky, R. Karlicek, *Anal. Chim. Acta* 494 (2003) 133.

[12] H.M. González-San Miguel, J.M. Alpízar-Lorenzo, V. Cerdà, *Anal. Bioanal. Chem*. 387 (2007) 663.

[13] P. Chocholous, D. Satinsky, P. Solich, *Talanta* 70 (2006) 408.

[14] D. Satinsky, P. Chocholous, M. Salabova, P. Solich, *J. Sep. Sci* 29 (2006) 2494.

[15] J. Klimundova, D. Satinsky, H. Sklenarova, P. Solich, *Talanta* 69 (2006) 730.

[16] H. Kobayashi, T. Ikegami, H. Kimura, T. Hara, D. Tokuda, N. Tanaka, *Anal. Sci*. 22 (2006) 491.

[17] P. Chocholous, P. Solich, D. Satinsky, *Anal. Chim. Acta* 600 (2007) 600 (2007) 129.

[18] G. Vivó-Truyols, J.R. Torres-Lapasió, A.M. van Nederkassel, Y. Vander Heyden, D.L. Massart, *J. Chromatogr. A* 1096 (2005) 133.

[19] G. Vivó-Truyols, J.R. Torres-Lapasió, A.M. van Nederkassel, Y. Vander Heyden, D.L. Massart, *J. Chromatogr. A* 1096 (2005) 146.

[20] A. Cladera, E. Gómez, J.M. Estela, V. Cerda, *J. Chromatogr. Sci*. 30 (1992) 453.

[21] M. Daszykowski, B. Walczak, *Trends Anal. Chem*. 25 (2006) 1081.

[22] R. Tauler, A. Smilde, B.R. Kowalski, *J. Chemom*. 9 (1995) 31.

[23] R. Tauler, *Chemom. Intell. Lab. Syst*. 30 (1995) 133.

[24] A. de Juan, R. Tauler, *Anal. Chim. Acta* 500 (2003) 195.

[25] V. Gómez, I. Ruisánchez, M.P. Callao, *Anal. Chim. Acta* 600 (2007) 233.

[26] R. Gargallo, R. Tauler, F. Cuesta-Sánchez, D.L. Massart, *Trends Anal. Chem*. 15 (1996) 279.

[27] M.J. Rodríguez-Cuesta, R. Boqué, F.X. Rius, J.L. Martínez Vidal, A. Garrido Frenich, *Chemom. Intell. Lab. Syst.* 77 (2005) 251.

[28] E. Peré-Trepat, A. Hildebrandt, D. Barceló, S. Lacorte, R. Tauler, *Chemom. Intell. Lab. Syst.* 74 (2004) 293.

[29] S. Mas, G. Fonrodona, R. Tauler, J. Barbosa, *Talanta* 71 (2007) 1455.

[30] E. Comas, R.A. Gimeno, J. Ferré, R.M. Marcé, F. Borrull, F.X. Rius, *J. Chromatogr. A* 1035 (2004) 195.

[31] H. Li, J. Hou, K. Wang, F. Zhang, *Talanta* 70 (2006) 336.

[32] J. Jaumot, R. Gargallo, A. de Juan, R. Tauler, *Chemom. Intell. Lab. Syst*. 76 (2005) 101.

[33] A. de Juan, Y. Vander Hieden, R. Tauler, D.L. Massart, *Anal. Chim. Acta* 346 (1997) 307.

[34] M. Amrhein, B. Srinivasan, D. Bonvin, M.M. Schumacher, *Chemom. Intell. Lab. Syst*. 33 (1996) 17.

[35] J. Saurina, S. Hernández-Cassou, R. Tauler, A.J. Izquierdo-Ridorsa, *J. Chemometr.* 12 (1998) 183.

[36] A. Pasamontes, M.P. Callao, *Anal. Chim. Acta* 515 (2004) 159.

[37] Matlab, The Mathworks, South Natick, MA, 2000.

[38] R. Tauler, A. de Juan, A. Multivariate Curve Resolution home page, http://www.ub.es/gesq/mcr/mcr.htm.

[39] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. de Jong, P.J. Lewi, J. Smeyers-Verbeke, *Handbook of Chemometrics and Qualimetrics*. Part A, Elsevier: New York, 1997.

[40] J. Riu, F.X. Rius, *Anal. Chem*. 68 (1996) 1851.

[41] M. Cledera-Castro, A. Santos-Montes, R. Izquierdo-Hornillos, *J. Chromatogr. A* 1087 (2005) 57.

[42] R.D. Thompson, *J. AOAC Int.* 48 (2001) 815.

5.4. CONCLUSIONS

The future of multicomponent analysis with flow systems may lie in increasing the implementation of some of the strategies described in the paper above and, in general, in combining the various strategies presented: solid phase extraction, sequential injection chromatography, multichannel manifolds, selective detectors, hyphenations and chemometrics. In particular, the coupling of chemometric treatments with the other strategies may be one of the most attractive choices, because of its low experimental cost and the simple configurations. Flow systems could be hyphenated with high selectivity techniques (ICP-MS, capillary electrophoresis) when preconcentration/separation or small volumes are needed.

The second paper, in particular, presents a low experimental cost strategy that couples SIC with MCR-ALS.

This novel method increases the possibilities of SIC systems because flow rates can be increased by instrumentally modifying the solenoid valve on the top of the syringe, living ruggedness to the SIC systems. In this way, quick analyses are achieved (less than one minute). The scope of low-pressure chromatographic separations that use sequential injection instrumentation can be expanded from the traditional assays of drugs in relatively simple multicomponent matrices (e.g. pharmaceutical preparations) to more complex matrices (e.g. disinfectant products) as demonstrated in this work.

What is more, coupling it to curve resolution methods considerably increases the number of analytes to be resolved. In the paper presented, we studied mixtures of five components that eluted in a single peak (i.e. they presented strong signal overlapping and very similar UV-visible spectra). The coupling of SIC with the second order calibration method MCR-ALS reduces not only the analysis time but also solvent expenses.

This novel approach has great potential. SIC applications can be used in other fields, such as environmental matrices or foodstuffs, and even at trace levels, by coupling with an on-line preconcentration technique, such as a lab on valve (LOV) module.

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6. General conclusions

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This chapter contains the general conclusions of the work presented in this thesis. Specific conclusions have been drawn at the end of each paper and chapter.

Second-order methods are a practical solution to complex resolution and calibration analytical problems. Multivariate curve resolution with alternating least squares (MCR-ALS) may save time (sample pretreatments) and resources if it is properly implemented. Analytes of interest do not need to be totally selectively resolved and multicomponent analysis can be carried out in a single analysis. MCR-ALS requires the signal from the analyte of interest to be a data matrix. Anyway, the data from the measured standards and the measured test sample must follow a particular mathematical structure (bilinear) and the number of factors needed to build the model has to be properly selected.

SIA systems have some advantages: for example, the high frequency of analysis, the low consumption of reagents and samples, the short analysis time and its adaptability to complementary modules. An analytical sequence can be designed (flow, volume and concentration of sample and reagents) that gives rise to a total or partial reaction between analytes and reagents. When the reaction is partial, an evolving system is obtained that provides second-order data, i.e. a data matrix per sample.

In this thesis, the SIA-MCR-ALS combination has been used for the development of various methodologies to determine subproducts of the tanning industry. The second-order advantage (i.e. the ability to predict the concentration of an analyte in a sample even in the presence of unknown interferents which were not present in the calibration standards) was assessed by studying matrices of different complexities and in the presence of interferents. Although matrix effect can not be afforded with second-order data treatments, different strategies have been proposed. Methods have been developed that enable up to three analytes to be determined in a single analysis, so this combination has considerable potential in multicomponent analysis. Methods were developed in a wide range of applications for both analytes and sample types by choosing a reagent that prompted a chemical evolution of the main species of interest in the system measured.

The resolution capacity of these systems may be limited by the necessary selectivity of the analytes toward the reaction proposed in some of the measurement orders (time or

absorbances), so it is interesting to explore other strategies for obtaining second-order data with flow systems. We have demonstrated that a new generation of flow systems, Sequential Injection Chromatography (SIC) coupled to multivariate curve resolution with alternating least squares, can enhance resolution capabilities and increase the number of species to be determined. This novel approach could have great potential in the future. SIC applications can be extended to such other fields as environmental matrices or foodstuffs, and even at trace levels, by coupling an on-line preconcentration technique, such as a lab on valve (LOV) module.

The working experimental conditions of the chemical variables (reagents, volume of sample and volume of reagents) and the instrumental variables (flow, range of wavelengths and number of measurements) are critical for obtaining correct second-order data. In most of the experiments, we used experimental design methodology to establish experimental conditions.

This thesis has mainly focused on the field of the environment, and specifically to the tanning industry. Making use of skins and hides, a by product of human food production, is one of the most ancient transformation activities, and it has become particularly relevant in a society that has acquired particular sensitivity to environmental issues: maximum use and reuse of resources with the minimum impact possible on nature. In this context, research is essential if the tanning industry is to innovate and improve in an attempt to increase quality and productivity and overcome the ecological problems that environmental legislation is making increasingly clearer. This thesis, then, is a contribution to this field.

This thesis focuses principally on the application of MCR-ALS to SIA-DAD data. The work presented here points out a number of areas that still need to be studied in more detail:

a) The qualimetric studies and figures of merit obtained with MCR-ALS need to be developed.

b) MCR-ALS needs to be applied to other more complex SIA systems (i.e. with preconcentration columns, on-line derivatizations, etc.).

c) Second-order calibration methods need to be applied to other techniques and other fields.

b) Second-order calibration algorithms need to be applied to dynamic processes in which the signal (e.g. a spectrum) is measured within a process over time.

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Appendix

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List of papers by the author presented in this Thesis:

1. V. Gómez, M.P. Callao. *Use of multivariate curve resolution for determination of chromium in tanning samples using sequential injection analysis*, Analytical and Bioanalytical Chemistry 382 (2005) 328-334.

2. V. Gómez, A.Pasamontes, M.P. Callao. *Factorial design for optimizing chromium determination in tanning wastewater*, Microchemical Journal 83 (2006) 98-104.

3. V. Gómez, M.S. Larrechi, M.P. Callao. *Chromium speciation using sequential injection analysis and multivariate curve resolution*, Analytica Chimica Acta 571 (2006) 129-135.

4. V. Gómez, M.P. Callao. *Chromium determination and speciation since 2000*, Trends in Analytical Chemistry 25 (2006) 1006-1015.

5. V. Gómez, J. Font, M.P. Callao. *Sequential injection analysis with second-order treatment for the determination of dyes in the exhaustion process of tanning effluents*, Talanta 71 (2007) 1393-1398.

6. V. Gómez, I. Ruisánchez, M.P. Callao. *Matrix effect in second-order data. Determination of dyes in a tanning process using vegetable tanning agents*, Analytica Chimica Acta 600 (2007) 233-239.

7. V. Gómez, M.S. Larrechi, M.P. Callao. *Kinetic and adsorption study of acid dye removal using activated carbon*, Chemosphere 69 (2007) 1151-1158.

8. V. Gómez, M.P. Callao. *Experimental designs for optimizing and fitting the adsorption of dyes onto activated carbon*, Submitted.

9. V. Gómez, M.P. Callao. *Multicomponent analysis using flow systems*, Trends in Analytical Chemistry 26 (2007) 767-774.

10. V. Gómez, M. Miró, M.P. Callao, V. Cerdà. *Coupling of sequential injection chromatography with multivariate curve resolution-alternating least squares for enhancement of peak capacity*, Analytical Chemistry 79 (2007) 7767-7774.

List of papers by the author which their content is not related to the scope of the thesis:

V. Gómez, M.P. Callao. *Analytical applications of second-order calibration methods* Anal. Chim. Acta, in preparation

M. Fernández, V. Gómez, M. Miró, M.P. Callao, V. Cerdá.
Determinación simultánea de vitaminas hidrosolubles empleando cromatografía de inyección secuencial (SIC) y resolución de curvas multivariante con mínimos cuadrados alternados (MCR-ALS)
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Contributions to international meetings related with the Thesis:

V. Gómez, A. Pasamontes, M.P. Callao.
A study to analyse chromium in tannery samples using sequential injection analysis (SIA) and multivariate curve resolution (MCR).
9th Chemometrics in Analytical Chemistry – Lisboa (Portugal) (2004).
Poster communication.

V. Gómez, M.P. Callao.
Multivariate curve resolution (MCR) application for the determination of chromium in tanning samples using sequential injection analysis (SIA).
Euroanalysis – Salamanca (Spain) (2004)
Poster communication

V. Gómez, M.P. Callao.
Chromium speciation using sequential injection analysis and multivariate curve resolution.
11as Jornadas Análisis Instrumental – Barcelona (Spain) (2005)
Poster communication

V. Gómez, A. Pasamontes, M. P. Callao.
Análisis por inyección secuencial: diseño y optimización de las condiciones experimentales para determinar cromo en agua de curtido de pieles.
1er Workshop de la Xarxa Catalana de Quimiometria - Barcelona (Spain) (2005).
Poster communication.

V. Gómez, J. Font, M.P. Callao.
Sequential injection with second-order treatment for determination of dyes in tanning effluents.
10th International Conference on Flow Analysis – Porto (Portugal) (2006)

ISBN: 978-84-691-0990-8/D.L: T.2293-2007

Oral communication

V. Gómez, M.P. Callao.

Acid dyes removal using activated carbon.

2º Workshop de Jóvenes Investigadores en Quimiometría – Tarragona (Spain) (2006)

Poster communication

V. Gómez, M.P. Callao.

Experimental designs for optimizing and fitting the adsorption of dyes onto activated carbon.

VI Colloquium Chemometricum Mediterraneum - Saint Maximim (France) (2007)

Oral communication

V. Gómez, M. Miró, V. Cerdà, M.P. Callao.

Sequential injection chromatography coupled to multivariate curve resolution with alternating least-squares for the determination of phenolic compounds.

IX International Symposium on Analytical Methodology in the environmental field – Mallorca (Spain) (2007)

Oral communication

M. Fernández, V. Gómez, M. Miró, M.P. Callao, V. Cerdá.

Determinación simultánea de vitaminas hidrosolubles empleando cromatografía de inyección secuencial (SIC) y resolución de curvas multivariante con mínimos cuadrados alternados (MCR-ALS)

XIV Reunión nacional de la sociedad española de química analítica - Mallorca (Spain) (2007)

Poster communication