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EXPLOITING THE USE OF A LIQUID WAVEGUIDE CAPILLARY CELL FOR SPECTROPHOTOMETRIC DETERMINATIONS IN FLOW-BASED SYSTEMS

Ricardo Nuno Mendes de Jorge Páscoa

Thesis submitted to the Universidade Católica Portuguesa to attain the degree of PhD in Biotechnology – Chemistry

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by

Ricardo Nuno Mendes de Jorge Páscoa

Under the supervision of António O.S.S. Rangel, Professor, and co-supervision of Ildikó V. Tóth, PhD

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Abstract

In this thesis, the use of a liquid waveguide capillary cell (LWCC) was exploited for the spectrophotometric determination of several analytes in different types of water. With the purpose of in-line sample handling, different flow approaches were used for the development of simple, robust, cheap and automated analytical procedures.

The first procedure was based on a sequential injection analysis (SIA) system for the determination of iron in coastal waters. With the goal of reaching low levels of iron, a LWCC was coupled to the system. This procedure used a doubled-line SIA system to improve mixing conditions between sample and reagents. The detection was based on a colorimetric reaction and two different reagents (ferrozine and 1,10-phenanthroline) were tested. The absorbance measurements were carried out at the wavelengths of 512 and 562 nm for the detection of iron-1,10-phenathroline and iron-ferrozine complex, respectively. An interference study was performed for both reagents. The developed method was also applied to natural waters (river, well, ground, potable and sea waters) and then compared with the reference procedure. One certified reference water sample was used to test the accuracy of the developed method.

The objective of the second work was to determine iron at lower levels than the previous work and, as a consequence, to measure the levels of iron in ocean waters. With this in mind, a LWCC and a pre-concentration resin were coupled to a multi-syringe flow injection analysis (MSFIA) system. Two different pre-concentration resins (Chelex 100 and NTA Superflow) were tested and evaluated. The determination of iron was also based on a colorimetric reaction and two reagents were tested, ferrozine and ammonium thiocyanate. The reactions were monitored at the wavelengths of 480 and 562 nm for the detection of iron-ammonium thiocyanate and iron-ferrozine complex, respectively. The accuracy was assessed using a certified reference water sample.

A multi-parametric system for the spectrophotometric determination of zinc and copper at low levels in waters was the third work of this thesis. To attain this objective, a LWCC was coupled to a MSFIA system. The developed procedure for both analytes was based on a colorimetric reaction with zincon reagent at different pH values and monitored at 620 nm. Zincon reagent reacts only with copper at pH 5 and with copper and zinc at pH 9. An interference study for both determinations was carried out. The developed work was also applied to natural waters and three certified reference water samples.

Subsequently, a multi-pumping flow system (MPFS) coupled with a LWCC was developed for the determination of titanium. This determination was based on the colorimetric reaction of titanium with chromotropic acid and the absorbance measurements were carried out at 425 nm. An interference study was performed in order to evaluate possible interferences. The developed procedure was applied to natural waters as well as to sunscreen formulations (the results were compared with the reference procedure). The accuracy was assessed with one certified lake sediment.

The development of a spectrophotmetric method for bromate determination in waters at trace levels was the last work of this thesis. With this objective, a LWCC was coupled to a MPFS. The proposed methodology was based on a colorimetric reaction and two different colour reagents were tested, chlorpromazine and trifluoperazine. The lack of repeatability detected in this approach led to the development of a FIA approach in order to find out the reasons of this occurrence.

Resumo

Nesta tese, utilizou-se uma célula de fluxo de percurso óptico longo na determinação espectrofotométrica de vários analitos em diferentes tipos de água. Com o propósito de manusear/transportar as amostras, vários sistemas de fluxo foram utilizados de forma a desenvolver procedimentos analíticos mais simples, robustos, automatizados e de baixo custo.

O primeiro procedimento baseou-se no uso de um sistema de injecção sequencial para a determinação de ferro em águas estuarinas. Com o objectivo de determinar os níveis baixos de ferro presente neste tipo de amostra, acoplou-se ao sistema uma célula de fluxo de percurso óptico longo. Este procedimento utilizou um sistema de injecção sequencial de duplo canal de forma a melhorar a mistura entre amostra e reagentes. A detecção baseou-se numa reacção colorimétrica e dois reagentes foram testados (ferrozina e 1,10-fenantrolina). As medidas espectrofotométricas foram realizadas aos comprimentos de onde de 512 e 562 nm para a detecção dos complexos formados de ferro-1,10-fenantrolina e ferro-ferrozina, respectivamente. Realizou-se um estudo de possíveis interferentes para ambos os reagentes. O método desenvolvido foi igualmente aplicado a diferentes tipos de água (águas de rio, poço, mina, mar e amostras de água potáveis) e os resultados obtidos foram comparados com o procedimento de referência. Usou-se uma amostra de água certificada de forma a comprovar a exactidão do método desenvolvido.

O segundo método desenvolvido teve como principal objectivo determinar ferro em concentrações mais baixas do que no trabalho anterior e assim atingir os valores de ferro presentes em águas do mar. De forma a poder atingir este objectivo, uma célula de percurso óptico longo e uma coluna de pré-concentração foram acopladas a um sistema de fluxo baseado numa multi-seringa. Dois tipos de colunas de pré-concentração, Chelex 100 e NTA Superflow, foram testadas e avaliadas. A determinação de ferro baseou-se numa reacção colorimétrica e foram testados dois reagents (ferrozina e tiocianato de amónio). As medidas espectrofotométricas foram realizadas aos comprimentos de onda de 480 e 562 nm para a detecção dos complexos formados de ferro-tiocianato de amónio e ferro-ferrozina, respectivamente. A exactidão deste procedimento foi avaliada através de uma amostra de água certificada.

O terceiro procedimento realizado nesta tese envolveu um sistema multi-paramétrico para a determinação espectrofotométrica de zinco e cobre em águas a concentrações baixas foi. Com este intuito, acoplou-se uma célula de fluxo de percurso óptico longo a um sistema de fluxo baseado numa multi-seringa. O procedimento

desenvolvido para ambos os analitos baseou-se numa reacção colorimétrica com o reagente zincon a diferentes valores de pH e monitorizada a 620 nm. A pH 5 o reagente zincon reage apenas com o cobre e a pH 9 com ambos. Foi efectuado para ambos os analitos um estudo de possíveis interferentes. O procedimento desenvolvido foi igualmente aplicado a diferentes tipos de amostras de água e a três amostras de águas certificadas.

O quarto método desenvolvido visou determinar titânio a concentrações muito baixas. Neste procedimento utilizou-se um sistema de fluxo baseado em micro-bombas com uma célula de fluxo de percurso óptico longo acoplada. A determinação baseou-se novamente numa reacção colorimétrica entre o titânio e o ácido cromotrópico e a detecção foi realizada a 425 nm. Foi efectuado um estudo de interferentes com o intuito de avaliar possíveis interferências. O procedimento desenvolvido foi aplicado a diferentes tipos de água e a amostras de cremes solares (os resultados obtidos para os cremes solares foram comparados com o procedimento de referência). A exactidão deste método foi avaliada através de uma amostra certificada de um sedimento de lago

O desenvolvimento de um método espectrofotométrico para a determinação de bromato em águas a concentrações baixas foi o último trabalho desta tese. Com este objectivo utilizou-se uma célula de fluxo de percurso óptico longo acoplada a um sistema de fluxo baseado em multi-bombas. A metodologia proposta baseou-se igualmente numa reacção colorimétrica e foram testados dois reagentes (cloropromazina e trifluoperazina). A falta de repetibilidade detectada neste sistema levou ao desenvolvimento de um sistema de análise por injecção em fluxo com o objectivo de determinar as causas deste acontecimento.

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Abbreviations

AAS – atomic absorption spectrometry

BTB – bromothymol blue

CR – chelating resin

CRM – certified reference material

EPA – Environmental Protection Agency

EU – European Union

FEP – fluorinatedethylene copolymer

FIA – flow injection analysis

HC – holding coil

HPLC – high performance liquid chromatography

IC – ion chromatography

ICP-AES – inductively coupled plasma atomic emission spectrometry

ICP-MS – inductively coupled plasma mass spectrometry

IUPAC – International Union of Pure and Applied Chemistry

IV – injection valve

LC_{50} – lethal concentration

LOD – limit of detection

LOQ – limit of quantification

LWCC – liquid waveguide capillary cell

MCFIA – multi-commutation flow injection analysis

MPFS – multi-pumping flow systems

MSFIA – multi-syringe flow injection analysis

PDD – 2,2-bis(trifluoro-methyl)-4,5-difluoro-1,3-dioxole

PTFE – polytetrafluoroethylene

PVC – polyvinyl chloride

rFIA – reversed flow injection analysis

RC – reaction coil

RD – relative deviation

RI – refractive index

RSD – relative standard deviation

SFA – segmented flow analysis

SIC – sequential injection chromatography

SIA – sequential injection analysis

SI-LOV – sequential injection lab-on-valve

SL – sample loop

SV – selection valve

TIR – total internal reflection

TFE – tetrafluoroethylene

UTEVA – uranium and tetravalents actinides

UV – ultra-violet

W – waste

WFD – Water Framework Directive

WHO – World Health Organisation

Table of contents

List of figures

List of tables

Chapter 1

General introduction

In this chapter, a brief summary of some aspects of water monitoring is given. The description of flow analysis systems including the principal characteristics of each system is also presented. The history of the liquid waveguide capillary cells as well as its characteristics and working modes are provided in this section. Moreover a description of several applications of this equipment using different detection modes is given.

1.1. Objectives

The main objective of this work was to explore the application of a LWCC in flow-based spectrophotometric determinations. The idea was to avoid off-line or in-line complex physical or chemical treatments (solid-phase or liquid-liquid extraction) to significantly improve the sensitivity (and lower the detection limit) of molecular absorption spectrophotometric methods for environmental analysis.

The exploitation of different flow modes coupled with the LWCC was another important objective, and their application to the determination of different analytes at trace levels in different types of waters. The use of different flow systems approaches allowed a real overview of all the advantages and drawbacks of each used technique.

Another important objective was the reduction of the reagents consumption and effluent production.

The multi-parametric analysis using the same manifold and the direct introduction of sample were other important purposes of the developed methodologies.

The following chapters present new analytical procedures developed throughout this thesis aiming to assemble all the abovementioned objectives.

1.2. Water monitoring

Water is a vital resource to humankind and, with the estimated increase of world population, more important it will become in the future. Throughout the last century and unfortunately in this century, drinking water resources have been decreasing due to the increase of the pollution related to human activity. With the aim of monitoring and

control the exposure to dangerous chemical substances, water analysis is essential (Anastas 1999).

Since many diseases are related to the exposure of some hazardous substances, the developed countries have increased the water control through tighter regulations. This causes a great increase in the healthcare costs of a country and moreover a healthy population should be a moral imperative to every country government.

With the implementation of tighter regulations, the impact of pollution in European surface waters caused by industrial discharges of toxic substances has decreased around 70% over the past 30 years (Greenwood et al. 2007).

In order to improve, protect and prevent further deterioration of water quality across Europe, the European Union (EU) has created the Water Framework Directive (WFD) (Madrid and Zayas 2007). This framework involved different water elements such as:

- Physicochemical properties (temperature, density, colour, turbidity, pH value, redox potential, conductivity, surface tension, suspended solids and total/dissolved organic carbon)
- Hydromorphological status (erosion and bench river characteristics)
- Biological (distribution and composition of the species and biological effects)
- Chemical monitoring (with particular emphasis on the contaminants in the list of priority pollutants)

Water monitoring depends on the development of analytical methodologies using modern techniques. As a result, the characterisation and understanding of some dangerous substances and their metabolites became possible.

Water monitoring can be classified in three main categories of analysis (Greenwood et al. 2007):

- In-situ analysis (it normally involves the use of a sensing device directly in contact with the water body)
- On-line analysis (in this case the sample is transported through an automatic mechanism to the analytical instrumentation near the water body, the sample can be pretreated)
- Off-line analysis (the water is collected, stored and then analysed)

These categories can be illustrated in Fig 1.1.

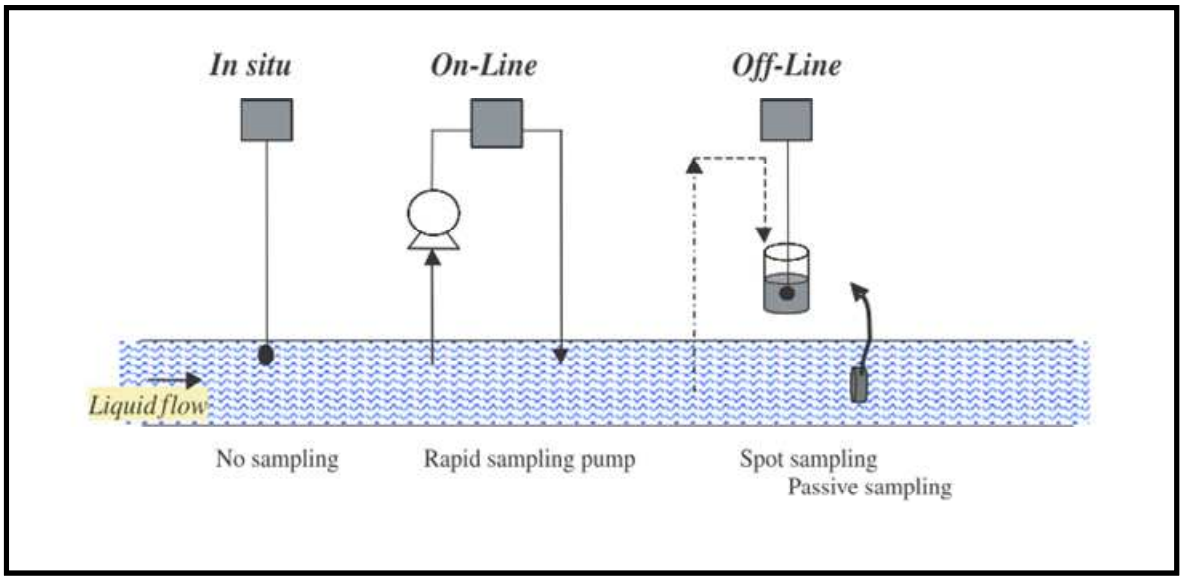

Figure 1.1. Representation of the three main categories of water sampling/monitoring (Greenwood et al. 2007).

Most of the developed analytical methodologies involve hazardous chemical substances. The new analytical procedures to be developed should be focused in reducing the use of these substances ("Green Chemistry" procedures) with the goal of not contributing to further environmental problems (Anastas 1999).

The new concept named “Green Chemistry” can be defined as the design of chemical products and chemical processes that reduce/eliminate the use and generation of hazardous substances (Anastas 1999). Nowadays, “Green Chemistry” methodologies are, more than ever, a moral obligation when a new method is proposed. These methodologies should also take in consideration the other principal characteristics of an analytical procedure such as sensitivity, accuracy, efficiency and cost (Anastas 1999).

Aiming to accomplish reliable analysis, a good sampling and handling mode is essential. An erroneous analysis could cause the identification of unreal hazards or a miss trace of some dangerous substances (Madrid and Zayas 2007).

Flow analysis techniques emerge as an attractive replacement tool for conventional analysis procedures. By adopting these techniques, a large number of analysis errors are reduced since human sampling handling is minimised.

1.3. Flow analysis

With an increasing demand for analysis, flow techniques have been extensively used and improved for this purpose. Flow analysis techniques, among other important advantages that will be further discussed in the subsequent sections, allow reduced human sample handling as both samples and reagents are transported through a tubes system. Moreover, a reduction in sample/reagents consumptions is achieved as well as low effluent production.

These systems permit also many different applications due to their versatility.

In the following sections, a brief discussion of the main advantages/disadvantages of several flow analysis techniques will be given, with particular focus to the flow techniques used throughout this thesis.

1.3.1. Segmented flow analysis

Segmented flow analysis (SFA) can be considered the first flow analysis technique and was described by Skeggs et al. (1957). SFA (Fig. 1.2) is characterised by a segmented flow caused by the introduction of air bubbles in the flow stream. This generates different segments of sample and reagent separated by air bubbles with low sample dispersion in a turbulent flow. The analytical signal is registered after physical and chemical equilibrium. This technique requires an air debubbler before the detector in order to remove all the air bubbles.

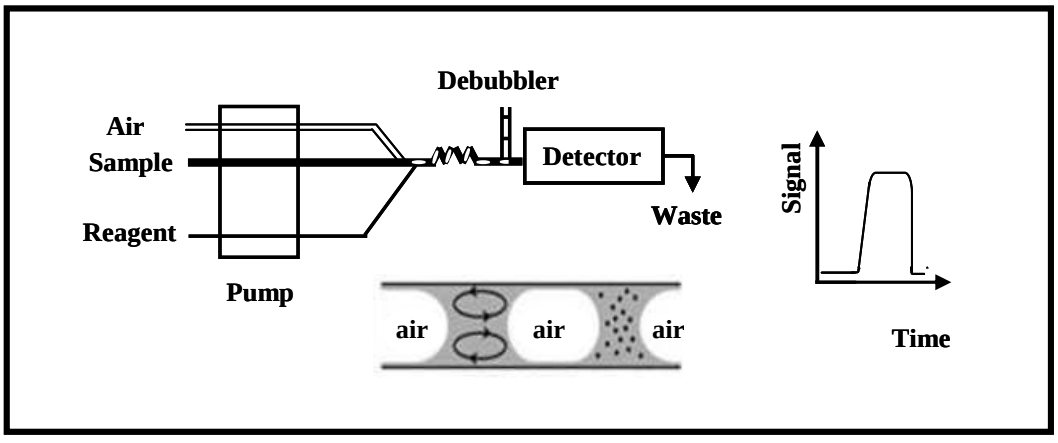

Figure 1.2. Schematic representation of a typical SFA manifold.

1.3.2 Flow injection analysis

In 1975, flow injection analysis (FIA) was introduced by Ruzicka and Hansen as a new flow analysis technique. This new technique produces a laminar flow since the flow stream is not segmented by air bubbles and the sample is injected in the flow stream by an injection valve (described in Chapter 2). The FIA system (Fig. 1.3) is also characterised by the reproducible sample injection, controlled dispersion of the sample zone and reproducible timing of the analyte zone from the injection to the detection point. Therefore, the concept of physical and chemical equilibrium was discarded and the analytical signal obtained is transient. Moreover, it has allowed the implementation of gas diffusion and dialysis units as well as flow cells with increased pathlengths aiming to the reducing of matrix interference or/and to enhancing the sensitivity (Ruzicka 1992).

Considering all these features, FIA technique presents a higher sampling rate and lower reagent consumption as principal advantages over SFA.

The more important drawbacks attributed to this technique are the continuous consumption of carrier and reagents throughout the analytical cycle and the difficulty to perform multi-parameter analysis.

Nevertheless, this flow analysis technique is the most used over the scientific community since 17,200 publications were obtained by the search engine ISI Web of Knowledge using “flow injection analysis” as topic (12/04/2011).

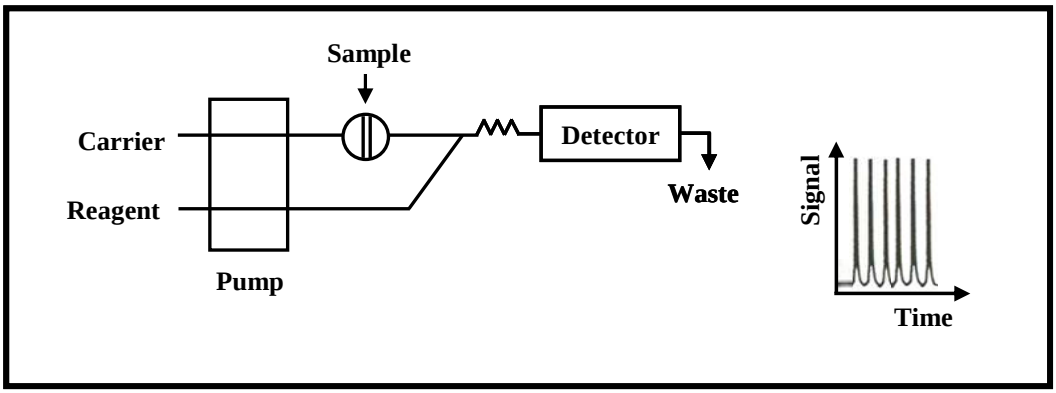

Figure 1.3. Schematic representation of a typical FIA manifold.

1.3.3. Sequential injection analysis

Some years later, Ruzicka and Marshall (1990) presented the sequential injection analysis. With SIA (Fig. 1.4), the more important drawbacks of FIA were avoided and some of the above described advantages were maintained (reproducible sample injection, controlled dispersion, reproducible timing and physical/chemical equilibrium is not necessary). This approach is characterised by the sequential aspiration of well defined sample and reagent plugs into the holding coil by means of a selection valve. Then, by reversing the flow this mixture is transported to the detector. Another important feature of SIA systems is the use of a computer, which controls the selection valve and pump, in order to manage the introduction of precise amounts of sample/reagents, the flow stream direction and precise timing procedures.

Under the above mentioned conditions, the reduction in the reagents consumption and waste generation as well as the possibility of performing multi-parameter analysis (more versatile) are the principal improvements achieved by this

technique in comparison to FIA. The possibility of using procedures with slow reactions or even the stopped flow technique are other important features.

Another important characteristic of SIA is that the mixture between sample and reagent plugs occurs by reversing the flow to the detector, resulting in sinusoidal flow. With reversing the flow, only a partial overlap of sample/reagents plugs is achieved.

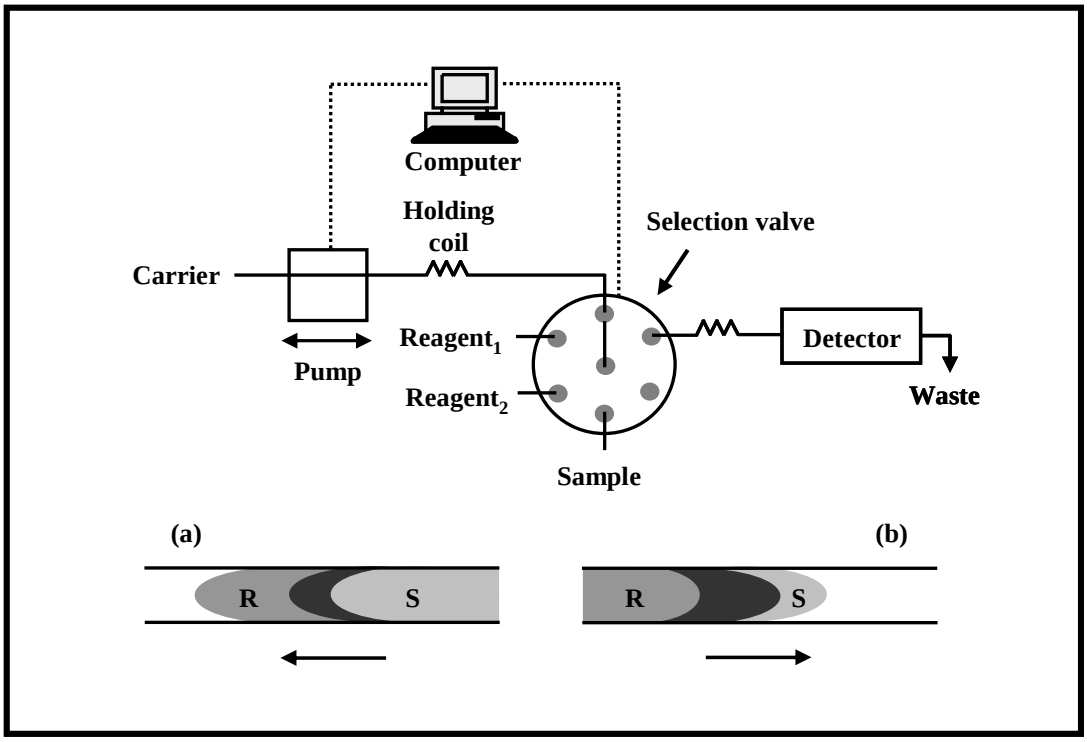

Figure 1.4. Schematic representation of typical SIA manifold with the flow stream in the aspiration phase of reagent/sample plugs to the holding coil (a) and the propelling of the same plugs to the detector (b) after flow reversal. The darker zones represent the reagent (R) and sample (S) overlapping zone.

This partial overlap of reagent and sample plugs may be considered an important drawback of SIA when compared to FIA, leading to poorer mixing conditions. This can cause lower sensitivities in SIA manifolds when compared to FIA manifolds for the same procedures, and produce erratic signals due to the schlieren effect (Dias et al. 2006); this effect will be detailed in section 1.5.7.

This technique comprises 1,618 publications, obtained by the search engine ISI Web of Knowledge using “sequential injection analysis” as topic (12/04/2011), revealing a good acceptance over the scientific community.

1.3.4. Multi-commuted flow injection analysis

The multi-commuted flow injection analysis (MCFIA) was introduced by Reis et al. (1994). However, it was Malcolme-Lawaes et al. (1987) that firstly applied the MCFIA approach but presented it as a FIA procedure. MCFIA is based on the use of three-way solenoid valves (Fig. 1.5) associated with the binary sampling mode (Reis et al. 1994).

These solenoid valves work simultaneously or sequentially in a fast switching mode usually denominated “on” or “off” according to the path used by the solution. These modes are selected through the activation of the solenoid device and the paths used by the solutions are based on the position of two polytetrafluoroethylene (PTFE) membranes located in the central part of this valve. By activation of the solenoid device (“on” mode), one of the PTFE membranes is pressed and the solution passes through one path. When the solenoid valve is not activated (“off” mode) the PTFE membrane returns to the original position and the solution passes through another path.

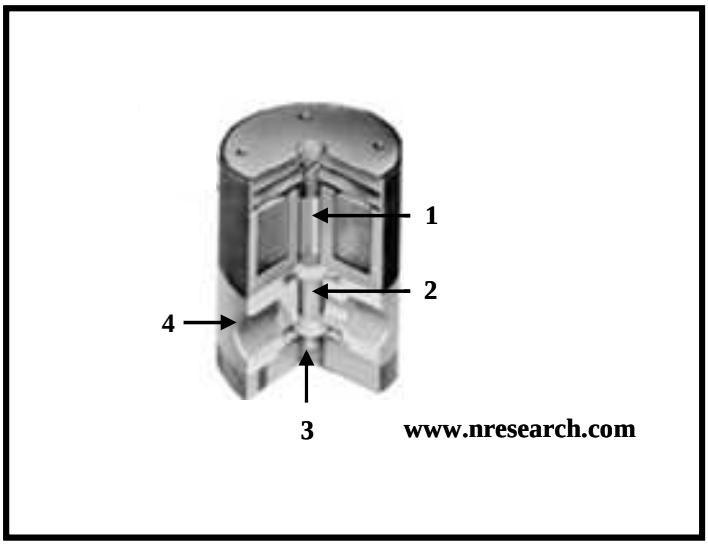

Figure 1.5. Representation of the interior of a three-way solenoid valve: 1 -nucleous; 2 - PTFE membranes; 3 -helical spring; 4 -tubing connection.

These devices may introduce the sample and reagent independently (one valve each) or both reagent and sample with the same valve. This last option provides the sequential introduction of small plugs of sample and reagent resulting in a fast mixing between both plugs; this approach was named binary sampling.

The initial MCFIA systems worked with a single-channel propulsion pump placed after the detection system (Silva et al. 2007). However, the formation of bubbles due to the aspiration of the solutions trough these devices (Fig. 1.6a) were sometimes observed. In order to avoid this problem a multi-channel propulsion pump should be placed before the detector (Fig. 1.6b) (Oliveira et al. 2007).

Using the propulsion pump before the detector (dispense mode), this technique becomes very similar to the FIA technique since sample and reagents are introduced in the flow stream and mixed in a confluence. However, it presents a reduced consumption of sample/reagents and waste generation due to the possibility of controlling the introduced volumes by activation/deactivation of the solenoid valves.

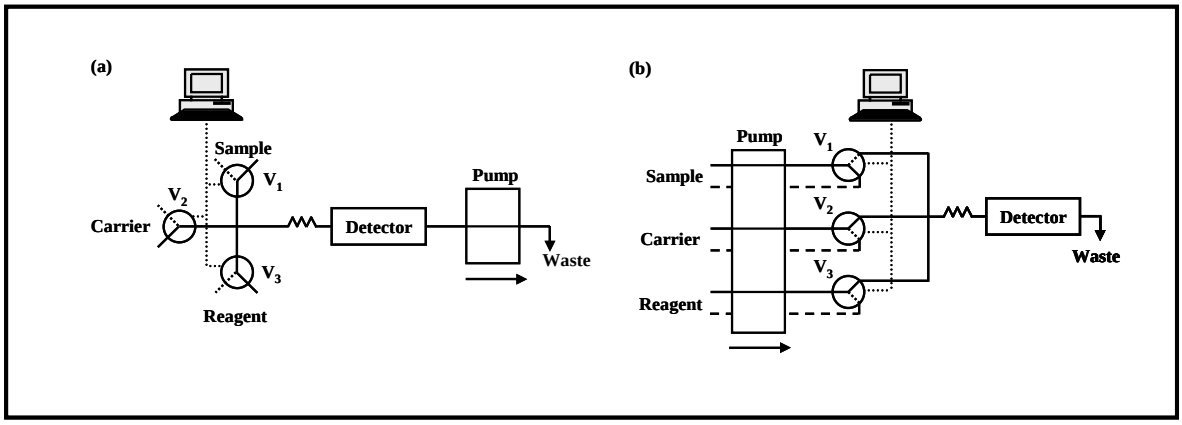

Figure 1.6. Schematic representations of two MCFIA manifolds using the aspiration mode (a) and the dispense mode (b).

Another difference between this technique and FIA is the sample injection mode. In MCFIA, the sample injection is mainly time-based (controlled by the respective solenoid valve) while in FIA is volume-based (determined by the sample loop size).

The major drawbacks of this technique are the difficulty to perform multi-parameter analysis using the same manifold and the over-heating of the solenoid valves caused by long periods of activation. This last negative aspect can lead to the deformation of the internal PTFE membranes.

1.3.5. Multi-syringe flow injection analysis

In 1999, Cerdà et al. presented the multi-syringe flow injection analysis based on the multi-commutation approach although using a different propulsion unit, the multi-syringe. This device propels all the solutions through the flow network and consists in

up to four syringes with a three-way commutation valve connected at the top of each syringe.

All the syringes are connected to a single motor and controlled through a serial port by computer software. These syringes are made of glass and are very resistant to strong acids and bases and also organic solvents. This characteristic presents an important advantage in comparison to the propulsion tubes used in the peristaltic pumps since they are not very resistant against some solutions and require a periodic replacement (Miró et al. 2002).

Another important benefit of this technique comes from the combination of syringes with different capacities (0.5-25 mL) along with the high efficiency of the burette motor which enables the exploitation of several flow rates and volumes with a great precision.

As a result, MSFIA gathers some advantages of FIA systems such as robustness and versatility along with the reduced consumption of sample/reagent of SIA systems and also the high speed of the commutation valves of MCFIA (Cerdà 2003).

Up to four extra commutation valves may be connected to the multi-syringe model.

MSFIA also presents some limitations since all four syringes move simultaneously and the flow rates/volumes selected are related to one syringe causing the reduction of the options range of the flow rates/volumes of the others syringes (Miró et al. 2002).

Moreover, it is not advisable to use a syringe as sample reservoir because it would require cumbersome washing steps to avoid contamination between consecutive solutions, increasing the analytical cycle time. The essential refill of the syringes is

another important feature that causes lower sampling rates of MSFIA when compared to FIA or MCFIA systems.

Fig. 1.7 represents an usual MSFIA manifold.

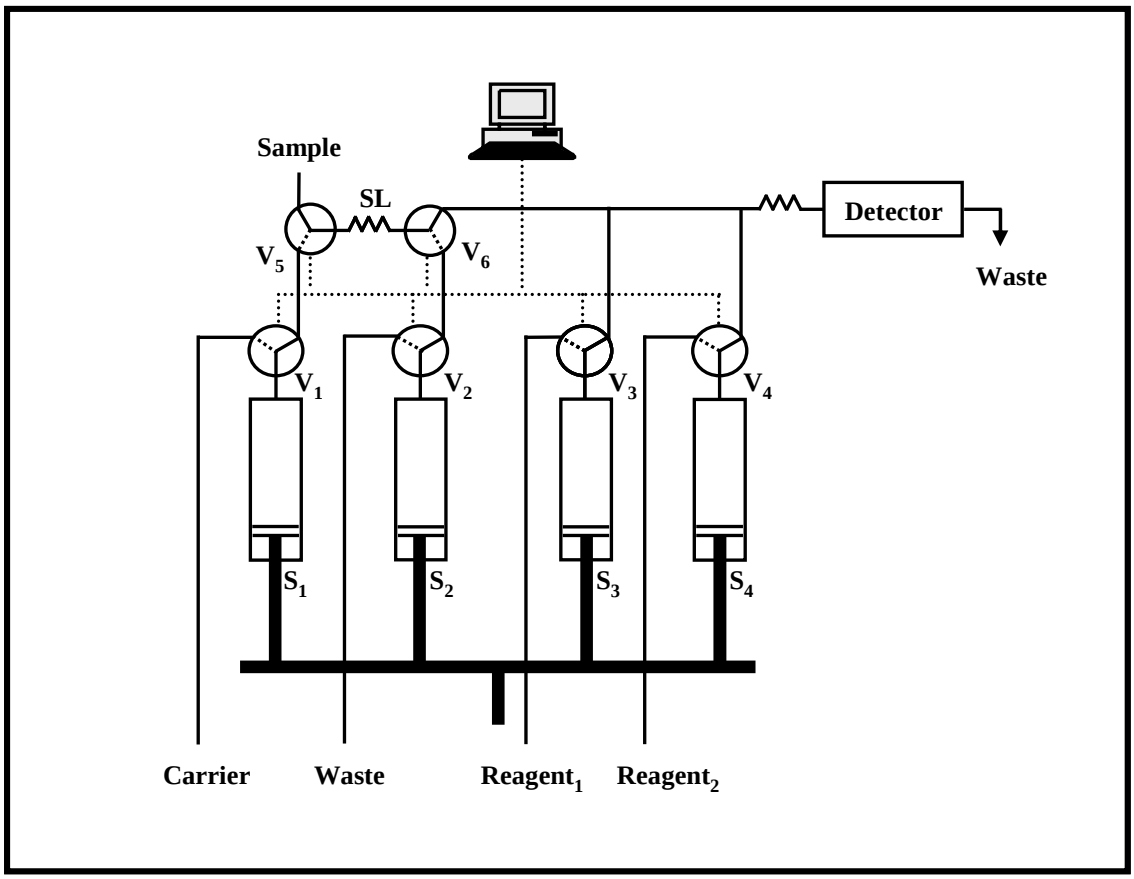

Figure 1.7. Schematic representation of a MSFIA manifold.

1.3.6. Multi-pumping flow systems

More recently, the multi-pumping flow system (MPFS) (Fig. 1.8a) was described by Lapa et al. (2002). The main characteristic of this technique is the use of solenoid micro-pumps (Fig. 1.8b) as both liquid propulsion units and commutation valves.

These micro-pumps require a low power source and work with a similar mechanism of the aforementioned commutation valves. Their activation through a voltage pulse will generate a negative pressure enabling the aspiration of the solution. When the voltage pulse ends, the solution located inside the micro-pump is propelled to the flow network.

The flow rate generated by these devices depends on two factors, the frequency of stroke and the volume propelled in each stroke.

By their activation, a pulsed flow is achieved thus improving the mixing and reaction zone homogenisation even in limited dispersion conditions (Lapa et al. 2002) and also the reduction of schlieren effects (Dias et al. 2006). This technique also enables binary sampling and merging zones approaches and the introduction of sample can be based on time or volume.

The small size and the low power supply voltage required to work in comparison to the peristaltic pumps are other important advantages of this flow technique.

The more important drawbacks of MPFS are the lack of robustness and also the low stability of the stroke volumes attained in each micro-pump.

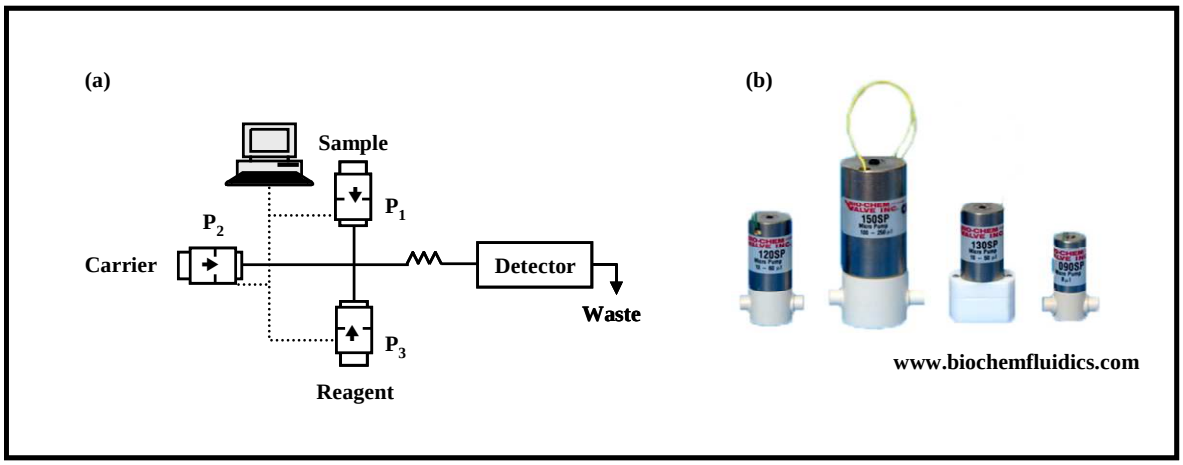

Figure 1.8. Schematic representation of a MPFS manifold (a) and photograph of different micro-pumps (b).

1.3.7. Sequential injection lab-on-valve

In 2000, Ruzicka (2000) introduced the sequential injection lab-on-valve (SI-LOV). Although this approach has been presented to the scientific community before the MPFS, it is only described at the end of this thesis introduction since it assembles different characteristics of the aforementioned flow approaches. It is characterised by gathering the mixing coils, the selection valve and the detection system in a portable miniaturised automatic unit (Fig. 1.9a). The incorporation of the detection system in the selection valve (Fig. 1.9b) is also an important feature of this approach.

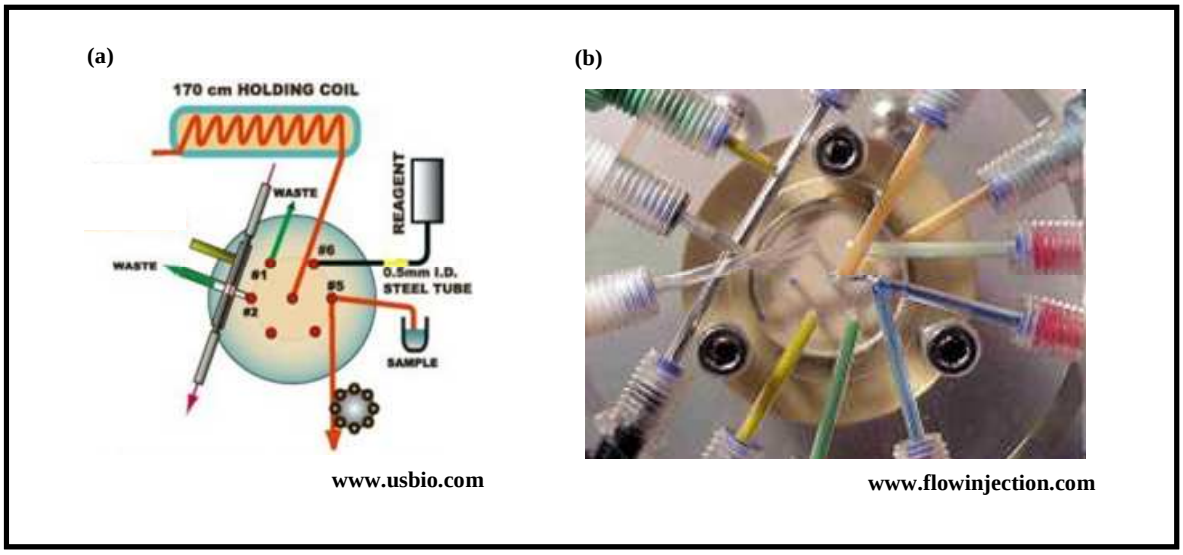

Figure 1.9. Schematic representation of a SI-LOV manifold (a) and photograph of a selection valve of a SI-LOV manifold (b).

The down scale of this approach allowed the substantial reduction of reagents consumption and effluent generation in a versatile feasible way. With this, the SI-LOV

approach is perfectly adequate to determinations involving enzymes and in immunoassays.

The major drawback of this approach is the cost of the commercially available equipment.

1.4. Strategies to increase the sensitivity in spectrophotometric methods

As it was mentioned in the Objectives section, in this work a LWCC was applied to significantly increase the sensitivity of the molecular absorption spectrophotometric methods.

It is well known that molecular absorption spectrophotometry is one of the most used detection analytical techniques in many different fields because it is simple, robust, accurate, versatile and fundamentally, a low cost technique (Rocha and Teixeira 2004).

Spectrophotometry is also the most applied technique as flow analysis detection system. However, the limits of detection achieved with spectrophotometric methods do not reach such low levels necessary for some analytical applications. In this scenario, different strategies can be used in spectrophotometric flow analysis to improve the sensitivity and the detection limit. Increasing the optical pathlength and decreasing the sample dispersion in the flow cell at the same time, is an important approach to improve the sensitivity in spectrophotmetric methods (Ellis et al. 2009). Besides this approach, several others have been reported such as chemical derivatization, preconcentration/separation of the analyte and solid-liquid extraction or liquid-liquid extraction (Infante and Rocha 2008; Rocha and Teixeira 2004). The preconcentration is

the most used approach although it normally employs toxic effluents and involves low sampling rates (Infante and Rocha 2008). The chemical derivatization often requires toxic reagents (Rocha et al. 2009).

Considering the described strategies to increase the sensitivity, the increase of the optical pathlength of the flow cell is the most appealing. This strategy avoids laborious pretreatment steps and may be considered a chemical greener alternative since it involves lower reagent (most of the reagents are toxic to the environment) consumption. Nowadays, “Green Chemistry” is in focus and the minimization of the use of toxic substances and waste generation as well as the energy consumption is a very important feature of any developed method. The toxic reagents should be replaced for less toxic reagents or even discarded since the waste treatment is difficult, time consuming and very expensive. Nevertheless, the greener methods developed should maintain the principal analytical characteristics such as, sensitivity, accuracy and precision (Rocha et al. 2009).

Therefore, the developed methods using flow cells with long optical path such as the liquid waveguide capillary cells (LWCCs), may attain the analytical characteristics above referred. Nanomolar determinations become easy to reach in a very simple, low cost and versatile way (Gimbert and Worsfold 2007). A detailed description of the LWCCs is given in the subsequent section.

1.5. Liquid waveguide capillary cell

1.5.1. Characteristics of LWCCs

The first LWCC was used in the 70's with Raman spectroscopy and the capillaries used were made of borosilicate or fused silica with a refractive index (RI) of 1.51 and 1.45, respectively. Therefore, it was only suitable for samples with RI higher than the material employed in these LWCCs such as organic solvents (Gimbert and Worsfold 2007). Fig. 1.10 represents typical experimental setup using a LWCC.

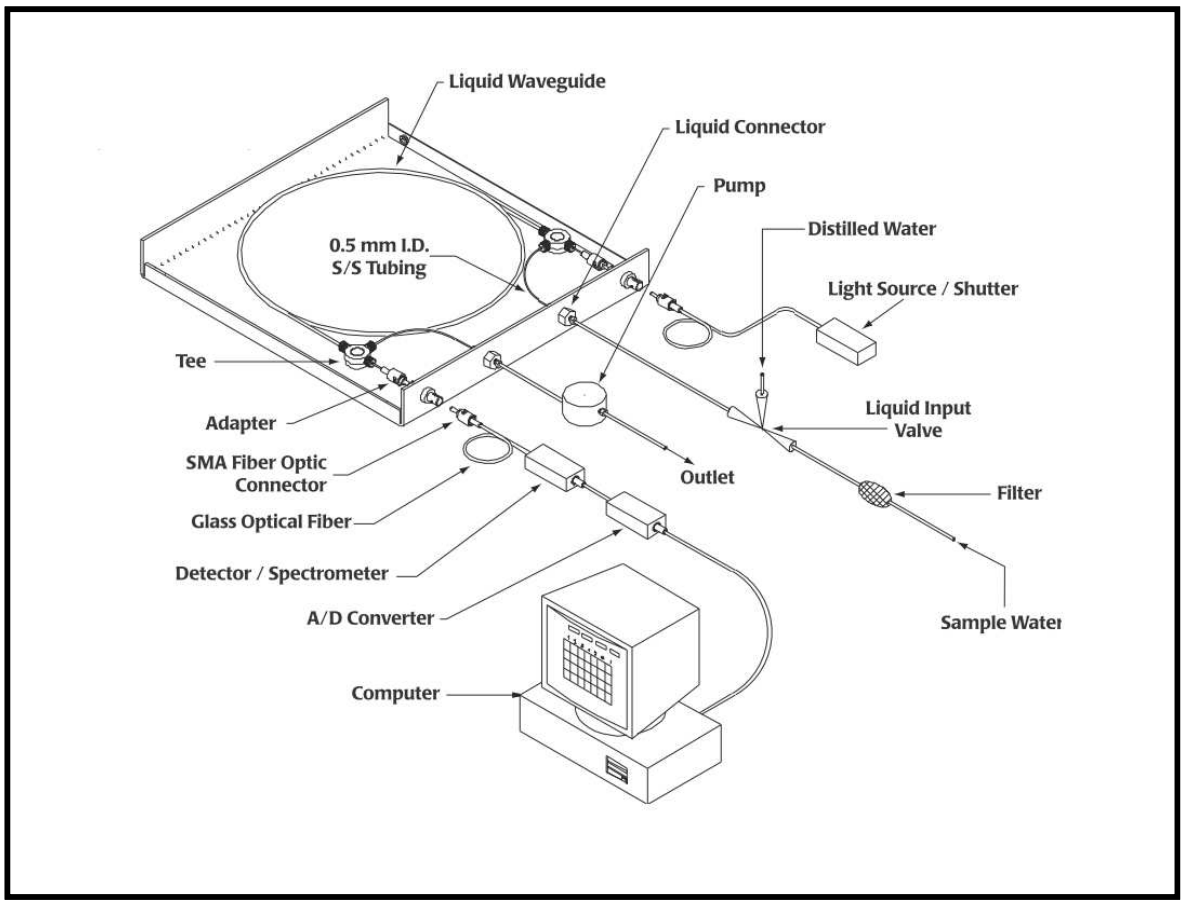

Figure 1.10. Typical experimental setup using a LWCC (World Precision Instruments 2011).

Consequently, these types of LWCCs were not very suitable for spectrophotometric measures because great part of analytical procedures is performed in diluted aqueous solutions (Rocha and Teixeira 2004).

For this reason, there was a great demand to develop a material with a RI lower than water. The solution appeared with a new polymer called Teflon AF.

Teflon AF is an amorphous fluoropolymer of tetrafluoroethylene (TFE) and 2,2-bis(trifluoro-methyl)-4,5-difluoro-1,3-dioxole (PDD) which presents a refractive index (RI) (1.29-1.31) lower than that of the water (1.33) (Belz et al. 1999; Dress and Franke 1997; Resnick and Buck 1993). This new polymer is patented by DuPont and is commercially available since late 1980 (Gimbert and Worsfold 2007) in two grades: Teflon AF 1600 and Teflon AF 2400. The most important distinction between these grades relies in the RI of 1.29 and 1.31 for Teflon AF 2400 and AF 1600, respectively (Dress and Franke 1996; Dress and Franke 1997; Takiguchi et al. 2006). With these RIs, the construction of LWCCs using water as the optical core was enabled (Christiansen et al. 2010; Dress et al. 1998), allowing light guidance inside the Teflon capillary and broadening a field of applications. The Teflon AF presents also a porous structure (Dasgupta et al. 1998; Li and Dasgupta 1999(b)) and has high transparency permitting working in a wide range of wavelengths (200-2000 nm) (Dasgupta et al. 1998; Li and Dasgupta 1999(b); Okada 2007).

In spite of all this positive features, Teflon AF is quite difficult to functionalize chemically (Christiansen et al. 2010) and is only soluble in an highly fluorinated solvent called “Fluorinert” (Dress and Franke 1996; Dress and Franke 1997). This solvent allows the adhesion of Teflon AF with glass (Dress and Franke 1996) which constitutes an interesting possibility of LWCC configurations and will be further explained.

In 1998, Dress et al. (1998) demonstrated that 5 µm layer thickness of Teflon AF 2400 was enough to confine the optical intensity to the liquid core avoiding any influence of the outside environment and any absorption or scattering by the capillary material. A comparison between Teflon AF 2400 and other tested capillary materials confirmed that there is no light absorption in Teflon AF 2400 in opposition with the other materials (Dress and Franke 1996; Dress et al. 1998). Despite this, Sugiya et al. (2009) stated that Teflon AF 2400 may be damaged by weak mechanical contact, reducing the light guiding efficiency inside the capillary.

Throughout this thesis, a liquid waveguide capillary cell (LWCC) made of Teflon AF 2400 was used, therefore this material will be more in focus than Teflon AF 1600.

The LWCCs can be classified in two types, type I (Fig. 1.11a) and type II (Fig. 1.11b). The type I consists of solid Teflon AF tubing with low refractive index (Byrne and Kaltenbacher 2001; World Precision Instruments 2011), as referred above. The light travels down the capillary and is totally internally reflected at the Teflon AF interface with the condition that the incident angle exceeds the critical angel; the light passes from an optically denser medium that is water to a less dense medium that is Teflon AF (Zhang and Chi 2002). The type II cell consists of a fused silica capillary tubing (RI=1.46) with an outer coating of Teflon AF with low refractive index (Byrne and Kaltenbacher 2001; Gimbert and Worsfold 2007; Infante and Rocha 2008; World Precision Instruments 2011). The light travels down the capillary and is totally internally reflected at the interface between the outer fused silica capillary surface and the Teflon AF coating with the same condition referred above; the incident angle has to exceed the critical angel (Takiguchi et al. 2006; Zhang and Chi 2002).

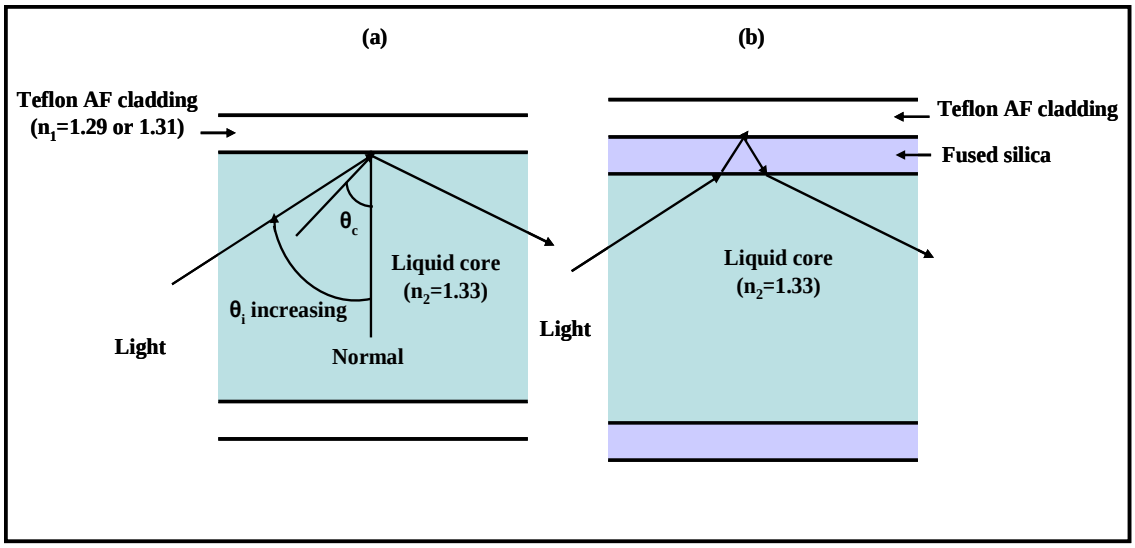

Figure 1.11. Scheme of the light path in the LWCC type I (a) and type II (b). This scheme is accurate for angles larger than the critical angle for total internal reflection.

Teflon AF with its porous structure is permeable to gases and the adsorption of some reactive species in aqueous samples can occur in its surface (Li and Dasgupta 2001; World Precision Instruments 2011; Zhang 2006). The porous structure can be a positive feature in the determination of gaseous analytes (Li and Dasgupta 2001) although some air bubbles can adhere to it and cause signal distortion/baseline shifts (World Precision Instruments 2011; Zhang 2006) and some samples might require pH buffering due to CO_2 penetration through Teflon AF (World Precision Instruments 2011). These effects concerns to type I cell and can be eliminated in type II cell.

The use of a thin layer of fused silica in type II cell protects the Teflon AF from the undesirable effects abovementioned since fused silica is impermeable to gases and avoids the direct contact among the sample and Teflon AF (Belz et al. 1999; World Precision Instruments). Therefore, type II cell has low adsorption of analytes in cell surface, less air bubbles retention, (Belz et al. 1999; Gimbert and Worsfold 2007; Takiguchi et al. 2006) is less vulnerable to light scattering and schlieren effects, (Floge

et al. 2009) easier to clean (Zhang 2006) and consequently has an improved signal stability (World Precision Instruments 2011).

The effective optical pathlength of type I cells is statistically indistinguishable from the physical pathlength. In type II cells, the theoretical basis to calculate the effective pathlength has not been established until now and it is experimentally determined. It was confirmed to be slightly shorter than the physical pathlength (0.94 ± 0.01 times of its physical pathlength) because of the fused silica layer and also dependent on the wall thickness and inner diameter of the LWCC (World Precision Instruments 2011).

1.5.2. Total internal reflection phenomenon

In order to occur total internal reflection, the ray of light must pass from a material with a higher RI to one of a lower RI and it must hit the medium interface at an angle greater than the critical angle (θ_c) (Fig. 1.12). The critical and incident angles are measured according to the normal of the interface (perpendicular to the surface of the capillary), and the critical angle is specific to the Teflon AF grade used (9.9º and 14.1º for Teflon AF 1600 and 2400, respectively). These values can be calculated from the following equation:

$$\theta_c = \cos^{-1}\left(\frac{n_1}{n_2}\right)$$

where n_1 is the RI of the cladding material and n_2 is the RI of fluid core (Dallas and Dasgupta 2004; Ellis et al. 2009; Gimbert and Worsfold 2007).

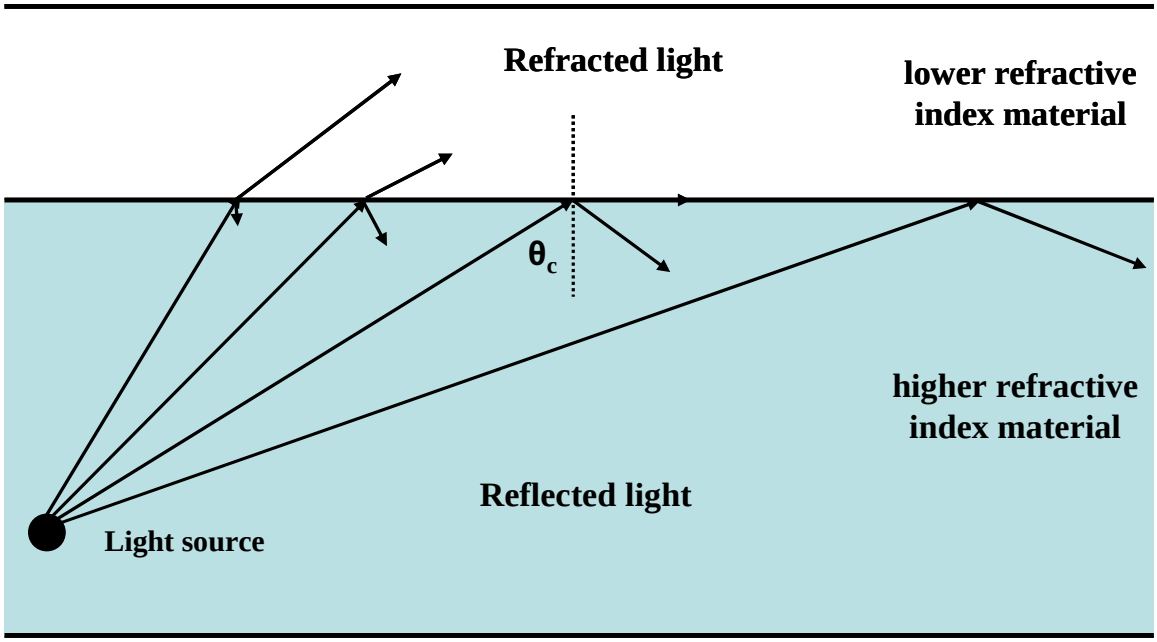

Figure 1.12. Scheme of total internal reflection. If the incidence angle is less than the critical angle, the major part is refracted and a little part is reflected. If the incidence angle is higher than the critical angle, all the light is completely reflected.

This means that the sample solutions act as the core of a fluid-filled light waveguide and the light is confined and propagated within the liquid core (D'Sa et al. 1999). Using liquids as optical core opens a wide field for different application (Dress and Franke 1997).

1.5.3. Working modes of the LWCCs

LWCCs are able to be connected through optical fibers to a light source and a detector, and the illumination in LWCC may be performed in two modes (Fig 1.13).

In the axial mode (Fig 1.13a), light is transported in and out of LWCC by optical fibers. This mode is commonly used for absorbance measurements (Gimbert and Worsfold 2007). In transverse mode (Fig. 1.13b), the LWCC is illuminated through the sidewall and at the end of LWCC the light is transported to the detector by optical

fibers. This mode is generally used for fluorescence measurements (Dallas and Dasgupta 2004).

The optical fibers will transport the light to the LWCC in the axial mode and from the LWCC in either axial or transverse mode.

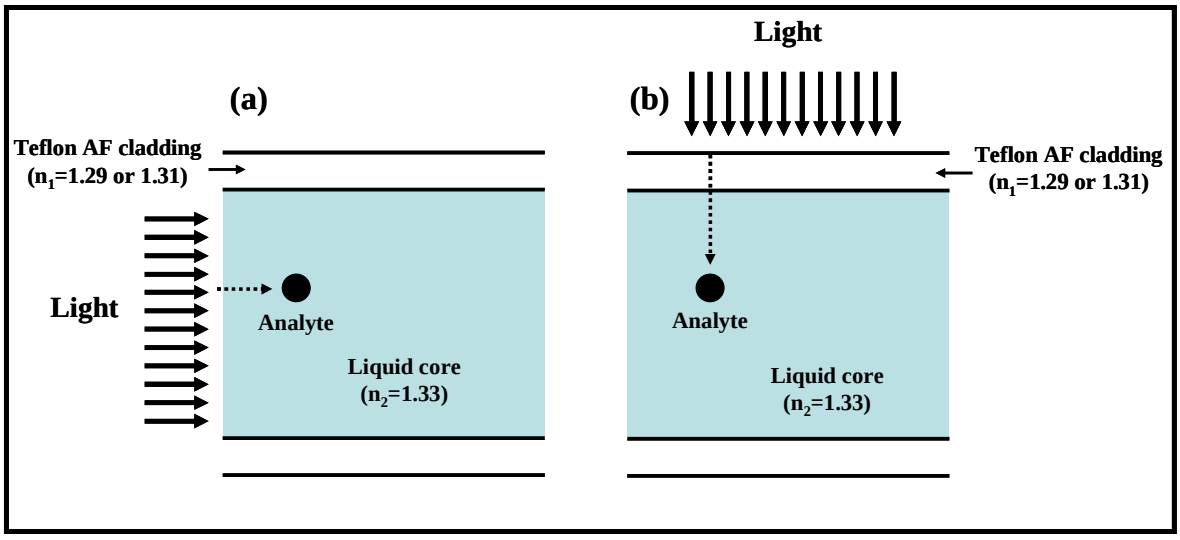

Figure 1.13. Scheme of the illumination modes in LWCC of type I; Axial illumination (a) and transverse illumination (b).

LWCC employing Teflon AF material was introduced by World Precision Instruments (WPI; Sarasota, FL, USA) in 1997 (Belz et al. 1999; Floge et al. 2009) and is commercially available in different pathlengths from 5 to 500 cm with respective inner volumes of 5 up to 1250 µL. These instruments were invented, patented and optimised by WPI during several years (World Precision Instruments 2011).

Several detection techniques such as spectrophotometry, chemiluminescence, fluorescence and Raman spectroscopy are attainable with the LWCCs (Dallas and Dasgupta 2004). Turbidimetric measures might also be exploited in the LWCCs however these systems should guarantee no accumulation of solids inside the equipment (Infante and Rocha 2008).

With these characteristics, LWCCs can be employed in the flow systems, used with manual sample injection, as gas sensors (type I), for in situ analysis (Gimbert and Worsfold 2007) and with most liquids with the exception of perfluorinated solvents (RI lower than 1.29) (World Precision Instruments 2011).

In spite of the above referred important advantages of LWCCs it is also true that some important drawbacks are related to them. The amplification of the blank signal is one of the major drawbacks and causes some limitations in the reagents/reactions assortments as well as in the sensitivity and linear range of the methods employed (Infante and Rocha 2008). This problem can be overcome by using immobilised reagents (Rocha et al. 2009). The air bubbles, the effect of solids in suspension and RI differences in the sample zone (schlieren effects) may also origin incorrect signals (Infante and Rocha 2008).

It should be emphasized that by increasing the pathlength of the LWCC, a lower percentage of light transmittance (World Precision Instruments 2011) will be obtained as well as a greater possibility of bubble formation. Therefore, a 100 cm LWCC is easier to work with than a 200 or 500 cm LWCC (Gimbert and Worsfold 2007).

1.5.4. Applications of the LWCCs

It can be stated that the large increase and implementation of the LWCCs in analytical chemistry is due to the development of Teflon AF polymer. In the following section, a brief description of some applications of the LWCC using different detection modes will be given.

1.5.4.1. Molecular absorption spectrophotometric detection mode

Table 1.1. Different applications of liquid waveguide capillary cells with spectrophotometric detection (continuing).

Analyte	System mode	Limit of detection	Linear range	Matrix	LWCC type	Preconcentration unit	Extra features	Reference
Phosphorous	Batch mode	5 ng L^{-1}	n.g.	Lake and sea waters	LWCC silver mirror (100 cm)	Without preconcentration unit	The first developed works employing LWCC	Lei et al. 1983
Iron (II)	Continuous flow	11 ng L^{-1}	Up to 0.6 ng L^{-1}	Aqueous standards	LWCC AF-2400 (450 cm)	Without preconcentration unit	-----	Waterbury et al. 1997
Nitrate (NO_3^-)	Batch mode	22 µg L^{-1}	Up to 408 µg L^{-1}	Aqueous standards	LWCC AF-2400 (20.3 cm)	Without preconcentration unit	The measures were performed at UV region.	Belz et al. 1998
Atrazine, diuron and linuron (pesticides)	Batch mode	0.3 -0.5 µg L^{-1} for the three analytes	Up to 1 µg L^{-1} for the three analytes	Tap water	LWCC AF-2400 (90 cm)	Without preconcentration unit	HPLC system coupled with LWCC	Gooijer et al. 1998
Nitrite (NO_2^-) and Nitrate (NO_3^-)	Continuous flow	23 ng L^{-1} for NO_2^- and 93 ng L^{-1} for NO_3^-	Up to1.4 µg L^{-1} for NO_2^- and 1.9 µg L^{-1} for NO_3^-	Surface sea waters	LWCC AF-2400 (450 cm)	Without preconcentration unit	Work able of performing in situ measurements	Yao et al. 1998
Coloured dissolved organic matter (CDOM)	Batch mode	n.a.	n.a.	Sea waters	LWCC AF-2400 (50 cm)	Without preconcentration unit	-----	D'Sa et al. 1999

Table 1.1. Different applications of liquid waveguide capillary cells with spectrophotometric detection (continuing part 1).

Analyte	System mode	Limit of detection	Linear range	Matrix	LWCC type	Preconcentration unit	Extra features	Reference
Chromium (VI) and Molybdenum (VI)	Continuous flow	10 ppt for Cr (VI) and 58 ng L^{-1} for Mo (VI)	Up to 1.6 µg L^{-1} for Cr (VI) 3 µg L^{-1} for Mo (VI)	Sea, rain and commercial waters	LWCC AF-2400 (500 cm)	Without preconcentration unit	With interference studies and method able of performing in situ measurements	Yao and Byrne 1999
Hydrogen sulfide	Continuous flow	5 nM	n.g.	Standards	LWCC AF-2400 (160 cm)	Without preconcentration unit	Flow cell able to carry out fluorimetric measures	Byrne et al. 2000
Phytoplankton	Batch mode	n.a.	n.a.	Estuarine and sea waters	LWCC AF-2400 (50 cm)	Without preconcentration unit	-----	Kirkpatrick et al. 2000
Nitrite (NO_2^-) and Nitrate (NO_3^-)	SFA	5 ng L^{-1} NO_2^- and 0.1 µg L^{-1} for NO_3^-	Up to 2 µg L^{-1} NO_2^- and to 16 µg L^{-1} for NO_3^-	Sea waters	LWCC AF-2400 (200 cm)	Without preconcentration unit	An automated instrument for in-situ analyses	Zhang 2000
Iron (II)	SFA	6 ng L^{-1}	Up to 3 µg L^{-1}	Aqueous standards	LWCC AF-2400 (200 cm)	Without preconcentration unit	-----	Zhang et al. 2001
Total dissolved inorganic carbon	Continuous flow	n.g.	n.g.	Natural river waters	LWCC AF-2400 (10 cm)	Without preconcentration unit	Flow cell able to determine alkalinity, fugacity and pH	Byrne et al. 2002
Copper	Batch mode	0.025 µg L^{-1}	Up to 10 µg L^{-1}	Natural waters (sea, river and commercial drinking water)	LWCC AF-2400 (440 cm)	Without preconcentration unit	Flow cell very long (reagents and sample mixed manually and then injected into the system)	Callahan et al. 2002

Table 1.1. Different applications of liquid waveguide capillary cells with spectrophotometric detection (continuing part 2).

Analyte	System mode	Limit of detection	Linear range	Matrix	LWCC type	Preconcentration unit	Extra features	Reference
Hydrogen peroxide	FIA	0.14 µg L^{-1}	Up to 24 µg L^{-1}	Samples from a disinfection processes	LWCC AF-2400 (50 cm)	Controlled-pore glass beads	With interference studies	Pappas et al. 2002
Coloured dissolved organic matter (CDOM)	Continuous flow	n.a.	n.a.	Sea waters	LWCC AF-2400 (204 cm)	Without preconcentration unit	Work able of performing in situ measurements	Miller et al. 2002
Nitrite (NO_2^-)	Continuous flow	55-115 ng L^{-1}	Up to 6 µg L^{-1}	Sea waters	LWCC AF-2400 (100 cm)	Without preconcentration unit	Fully automated unit for in-situ measurements	Steimle et al. 2002
Carbon dioxide	Continuous flow	2-3 µatm $_pCO_2$	Up to 500 µatm $_pCO_2$	Gaseous samples and sea waters	LWCC AF-2400 (18 cm)	Without preconcentration unit	-----	Wang et al. 2002
Phosphate (PO_4^{3-})	SFA	47 ng L^{-1}	Up to 19 µg L^{-1}	Sea waters	LWCC AF-1600 (200 cm)	Without preconcentration unit	An automated instrument for in-situ analyses	Zhang and Chi 2002
Beryllium	Batch mode	30 ng L^{-1}	Up to 500 µg L^{-1}	Contaminated plexiglas surface	LWCC AF-2400 (50 cm)	Metallochromic chelating agent (Chromazurol S)	With interference studies	Deng et al. 2003
Benzene and toluene	Continuous flow	10 µg L^{-1}	n.g.	Aqueous standards	LWCC AF-2400 (100 cm)	Without preconcentration unit	Measures were taken in UV region	Larsson and Dasgupta 2003

Table 1.1. Different applications of liquid waveguide capillary cells with spectrophotometric detection (continuing part 3).

Analyte	System mode	Limit of detection	Linear range	Matrix	LWCC type	Preconcentration unit	Extra features	Reference
Hydrogen peroxide	Continuous flow	26 pptv	Up to 5 ppbv	Gaseous standards	LWCC AF-2400 (5 cm)	Without preconcentration unit	With interference studies	Li and Dasgupta 2003
Aluminium (III) and Chromium (VI)	FIA	5 µg L^{-1} aluminium and 0.25 µg L^{-1} chromium	Up to 800 µg L^{-1} for aluminium and up to 46.2 µg L^{-1} for chromium	Aqueous standards	LWCC AF-2400 (50 cm)	Without preconcentration unit	Portable sytem with a flow cell able to carry out fluorimetric and chemiluminescence measures	Li et al. 2003
Carbon dioxide	Continuous flow	2-3 µatm $_pCO_2$	Up to 1000 µatm $_pCO_2$	Surface sea waters	LWCC AF-2400 (21 cm)	Without preconcentration unit	Autonomous in-situ method	Wang et al. 2003
Copper	Continuous flow	0.2 µg L^{-1}	Up to 11 µg L^{-1}	Estuarine waters	LW CC AF-2400 (100 cm)	Without preconcentration unit	Automated unit for in-situ measurements	Callahan et al. 2004
Phenols	Multi-commuted	1 µg L^{-1}	Up to 100 µg L^{-1}	Natural and waste waters	LWCC AF-2400 (100 cm)	Without preconcentration unit	Low reagent consumption and effluent production	Lupetti et al. 2004
Mercury	Continuous flow	1.6 µg L^{-1}	n.g.	Aqueous standards	LWCC AF-2400 (160 cm)	Without preconcentration unit	The developed work can be used for in-situ determinations	Tao et al. 2004
Nitrite (NO_2^-)	Continuos flow	46 ng L^{-1}	up to 23 µg L^{-1}	Sea water	LWCC AF-2400 (97 cm)	without preconcentration unit	Portable and automated unit for in-situ measurements	Adornato et al. 2005
Ammonium	Segmented flow analysis	0.09 µg L^{-1}	Up to 18 µg L^{-1}	Sea water samples	LWCC AF-2400 (200 cm)	Without preconcentration unit	-----	Li et al. 2005

Table 1.1. Different applications of liquid waveguide capillary cells with spectrophotometric detection (continuing part 4).

Analyte	System mode	Limit of detection	Linear range	Matrix	LWCC type	Preconcentration unit	Extra features	Reference
Chromium (VI)	Continuous flow	48.4 µg L^{-1}	Up to 5 mg L^{-1}	Estuarine waters	LWCC AF-2400 (1 cm)	Without preconcentration unit	Portable unit for in-situ measurements	Pressman and Aldstadt 2005
p-arsanilic acid	SIA	21.2 µg L^{-1}	Up to 1 mg L^{-1}	Surface waters from a swine farm	LWCC AF-2400 (100 cm)	Without preconcentration unit	-----	Roerdink and Aldstadt 2005
Acetone (gaseous)	FIA	14 ppbv	Up to 1.21 ppmv	Breath analysis in humans	LWCC FEP (10 cm)	Without preconcentration unit	With interference studies	Teshima et al. 2005
Pu(III,IV,V and VI)	Continuous flow	Pu(II) 2 mg L^{-1}, Pu(IV) 1 mg L^{-1}, Pu(V) 4 mg L^{-1} and Pu(VI) 0.2 µg L^{-1}	n.g.	Perchloric acid	LWCC AF-2400 (100 cm)	without preconcentration unit	The system was not applied to real samples	Wilson et al. 2005
Nitrite (NO_2^-)	FIA	0.1 µg L^{-1}	Up to29 µg L^{-1}	River water	LWCC AF-1600 (80 cm)	Without preconcentration unit	-----	Takiguchi et al. 2006
Nitrite (NO_2^-)	FIA	92 ng L^{-1}	Up to 28 µg L^{-1}	Aqueous standards	LWCC AF-2400 (400 cm)	Without preconcentration unit	-----	Zhang 2006
Chloride	Multi-commuted	0.3 mg L^{-1}	Up to 100 mg L^{-1}	Natural waters	LWCC AF-2400 (100 cm)	Solid phase unit	With interference studies	Bonifácio et al. 2007
Organophos-phorus pesticides	Batch mode	6.7 ng L^{-1}	Up to 0.8 µg L^{-1}	Vegetables and fruit samples and River and tap waters	LWCC AF-2400 (9 cm)	Without preconcentration unit	With interference studies and able of determining in different matrixes	Cheng et al. 2007

Table 1.1. Different applications of liquid waveguide capillary cells with spectrophotometric detection (continuing part 5).

Analyte	System mode	Limit of detection	Linear range	Matrix	LWCC type	Preconcentration unit	Extra features	Reference
Phosphate (PO_4^{3-})	FIA	0.95 µg L^{-1}	Up to 95 µg L^{-1}	River water	LWCC AF-2400 (100 cm)	Without preconcentration unit	With interference studies	Gimbert et al. 2007
Magnesium	Batch mode	7 ng L^{-1}	n.g.	Sea waters	LWCC AF-1600 (200 cm)	Co-precipitation method	With interference studies	Li and Hansell 2008
Nitrate (NO_3^-) and Phosphate (PO_4^{3-})	FIA	0.12 µg L^{-1} NO_3^- and 0.15 µg L^{-1} for PO_4^{3-}	Up to 9 µg L^{-1} for $NO3^-$ and to 12 µg L^{-1} for PO_4^{3-}	Sea waters	LWCC AF-2400 (200 cm)	Without preconcentration unit	An automated instrument for in-situ analyses	Li et al. 2008
Carbon dioxide	Continuous flow	n. g.	Up to 800 µatm $_pCO_2$	Surface sea waters	LWCC AF-2400 (12 cm)	Without preconcentration unit	Autonomous in-situ method with low power consumption	Lu et al. 2008
Sulphate (SO_4^{2-})	Multi-pumping	150 µg L^{-1}	Up to 16 mg L^{-1}	River and rain waters	LWCC AF-2400 (100 cm)	Without preconcentration unit	Turbidimetric determination	Melchert and Rocha 2008
Phosphate (PO_4^{3-})	Continuous flow	20 µg L^{-1}	Up to 800 µg L^{-1}	Aqueous standards	LWCC AF-2400 (30 cm)	Without preconcentration unit	-----	Melchert et al. 2008
Phosphate (PO_4^{3-})	FIA	17 µg L^{-1}	Up to 500 µg L^{-1}	Surface and ground waters	LWCC AF-2400 (100 cm)	Without preconcentration unit	With interference studies	Neves et al. 2008
Phosphate (PO_4^{3-}) and nitrite (NO_2^-)	SFA	76 ng L^{-1} for phosphate and 69 ng L^{-1} for nitrate	Up to 57 µg L^{-1} for phosphate and up to 28 µg L^{-1} nitrate	Sea waters	LWCC AF-2400 (200 cm)	Without preconcentration unit	Automated method	Patey et al. 2008

Table 1.1. Different applications of liquid waveguide capillary cells with spectrophotometric detection (continuing part 6).

Analyte	System mode	Limit of detection	Linear range	Matrix	LWCC type	Preconcentration unit	Extra features	Reference
Silicate (SiO_3^-)	Syringe pump combined with SIA	8 µg L^{-1}	Up to 7.6 mg L^{-1}	Surface sea water	LWCC AF-2400 (200 cm)	Without preconcentration unit	The salinity effect was studied and different LWCC were tested	Amornthammarong and Zhang 2009
Iron (II) and Iron (III)	Reversed FIA	6 ng L^{-1} for Fe (II) and 11 ng L^{-1} for Fe (III)	Up to 11 µg L^{-1} for Fe (II) and up to 16 µg L^{-1} for Fe (III)	Rain water	LWCC AF-2400 (200 cm)	Without preconcentration unit	With interference studies	Huang et al. 2009
Phosphate (PO_4^{3-})	rFIA	47 ng L^{-1}	Up to 16 µg L^{-1}	Sea waters	LWCC AF-2400 (200 cm)	Without preconcentration unit	With interference studies	Ma et al. 2009
Chloride	MSFIA	60 µg L^{-1}	Up to 2 mg L^{-1}	Mineral, tap and well waters	LWCC AF-2400 (100 cm)	Without preconcentration unit	With interference studies	Maya et al. 2009
Iron (II)	Continuous flow	4.6 ng m^{-3}	n.g.	Urban atmospheric aerosols	LWCC AF-2400 (100 cm)	Without preconcentration unit	An automated instrument for in-situ analyses	Rastogi et al. 2009
Chlorine	Multi-commuted	7 µg L^{-1}	Up to 1.5 mg L^{-1}	Tap waters	LWCC AF-2400 (100 cm)	Without preconcentration unit	With interference studies	Salami et al. 2009
Chromium (III) and Chromium (VI)	Continuous flow	90 µg L^{-1} for Cr(III) and 0.7 µg L^{-1} for Cr(VI)	Up to 100 mg L^{-1} for Cr (III) and up to 1 mg L^{-1} for Cr (VI)	Aqueous standards	LWCC AF-2400 (37.6 cm)	Without preconcentration unit	Reagent free method with minimal waste effluent	Xin et al. 2009

Table 1.1. Different applications of liquid waveguide capillary cells with spectrophotometric detection (continuing part 7).

Analyte	System mode	Limit of detection	Linear range	Matrix	LWCC type	Preconcentration unit	Extra features	Reference
Nitrite (NO_2^-) and Nitrate (NO_3^-)	FIA	14 ng L^{-1} NO_2^- and 93 ng L^{-1} for NO_3^-	Up to 5 µg L^{-1} NO_2^- and to 12 µg L^{-1} for NO_3^-	Sea waters	LWCC AF-2400 (16 cm)	Solid phase extraction (Oasis® HLB cartridge)	-----	Zhang et al. 2009
Uranium (VI)	MPFS combined with MSFIA	12.6 ng L^{-1}	Up to 0.155 µg	Fresh, mineral, tap and sea water	LWCC AF-2400 (100 cm)	Solid phase unit (Transuranide resin)	With interference studies	Avivar et al. 2010
Plutonium (PuO_2^{2+})	Continuous flow	110 µg L^{-1}	Up to 27 mg L^{-1}	Aqueous standards	LWCC AF-2400 (100 cm)	Without preconcentration unit	-----	Cho et al. 2010
Ammonia (NH_3)	Micro-pumping	5 µg L^{-1}	Up to 255 µg L^{-1}	Urban air samples	LWCC AF-2400 (100 cm)	Without preconcentration unit	-----	Coelho et al. 2010
Nitrophenols	Continuous flow	12-60 ng L^{-1}	n.g.	Rain and air	LWCC AF-2400 (142 cm)	Solid phase unit (anion exchanger)	The developed work included also a reversed phase separation column	Ganranoo et al. 2010
Silicic acid	SFA	1 µg L^{-1}	Up to 192 µg L^{-1}	Sea water	LWCC AF-2400 (100 cm)	Without preconcentration unit	-----	Hashihama and Kanda 2010
Cyanide	FIA	0.8 µg L^{-1}	Up to 260 µg L^{-1}	Air samples and orange, pear and apple seeds	LWCC AF-2400 (50 cm)	Without preconcentration unit	With interference studies and able of determining in different matrixes	Ma et al. 2010
Carbaryl	Multi-pumping	1.7 µg L^{-1}	Up to 200 µg L^{-1}	Natural waters	LWCC AF-2400 (100 cm)	Cloud-point extraction	With waste treatment	Melchert and Rocha 2010

Table 1.1. Different applications of liquid waveguide capillary cells with spectrophotometric detection (continuing part 8).

Analyte	System mode	Limit of detection	Linear range	Matrix	LWCC type	Preconcentration unit	Extra features	Reference
Hypochlorite	Multi-pumping	6.8 µg L^{-1}	Up to 100 µg L^{-1}	River, lake and tap waters	LWCC AF-2400 (100 cm)	Without preconcentration unit	With interference studies	Melchert et al. 2010
Lead	Continuous flow	0.4 ng L^{-1}	Up to 6 µg L^{-1}	River water	LWCC AF-2400 (100 cm)	Without preconcentration unit	The method uses a coprecipitation method to remove interferences	Yabutani et al. 2010
Toltrazuril and lasalocid	SIC	19 µg L^{-1} for toltrazuril and 10 µg L^{-1} for lasalocid	Up to 1 mg L^{-1} for toltrazuril and up to 2 µg L^{-1} for lasalocid	Ground and commercial waters, pharmaceutical formulations and feed samples	LWCC AF-2400 (200 cm)	Without preconcentration unit	A monolithic column C18 was used	Björklund et al. 2011
Manganese	Multi-pumping	6 µg L^{-1}	Up to1500 µg L^{-1}	River, lake and commercial waters	LWCC AF-2400 (100 cm)	Solid phase unit	With interference studies	Silva et al. 2011
Uranium (VI)	Lab-on-valve combined with MSFIA	10.3 ng L^{-1}	Up to 0.3 µg	Sea, well, fresh, tap and mineral waters	LWCC AF-2400 (100 cm)	Solid phase unit (UTEVA resin)	With interference studies	Avivar et al. article in press

n.a.- not applied; n.g.- not given; SIC- sequential injection chromatography; rFIA- reverse flow injection analysis; FEP fluorinatedethylene copolymer; UTEVA- uranium and tetravalents actinides

Besides all the described applications of LWCCs using the spectrophotometric detection mode in Table 1.1, there are other works employing this specific equipment. Several works were not mentioned because they used small LWCC and/or were not applied to any specific determination.

Some of these not mentioned works in Table 1.1 should be pointed out. One of these works was performed by Dasgupta et al. (1998), where the potential use of LWCC for gaseous determinations was tested; the results obtained confirmed this potential and also the potential to determine volatile organic compounds dissolved in water. Milani and Dasgupta (2001) developed a method for the differentiation and determination of nitrogen dioxide and nitrous acid using a LWCC.

Tsunoda et al. (2003) developed a LWCC home made and tested it in the determination of sulphur in steel samples. Santana-Casiano et al. (2005) used a 5 m LWCC to study the oxidation of Fe(II) in seawater at nanomolar levels using a kinetic model that include speciation changes valid over a wide range of experimental conditions, and Liu et al. (2006) developed an automated instrument using a LWCC for in-situ pH measurements in water samples. Wada et al. (2006) tested a LWCC home made with different pathlengths to determine tegafur (anticancer agent) and Wang et al. (2007) described the first autonomous multi-parameter flow system using a LWCC for the simultaneous determination of pH, carbon dioxide fugacity and total inorganic carbon in seawater. Anastasio and Robles (2007) applied the LWCC to the determination of soluble chemical species in Arctic and Antarctic snow; in 2008, Sun et al. (2008) constructed an automated analyser using a LWCC for trace nutrients determination in sea waters and Mincher et al. (2008) applied a LWCC to verify the efficiency of an extraction method with the determination of oxidized americium; Robles et al. (2007) developed a LWCC home made for use in a commercial

spectrophotometer to measure very low light absorbance values for dilute aqueous solutions in both ultraviolet and visible wavelengths. Later on, Oakes et al. (2010) used the LWCC to characterize soluble iron in urban aerosols.

Another different application of LWCC was performed by Datta et al. (2003) where a LWCC impressed in silicon substrates in a chip-scale using Teflon AF was made and tested. Sun et al. (2006) integrated a 1.5 cm LWCC (Teflon AF 2400) in a micro fluidic liquid-liquid extraction system and applied it for sodium dodecyl sulphate determination. After that, Huang et al. (2008) developed a micro-FIA system with a 2 cm LWCC (Teflon AF 2400 with 50 µm i.d.) incorporated in the chip and tested for NO_2^- determination (this worked achieved consumptions of sample/reagents in the order of nL). In 2010, Pan et al. (2010) integrated also the LWCC in a micro fluidic system for DNA measurements.

Another interesting application of LWCC with spectrophotometric detection was carried out by Wada et al. (2008); a LWCC (Teflon AF 2400) home made was used for electrophoresis separation with kinetic absorption detection and tested with the decomposition of Cd^{2+} complex with 4-(2-pyridylazo)-resorcinol.

1.5.4.2. Chemiluminescence detection mode

Table 1.2. Different applications of liquid waveguide capillary cells with chemiluminescence detection.

Analyte	System mode	Limit of detection	Linear range	Matrix	LWCC type	Preconcentration unit	Extra features	Reference
Ammonium (NH_4^+)	FIA	2.2-4.7 µg L^{-1}	Up to 2.2 mg L^{-1}	Standards	LWCC AF-2400 (15 cm)	Without preconcentration unit	Very simple home-made fabrication and inexpensive	Li and Dasgupta 1999
Hydrogen peroxide	FIA	25 pptv	Up to 100 ppbv	Gaseous standards	LWCC AF-2400 (15 cm)	Without preconcentration unit	With interference studies	Li and Dasgupta 2001
Hydrogen peroxide	FIA	16 pptv	Up to 17 ppbv	Gaseous standards	LWCC AF-2400 (50 cm)	Without preconcentration unit	Portable sytem with a flow cell able to carry out fluorimetric and absorbance measures	Li et al. 2003
Salbutamol	FIA	24 µg L^{-1}	Up to 2.4 mg L^{-1}	Pharmaceutical samples	LWCC AF-2400 (100 cm)	Without preconcentration unit	-----	Sanchez and Rocha 2008(a)
Hypochlorite ($HClO^-$)	FIA	8 µg L^{-1}	Up to 3.8 mg L^{-1}	Pharmaceutical samples	LWCC AF-2400 (100 cm)	Without preconcentration unit	-----	Sanchez and Rocha 2008(b)

Besides all the described applications of LWCC using the chemiluminescence detection in Table 1.2, another different work should be pointed out. In 1999, Dasgupta and co-workers tested a LWCC for the determination of hypochlorite and also for nephelometric, phosphorimetric and fluorimetric determinations (more details are given in the fluorescence detection mode section). This work is a good example of the large versatility of the LWCC.

1.5.4.3. Fluorescence detection mode

Table 1.3. Different applications of liquid waveguide capillary cells with fluorsecence detection.

Analyte	System mode	Limit of detection	Linear range	Matrix	LWCC type	Preconcentration unit	Extra features	Reference
Ammonium/ Ammonia	FIA	0.6 µg L^{-1} for NH_3	Up to 1 mg L^{-1} for NH_3	Standards	LWCC AF-2400 (11.5 cm)	Without preconcentration unit	Very low cost method compared to commercially fluoremeters	Li et al. 1999
Formaldehyde	FIA	0.3 µg L^{-1}	Up to 160 µg L^{-1}	Urban gaseous	LWCC AF-2400 (11.5 cm)	Without preconcentration unit	With interference studies and an automated instrument	Li et al. 2000
Hydrogen peroxide	FIA	0.4 ppb aqueous 13.5 pptv for gaseous	Up to 3.4 mg L^{-1} aqueous and up to 1 ppbv gaseous	Urban gaseous	LWCC AF-2400 (11.5 cm)	Without preconcentration unit	With interference studies and capable of replace chromatrographic analyses of this analyte	Li and Dasgupta. 2000
Chlorophyll a and quinine sulfate	Continuous flow	0.05 µg L^{-1} quinine sulfate and 0.03 chlorophyll-a	Up to 5.12 µg L^{-1} quinine sulphate and up to 0.56 µg L^{-1} chlorophyll-a	Ocean waters	LWCC AF-2400 (160 cm)	Without preconcentration unit	Flow cell able to carry out spectrophotometric measures	Byrne et al. 2000
Hydrogen peroxide	FIA	8 pptv	Up to 5 ppbv	Gaseous standards	LWCC AF-2400 (50 cm)	Without preconcentration unit	Portable sytem with a flow cell able to carry out chemiluminescene and absorbance measures	Li et al. 2003

Along with all the described applications of LWCCs using the fluorescence detection mode in Table 1.3, there are other works that should be presented.

Dasgupta et al. (1999) applied the LWCC to different flow injection analysis determinations with fluorescence detection and also successfully to capillary electrophoresis. This work opened the field of LWCCs applications using fluorescence detection mode.

Another example of an application of LWCC with fluorescence detection is the work developed by Wang et al. (2001) in a capillary electrophoresis system used to determine arginine, phenylalanine and glycine. The results obtained are very interesting when compared to other sophisticated systems. Zhang et al. (2005), Kostal et al. (2005, 2006) developed similar works such as the above mentioned.

Another similar example is given by Olivares et al. (2002) and Wang et al. (2005), where the LWCC was used to separate (by capillary electrophoresis) and detect DNA fragments. Following this study, Xu et al. (2010) developed a miniaturized capillary electrophoresis system using the LWCC to detect DNA mutations. This system in the future could be applied in early diagnosis of cancer diseases.

The LWCC was also tested for liquid chromatography in flow analysis systems by Song et al. (2008). This study releases the potentiality of LWCC to be coupled in series with other detectors.

All the applications of LWCC with fluorescence detection described in the above section present several advantages when compared to commercial fluorometers such as, low price, similar detection limits, simple operation mode, versatile configurations and applications

1.5.5. Other examples of LWCCs made of different materials

Besides Teflon AF, other similar materials that can be incorporated in LWCCs has been recently presented. Some of them will be presented and discussed in this section.

Keller et al. (2007) evaluated a new fused silica capillary for absorbance measures. This material presents a RI of 1.441 and light guidance along the entire length (UV, visible and near infra red range) of the waveguide in a length up to 50 m. This material could also be employed in fiber optics presenting the great advantage of light guidance over the entire length of the waveguide. This material was also evaluated for fluorescence measures and the results are linear up to 20 m length (Paprocki et al. 2008).

Later on, Korampally et al. (2009) presented a new polymer constituted by organosilicate nano particules with an ultra low RI of 1.16. The results obtained show a superior light guidance performance over Teflon AF although the integration of this material to waveguide coatings is not simple. This polymer has a great potential especially for fluorescence measures because of a larger acceptance of incident angles when compared to Teflon AF. Further studies are needed to make a full comparison with Teflon AF.

In the same year, Sugiya et al (2009) used a water-ice chip as another option to Teflon AF 2400 in LWCCs. The ice chip has a RI of 1.309, higher than Teflon AF 2400 (RI=1.29) and water soluble organic solvents can not be used as a core because of the dissolution of water-ice therein.

Recently, Christinasen et al. (2010) presented a new type of polymer that can be incorporated in LWCCs. This new nano polymer presents a RI of 1.26 that is lower than Teflon AF 2400 (RI=1.29) and both cladding and core are made of the same material.

Despite all above mentioned, Teflon AF gives the impression to be the best component to be integrated in the LWCCs hitherto.

1.5.6. Other flow cells

There are other flow cells with different configurations commercially available for spectrophotometric analysis. The Z and U configuration with 1 cm of optical pathlength are commonly used. The pathlength restriction and occasionally large hydrodynamic dispersion, are the main attributed shortcomings of these configurations (Ellis et al. 2009).

Another different flow cell is the multi-reflective flow cell made of glass capillary coated externally with silver or aluminium. It has been reported that these type of cells present higher sensitivity than Z or U type flow cells because of the longer optical pathlength and lower dispersion. In this cell, the light beam is introduced with an incident angle and with this approach schlieren effects are reduced (Ellis et al. 2003). The major disadvantage of this type of cells is the absorption of a certain fraction of light upon each reflection by the external coating. The silver coat normally absorbs 5 to 10% of the incident light in the visible region although in UV region it absorbs a superior amount of the incident light. The aluminium coat is other option however 20% of absorption will occur in the UV region (Ellis et al. 2009). The silver coat absorbs less light than the aluminium coat (Lei et al. 1983).

An additional flow cell is the total internal reflection (TIR). The TIR flow cell consists of a fused silica quartz capillary and has no outer coat besides the air itself that has a RI of 1. To total internal reflection takes place in this cell the light beam must be introduced with an incident angle similar to the multi-reflective cell and in opposition to the LWCC (in absorbance measures) where the light is introduced in axial mode. This flow cell demonstrated a high tolerance to schlieren effects, low hydrodynamic dispersion, lower bubbles entrapment and mainly no absorption by the capillary wall when compared to the multi-reflective cell (Ellis et al. 2009).

1.5.7. Strategies to reduce the schlieren effects

The more important drawback related to LWCC is the so-called schlieren effect. It was stated by Yao and Byrne (1999) that the geometry of LWCC causes to be more susceptible to schlieren effects although in a smaller amount than the conventional flow cells. The schlieren effects or refractive index occurs when a sample with high ionic strength is introduced in a carrier stream with low ionic strength; the difference between the refractive indices of the sample and carrier will form a parabolic lens. This can cause “ghost” absorbance peaks even when no analyte-reagent is present and produce some errors in absorbance measures (Dias et al. 2006).

Several strategies have been described to reduce/compensate the schlieren effects including dual wavelength spectrophotometry (Mckelvie et al. 1997), in-line salinity compensation (Mckelvie et al. 1997), matrix matching between sample and carrier (Infante and Rocha 2008), absorbance measurement in the central part of analyte/reagent plug (Yamane et al. 1995) and introduction of light with an incident

angle (transverse to the flow cell) (Jambunathan et al. 1999). Dual wavelength spectrophotometry in a multi-channel spectrophotometer is an easy way to compensate the schlieren effects and this strategy was proven to be very efficient and also avoid baseline drifts (Li et al. 2003). The in-line salinity compensation and the matrix matching between sample and carrier are very difficult to be executed in samples containing high and variable concentrations of inert solutes. Improving mixing conditions between sample and carrier might reduce the schlieren effects although it is limited by the increase in sample dispersion (Infante and Rocha 2008). The introduction of light transverse to the flow cell causes lower sensitivity when compared to the introduction of light in axial mode (Ellis et al. 2009).

The dual wavelength measurement appears to be the simplest and best strategy. The principle of the signal compensation is to measure simultaneously the signal at two different wavelengths: one at which the reaction product absorbs predominantly the light and a reference wavelength at which no absorbance change due to the chemical reaction is observed.

Chapter 2

General materials and methods

In this chapter, a detailed overview of the reagents, samples and equipment used during this thesis is presented. Additionally, the optimisation procedures and statistical treatments employed are described.

2.1. Introduction

During the development of this doctoral thesis, different flow analysis systems (FIA, SIA, MSFIA and MPFS) were used. Herein a general description of all this systems including the devices and components used is provided.

Moreover, some details of the LWCC are presented since this device was the basis of all the developed work.

The general considerations related with the preparation of the reagents, laboratory water supply, manipulation and treatment of samples are also described in this chapter.

Additionally, several aspects of the optimisation procedures and statistical treatments as well as the data acquisition mode are fully described in this chapter.

2.2. Reagents and solutions

All chemicals used were of analytical grade quality with no further purification treatment. All solutions were prepared using deionised water with a specific conductance less than 0.1 $\mu S\ cm^{-1}$, except in Chapter 4, where Milli-Q water with resistivity superior to 18 MΩ cm was used. In Chapter 7, the standard solutions were prepared at room temperature with previously boiled deionised water.

In Chapter 3 and 4, standard stock solutions were obtained by weighing the respective reagent in a Sartorius (model SB 2105) analytical balance followed by dissolution in appropriate solutions (water or acidic solution). In Chapters 3, 4, 5, 6, and 7 a commercial solutions (atomic absorption standards) were used as standard stock

solutions. The working standard solutions were prepared by rigorous dilution of the stock solution using volumetric glass pipettes and volumetric flasks (class A) of different volumes.

When necessary, the pH of solutions was measured using combined glass electrode (Mettler, U420-S7/120, Columbus, USA) connected to a Crison potentiometer (model micro pH 2002).

2.3. Samples

Throughout this work several types of water were used. The majority of the samples used were gently provided by a water analysis laboratory. Water samples were acidified to pH < 2 and stored in a refrigerator at 4 ºC before analysis, as recommended by Clesceri et al. 1998.

2.4. Components of the flow systems

During the development of this thesis different flow analysis systems were employed. In this part, the main components of each flow technique are described.

2.4.1. Propulsion units

In Chapter 3 and 7 (FIA system), solutions were driven through two Gilson Minipuls 3 four channel (Fig. 2.1a) peristaltic pumps (Villiers-le-Bel, France) with

Tygon propulsion tubes. The propulsion tubes were replaced whenever malfunctions such as variation of the flow rate or loss of elasticity were detected (resulting in alterations of the analytical signals).

Volumes aspirated or dispensed were calculated experimentally by performing the respective operation step ten times and weighing the water aspirated from or propelled into a previous zeroed beaker. The volume aspirated per unit of time corresponded to the flow rate, expressed in mL min^{-1}.

In Chapter 4 and 5, solutions were propelled through a multi-syringe burette (Crison Instruments, Allela, Spain). The device (Fig. 2.1b) uses a multiple-channel piston pump, containing up to four syringes, driven by a single motor, controlled by computer software through a serial port. Three-way commutation valves (NResearch, Caldwell, NJ, USA) were connected at the head of each syringe. Two syringes of 10 mL were placed in position 1 and 2 and two syringes of 2.5 mL were placed in positions 3 and 4. Hamilton (ref. 81620 and 81420) glass syringes were used. The piston movement of the multi-syringe was divided in 16000 steps, therefore the minimum volume delivered was 0.62 µL for the 10 mL syringes and 0.16 µL for the 2.5 mL syringes.

In Chapter 6 and part of 7, micro-pumps (Bio-Chem Valve Inc., Boonton, NJ, USA, Ref. 120SP1220 and 120SP1230) with nominal dispensing volumes of 20 and 30 µL per stroke were used to propel the solutions (Fig. 2.1c). An I/O digital card (Sciware, Mallorca, Spain) with eight optocoupled digital input channels and eight digital relay output channels was used to control the micro-pumps. This card was connected to a personal computer through an RS485/RS232 interface and can independently control the operation of up to eight micro-pumps and/or commutation valves. These devices are set on a motherboard connected to a protection interface, which is connected to the relay outputs and an additional power source of 12 V is required to activate the solenoid

devices. In order to minimize the heat generation and extend the lifetime of the valves, a solenoid protection system (Sciware, Mallorca, Spain) was used. The power source, the RS serial interface and the I/O cards were integrated into a unique module (Ref. Module1 Sciware).

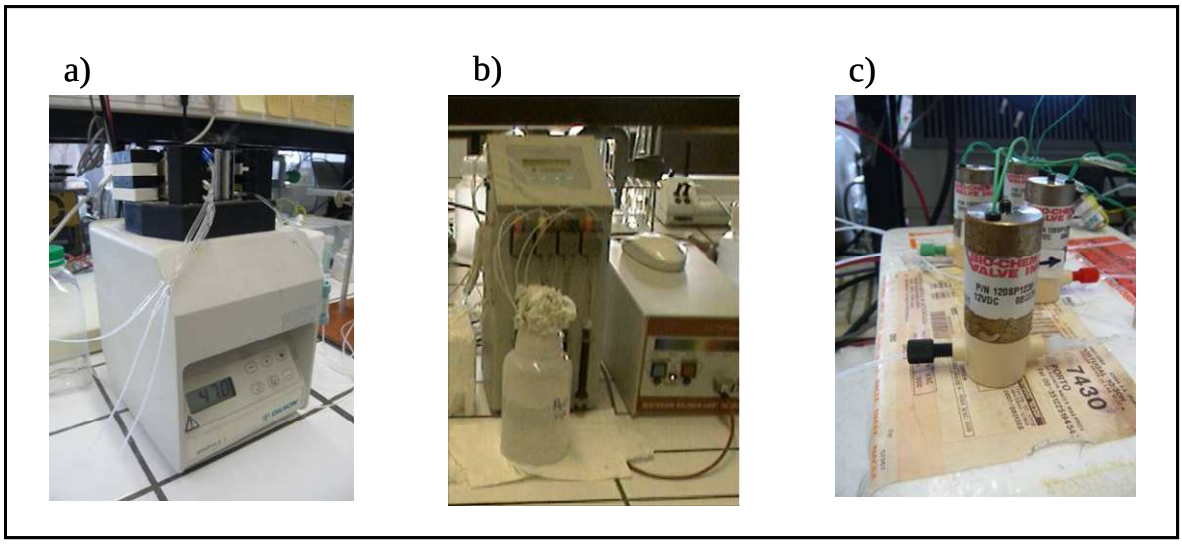

Figure 2.1. Different flow propulsion units: a) peristaltic pump; b) multi-syringe burette; c) micro-pumps.

2.4.2. Valves

2.4.2.1. Selection valves

In Chapter 3, two electrically actuated selection valves (Valco VICI C25-3188D, 8-port and Valco C25-3180D, 10 port, Houston, USA) were used (Fig. 2.2a).

2.4.2.2. Injection valve

In Chapter 7, a four-way injection valve (Rheodyne 5020) was used for injection of fixed volume samples and is represented in Fig. 2.2B. In this type of valve only two positions are available.

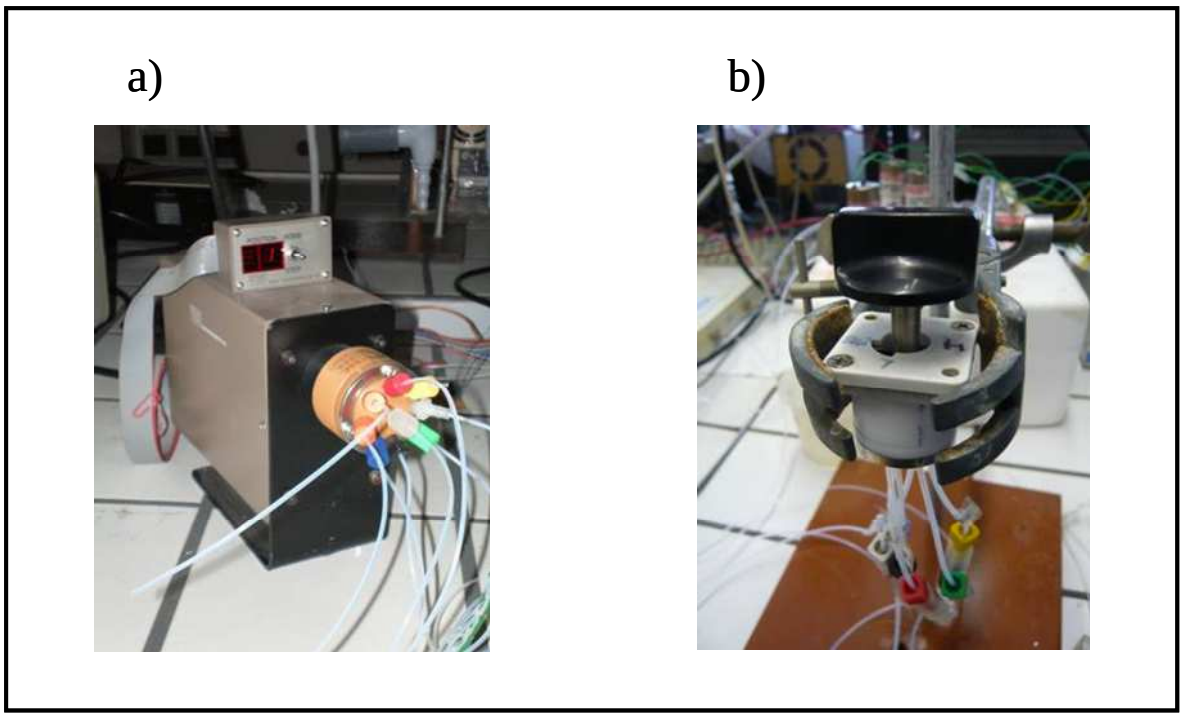

Figure 2.2. Different valve units: a) selection valve; b) injection valve.

2.4.2.3. Solenoid valves

In Chapter 4 and 5, three and four commutation valves (NResearch, Caldwell, NJ, USA, Ref 161T031) with an internal volume of 27 µL were used, respectively. The commutation valves (Fig. 2.3) were controlled through the multi-syringe referred above.

In Chapter 6 and Chapter 7, two commutation valves with the same characteristics of the last paragraph were used although these ones were controlled through an I/O digital card mentioned in the above section.

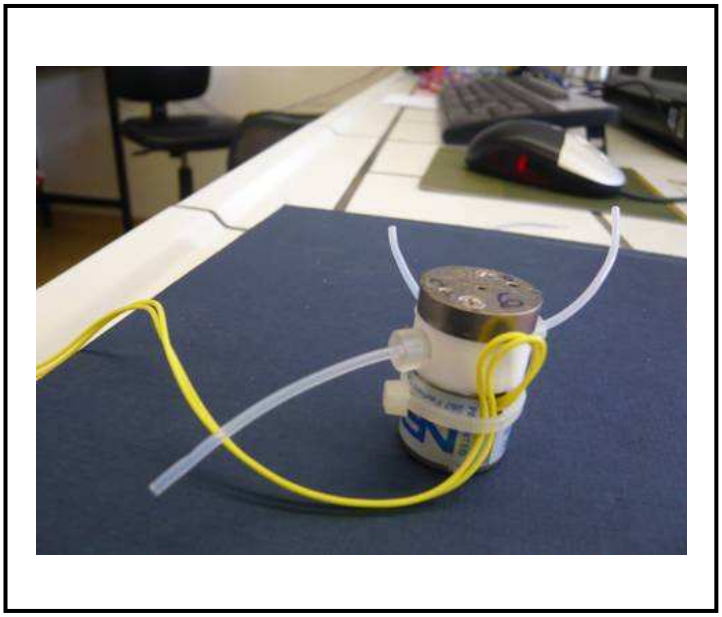

Figure 2.3. Commutation valve.

2.4.3. Flow tubes, connectors and other devices

Tubing connecting the different components of the developed flow systems were made of Omniift PTFE (0.8 mm inner diameter) with Gilson end-fittings and connectors.

The confluences were laboratory made acrylic “Y” connectors (Alegret et al. 1987).

In Chapter 4, Chelex 100 (Bio-Rad, 200-400 mesh, Sodium form) and NTA Superflow (Qiagen Inc., Valencia, SP) resins were packed intro columns (Fig. 2.4a). The resins were packed in PVC tubing (2.22 mm inner diameter and 2.5 cm length) and the suspensions introduced into the column by means of Pasteur pipette. Ordinary dishwashing foam was placed at both ends of the column to entrap the resins inside

(Fig. 2.4b). The Chelex 100 resin was suspended in a conditioning buffer and the NTA Superflow resin is available in a form of suspension, therefore it is ready to use.

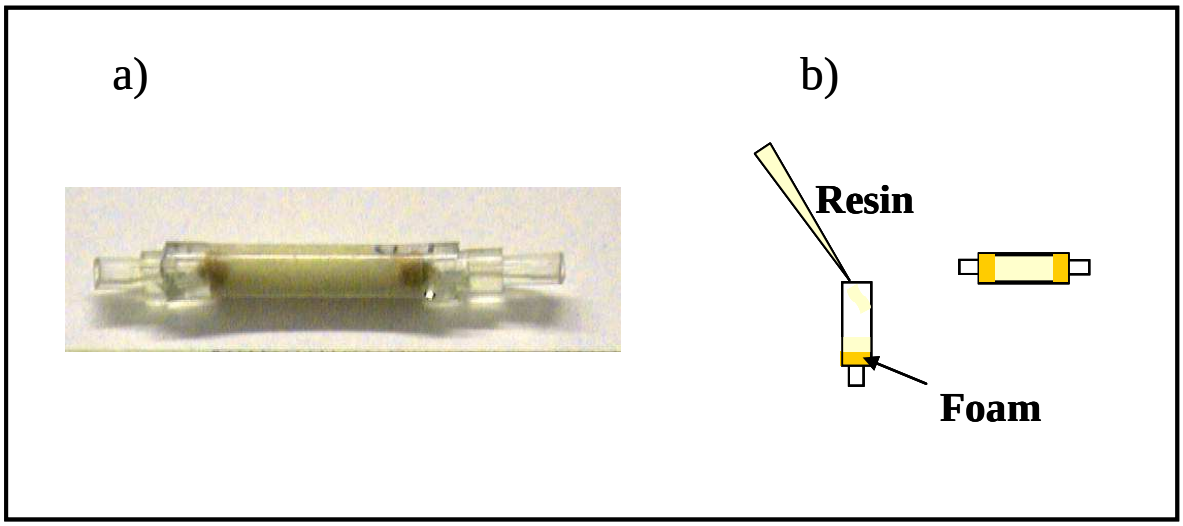

Figure 2.4. a) Photograph of the column; b) schematic representation of loading the column with the resin.

2.4.4. Detection system

As detection system, an Ocean Optics PC2000-ISA (Winter Park, USA) charge capacity detector, a pair of 200 µm fiber optic cable, a DH-2000 deuterium halogen light source (Top Sensor Systems, Eerbeek, The Netherlands) and a liquid waveguide capillary cell (LWCC 2100, World Precision Instruments, Sarasota, USA) (1.0 m pathlength, 250 µL inner volume, 550 µm inner diameter) was used. As it is a central point in this dissertation, a detailed description of the LWCC equipment is given in the subsequent section.

2.4.4.1. General details of LWCC's

Throughout this thesis, a LWCC 2100 (Fig. 2.5) commercially available from World Precision Instruments (Sarasota, USA) with 1.0 m of pathlength, 250 µL of inner volume and 550 µm of inner diameter was used. This flow cell belongs to the LWCC of type II, discussed in the Introduction Chapter. This LWCC is able of light guidance over the range of 230-730 nm, stands up to 160ºC and 2000 PSI of temperature and pressure, respectively. It also presents a small weight (1.4 kg), high resistance to most organic and inorganic solvents, although some perfluorinated solvents can affect the tubing. It presents a linear range from 0.01 to 2.0 absorbance units (limited only by noise and stray light form the measuring spectrophotometer) (World Precision Instruments 2011).

As referred above, the LWCC was connected to the light source and the detector through a pair of 200 µm fiber optic cable, as recommended by the manufacturer instructions.

When the waveguide is not in use for a long time, it should be filled with ultrapure water and the ends of the waveguide be sealed to avoid microbial growth within it and from drying out (Gimbert and Worsfold 2007).

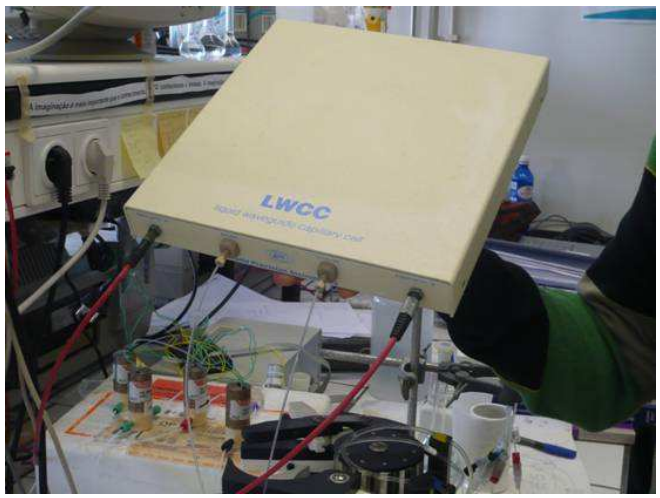

Figure 2.5. Photograph of the LWCC 2100 used throughout this thesis

2.5. Computer control

2.5.1. Connection to pumps, solenoid valves and multi-syringe burette

In Chapter 3, a 386 personal computer (Samsung, Korea) equipped with an Advantec PCL818L (Taipei, Taiwan) interface card, running in-house software written in QuickBasic 4.5, controlled the selection valve positions and the peristaltic pump directions and speed.

A personal computer Pentium II, running SCIWARE (Palmanyola, Mallorca, Spain) "Auto-analysis" software (version 5.0.3.5) controlled the multi-syringe operation (direction of piston displacement, number of steps and position of all commutation valves; Chapter 4 and 5) and the micro-pumps and all commutation valves (frequency

strokes of the micro-pumps, number of steps and position of all commutation valves; Chapter 6 and part of 7).

2.5.2. Data acquisition

In Chapter 3, the data acquisition was performed by SpectraWin software version 4.2 (Top Sensor Systems, Eerbeek, The Netherlands) through an external trigger signal made from the Advantec PCL818L interface card.

The data acquisition in Chapter 4, 5, 6 and part of 7 was performed by "Auto-analysis " computer software referred in the preceding section.

In the FIA system (part of Chapter 7), the data acquisition was carried out by the above mentioned SpectraWin software although employing a manual trigger.

Finally, the obtained data was analysed with Microsoft Excel 2003.

2.5.3. Programs

The programs used to control the selection valves and pumps in Chapter 3 were written in Microsoft QuickBasic 4.5. Throughout the remainder work (Chapter 4-7) "Auto-analysis" software was used to control the multi-syringe burette, the micro-pumps and commutation valves. For each work, one different program was applied.

2.6. Development and optimisation of flow systems

Throughout this thesis, the manifolds were devised to make possible the direct sample introduction, with no necessary pretreatment.

Initially, when a preliminary manifold configuration was set up, the parameters were roughly chosen on a trial and error basis to allow each determination to be performed between the expected concentration limits. Afterwards, the parameters were optimised to maximize sensitivity and determination rate and to minimize reagent consumption. This optimisation process was attained by using the univariate method where only one parameter was changed within a certain range while all the others were kept constant.

Following the optimisation, the developed methods were characterised in terms of limits of detection and quantification, working concentration range, sampling rate, reagent consumption and effluent volume.

The detection limit (LOD) was calculated according to IUPAC recommendations (IUPAC 1976) in Chapters 3 to 6 and expressed in concentration units. It derived from the smallest measure (x_{LOD}) that can be detected with reasonable certainty for the developed analytical procedure. The value of x_{LOD} was calculated with the following equation:

$$x_{LOD} = \bar{x}_b + 3s_b$$

where $\bar{x}_b$ is the mean of ten consecutive blank measurements, s_b is the standard deviation of those blank measures and 3 is a numerical factor determined assuming a 95% confidence level.

The limit of quantification (LOQ) was also calculated according to IUPAC recommendations (IUPAC 1976) in Chapters 3 to 6. When expressed in concentration units, it derived from the smallest measure (x_{LOD}) that can be quantified with reasonable certainty for the developed analytical procedure. The value of x_{LOQ} was calculated with the following equation:

$$x_{LOQ} = \bar{x}_b + 10s_b$$

where $\bar{x}_b$ is the mean of ten consecutive blank measurements, s_b is the standard deviation of those blank measures and 10 is a default value that represents a10% relative standard deviation.

In Chapter 7, the limits of detection (LOD) and quantification (LOQ) were calculated from the least-squares linear regression parameters (Miller and Miller 1993). The detectable absorbance limit (y_{LOD}) was assessed as:

$$y_{LOD} = b + 3s_{y/x}$$

where b is the intercept and $s_{y/x}$ is the standard error of the linear regression. The detection limit, C_{LOD} was calculated by interpolation of y_{LOD} on the equation:

$$y_{LOD} = mC_{LOD} + b$$

where m corresponds to the slope of the regression. The quantification limit, C_{LOQ} was calculated by interpolation of y_{LOQ} on the equation:

$$y_{LOQ} = mC_{LOQ} + b$$

where y_{LOQ} was achieved through the equation:

$$y_{LOQ} = b + 10s_{y/x}$$

The working concentration range was established by analysing a serious of working standard solutions with different concentrations and determining the range where the relation between the signal and the concentration corresponded to a liner polynomial function.

The sampling rate was established by the time required for a complete analytical cycle and expressed as number of determinations performed per hour. The complete analytical cycle was calculated by adding the time necessary for each operation step.

The reagent consumption was calculated based on the volume of each reagent spent per assay and the concentration of the same reagent and expressed in terms of quantity (mol) per assay.

The effluent volume corresponds to the total volume wasted per assay and expressed in terms of quantity (mL).

2.7. Analysis of water samples

Calibration curves were established by analysing working standard solutions in the developed system and registering the corresponding signal in absorbance units. The relation between signal and concentration was linear for all developed methodologies. The analytes concentration in the samples was determined by interpolation on the above mentioned calibration curves.

The accuracy of the developed methods was evaluated by comparison of the results obtained by the proposed flow methods (C_{FLOW}) with those achieved by the corresponding reference procedures (C_{REF}). Both sets of results were plotted against each other (C_{FLOW} versus C_{REF}) and a linear regression was established ($C_{FLOW} = C_0 + sC_{REF}$) between the two variables. The parameters of the regression line, intercept (C_0) and slope (s) were calculated and a perfect agreement of the methods would mean a C_0 equal to 0 and s equal to 1. Consequently, the methods are statistically comparable if C_0 and s do not differ from 0 and 1 respectively. This was verified by estimating the errors in the intercept and slope values through calculation of their confidence limits at 95% significance level (Miller and Miller 1993).

The relative deviation (RD) values were calculated based on the following expression and expressed as percentage:

$$RD = \frac{C_{FLOW} - C_{REF}}{C_{REF}} * 100$$

In interference studies, the difference values were calculate based on the subsequent expression and expressed as percentage:

$$Difference = \frac{C_{(St+I)} - C_{(S)}}{C_{(S)}} * 100$$

where ($C_{(St+I)}$) is the concentration obtained of the standard with the interference specie and ($C_{(S)}$) is the concentration of the standard.

In the recovery tests the samples were analysed before and after a standard addition of analyte and the recovery (R_A) was calculated, according to IUPAC (2002), using the following expression:

$$R_A = \frac{Q_A(0+S) - Q_A(0)}{Q_A(S)}$$

where $Q_A(0+S)$ is the quantity of the analyte *A* recovered, $Q_A(0)$ is the quantity of the analyte in the original sample and $Q_A(S)$ is the amount of the analyte added (spike value).

In Chapter 6, a paired *t*-test was performed on the data obtained with the developed flow method and reference method (ICP-MS) for five sunscreens samples. The null hypothesis is verified if the *t* value calculated for each sample is lower than *t* critical value (P=0.05); indicating that there is no significant difference between the results obtained for both methods (Miller and Miller 1993).

In all the developed works, except in Chapter 7, the accuracy was additionally validated by analysis of certified reference materials. Results were considered accurate

if the concentration values obtained by the flow proposed methods were within the acceptance limits mentioned in the certified reference material specifications.

Chapter 3

Sequential injection trace determination of iron in natural waters using a long-pathlength liquid core waveguide and different spectrophotometric chemistries

This PhD program began with the development of a sequential injection analysis method for the determination of iron in coastal, ground and surface fresh waters using two different reagents (ferrozine and 1,10-phenathroline). The methodology used a double-line SIA system to improve mixing conditions between sample and the reagent solutions coupled with a liquid waveguide capillary cell to enhance the sensitivity of the determination. The detection limit for the ferrozine and 1,10-phenanthroline reagent was of 0.15 and 0.35 µg L^{-1}, respectively. The system provided a linear response up to 20 µg L^{-1}, a high throughput rate (41 h^{-1}) and low reagent consumption. The developed method was applied to natural waters (river, well, ground, potable and sea waters) and one reference water sample.

3.1. Introduction

The understanding of the bioavailability and the geochemical cycling of iron in marine systems (Miller et al. 1995) has been in the focus of environmental research in the last decades. Iron is a vital constituent of plant life, where it is essential for photosynthetic and respiratory electron transport, nitrate reduction, chlorophyll synthesis and detoxification of reactive oxygen species (Sunda and Huntsman 1995). Being a limiting nutrient for phytoplankton growth, it presents a consequent critical role (Coale et al. 1996) on the fixation of carbon dioxide by photosynthesis and on the CO_2 exchange between atmosphere and seawater (Watson et al. 2000). These effects have implications on global carbon, sulphur cycles and climate change.

Iron(II) is highly soluble in seawater but thermodynamically unstable (Achterberg et al. 2001). It is rapidly oxidized to iron(III) in the euphotic zone by hydrogen peroxide, oxygen (Millero and Sotolongo 1989), superoxide (O_2^-) and hydroxyl radical (OH) (Millero 1989). Therefore, iron(III) is the dominant form of dissolved iron in surface seawater (Millero and Sotolongo 1989; Achterberg et al. 2001), however, only a small fraction of iron(III) occurs in a free hydrated (Fe^{3+}) form. The major fraction (80% to 99%) is strongly complexed by organic ligands (Gledhill and van der Berg 1994), possibly produced by phytoplankton (Rue and Bruland 1997) or bacteria (Granger and Price 1999). The fraction of iron(II) in natural waters can appear as a result of the combination of photoreductive dissolution of particulate iron(III) (hydr)oxides (Miller et al. 1995) and of inorganic iron(III) complexes (King et al. 1993) with thermal, enzymatic and microbial cycling pathways (Croot et al. 2001) as well as through ocean atmospheric deposition (Zhuang et al. 1992) and diffusion from reducing sediments (Hong and Kester 1986). The mechanisms linked to the biological

uptake of iron remain uncertain (Achterberg et al. 2001), although recent studies have indicated that iron(III) complexed by organic ligands (siderophores and porphyrins) is available for uptake by different types of phytoplankton (Hutchins et al. 1999). With this in mind and to better understand the biogeochemistry of iron in waters, the acquisition of high-quality analytical data are a prerequisite.

The determination of iron in water samples is usually carried out for routine quality control, as limits are imposed by legislation on their content (Clesceri et al. 1998). In drinking water, the European Union directive (CEU 1998) sets the limit of 200 $\mu g\ L^{-1}$ for iron. Trace element analyses in natural and sea waters are even more demanding because of the different physical, chemical and biological processes involved (Hanrahan et al. 2002). In the case of iron analysis in seawaters, matrix effects and low concentrations levels are the main difficulties. To overcome these difficulties and to increase the sensitivity, separation and/or preconcentration of the analyte is often necessary (Lohan et al. 2005) in spectrophotometric methods.

Iron is present in many materials, causing a risk of contamination during sampling, filtration, storage and analysis (Achterberg et al. 2001). Flow analysis techniques with increased accuracy, precision, sample throughput (Morais et al. 2005), reproducible sample injection, controlled dispersion of the sample zone (Segundo and Rangel 2002), low detection limits, low reagent and sample consumption, simplified sample handing and reduced contamination risks are suitable and attractive tools for both shipboard and in situ determinations (Lunvongsa et al. 2006). Of the flow methods, sequential injection analysis uses a simple, reliable and robust manifold to perform automated wet-chemical analysis, allowing further reduced reagent and sample consumption, minimized waste production (Segundo and Rangel 2002) and precise automatic control of reaction conditions (Ruzicka and Sacampavia 1999). In SIA

systems, flow is reversed and mutual dispersion of the stack of sample and reagent zones occurs, owing to axial and radial diffusion, although only a partial overlap of analyte and reagent zones is achieved (Segundo and Rangel 2002). To overcome this potential difficulty, various modifications in the flow configurations have been introduced, one of which is the double-line SIA system (Morais et al. 2005). This strategy makes use of the merging-zones approach, where good overlapping of the reagent and sample zone is achieved.

Various flow-based spectrophotometric methods have been presented for the determination of iron. A comparison of various figures of merit for the different methods can be found in Table 3.7. The FIA methods with no preconcentration step (Pascual-Reguera et al. 1997) present a very high detection limit, whereas other FIA methods with a preconcentration step (Blain and Treguer 1995) present low detection limits but still have some disadvantages such as high reagent consumption and high effluent generation. Other existing flow approaches such as SIA (Morais et al. 2005), multi-commutated flow (Feres and Reis 2005) and multisyringe flow injection (Gomes et al. 2005) present lower reagent consumption but still inadequate detection limits for natural water samples.

The objective of this work was to exploit the potential of the liquid waveguide capillary cells in a sequential injection manifold to provide sufficient sensitivity for the trace iron determination with no need to introduce off-line or in-line preconcentration steps. This technology allows increasing of the pathlength in spectrophotometric measurements without light attenuation (Fuwa et al. 1984). In optical fibers, the light undergoes total internal reflection at the walls and not only the light-conducting path is transparent in the wavelength of interest, but the core region of the fiber also has a refractive index higher than that of the cladding material; therefore the light is trapped

in the optically denser core. Since 1993, the use of Teflon AF-2400 (DuPont 127 Fluroproducts, DE, USA)-a polymer that is largely transparent throughout the UV and visible range, with refractive index (1.29) lower than water (1.33) (Li et al. 2003)-has made it possible to construct totally internally reflecting detection cells that suffer from minimal light loss by scattering in the detection cell equipment with no light dispersion. Teflon is also chemically stable and inert, allowing its universal application. Liquid waveguide capillary cells are obligatorily coupled with optical fibers for bringing light in and out of the cell (Dasgupta et al. 2003). This detection cell, unlike conventional flow cells, has a low dispersion factor and less schlieren effects derived from the reduced internal volume. This technology was presented by Waterbury et al. (1997), using a continuous flow method with a long (4.5-m) pathlength waveguide capillary cell for the determination of iron with low detection limit, although it had the same disadvantages mentioned for FIA methods. Nowadays, with all the concern and the information about environmental problems, developing analytical method with low effluents generation for a greener chemistry is crucial.

In this work, we developed a spectrophotometric SIA flow system for the determination of iron in different water matrices. For this purpose, two colorimetric reagents were tested (ferrozine and *o*-phenanthroline) and compared in terms of sensitivity and susceptibility to interferences when a long pathlength liquid waveguide capillary cell was used as a detection cell.

3.2. Materials and methods

3.2.1. Reagents and solutions

River water certified reference material (NRC-SLRS-4) was also analysed for the evaluation of the accuracy of the developed method as recommended by the National Research Council of Canada.

All solutions used in interference studies (Zn, Cd, Mn, Cu, Al) were prepared by diluting commercial atomic absorption standards (Spectrosol).

A 2% (w/v) ascorbic acid solution was prepared daily by dissolution of the solid in a 2 mol L^{-1} acetic acid-ammonium acetate solution and final pH was adjusted with acetic acid to 4.2. A solution of 0.013 mol L^{-1} 1,10-phenanthroline was also prepared in 0.05 mol L^{-1} HCl. Ferrozine stock solution of 0.0025 mol L^{-1} was prepared by dissolving 0.1231 g of ferrozine (5,6-Diphenyl-3-(2-pyridil)-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate, $C_{20}H_{14}N_4O_6S_2$) in 100 mL of water.

3.2.2. SIA procedure and system configuration

The manifold configuration used for the determination of iron is shown in Fig. 3.1.

The determination of iron includes three steps and uses a single analytical sequence that is listed in Table 3.1. The first step was aspiration of the sample or standard to HC_1. At the same time, the colour reagent was aspirated into HC_2. In the second step, the buffer solution was drawn up to HC_1 and at the same time, the colour

reagent was aspirated into HC_2. Finally, the flow was reversed and the HC_1 and the HC_2 contents were mixed in a confluence (c, Fig. 3.1) and propelled through reaction coil (RC, Fig. 3.1) to the detector, where the change in absorbance was measured. The absorbance measurements were carried out at the wavelengths of 512 and 562 nm for the detection of iron-1,10-phenathroline and iron-ferrozine complex, respectively.

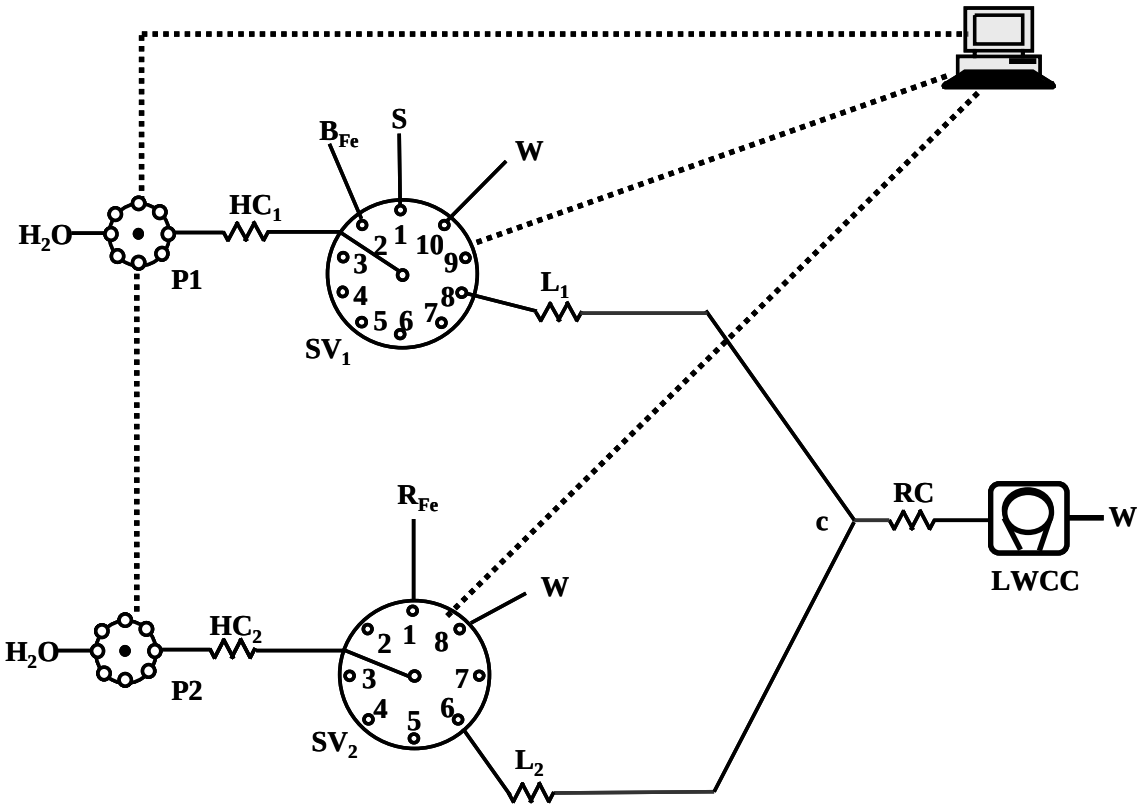

Figure 3.1. Sequential injection manifold for the determination of iron in natural waters. SV_1, SV_2: selection valves; P_1, P_2: peristaltic pumps; HC_1, HC_2: holding coils (2 m); RC: reaction coil (85 cm); L_1, L_2: reactors (25, 14 cm); c: confluence; LWCC: liquid waveguide capillary cell (1.0 m of pathlength) and CCD array spectrophotometer; W: waste; S: sample or standard; B_{Fe}: acetate buffer solution; R_{Fe}: colour reagent (ferrozine or 1,10-phenanthroline).

We compared SIA results with those provided by atomic absorption spectrometry (AAS) (Perkin Elmer, 4100 ZL, Uberlingen, Germany) with graphite furnace atomization (Clesceri et al. 1998) with Zeeman longitudinal background correction. We used 20 µL of sample and 5 µL of magnesium nitrate 3 g L^{-1} as matrix modifier per assay.

The primary objective of this work was the development of a sequential injection system for iron determination in waters using a LWCC, to achieve low detection/quantification levels.

Table 3.1. Sequential injection protocol sequence for the determination of iron in waters.

Step	Selection valve position		Operation time (s)	Flow rate (mL min^{-1})		Volume (mL)		Description
	SV_1	SV_2		P_1	P_2	P_1	P_2	
1	1	1	9.1	1.66	0.56	0.250	0.085	Aspirate sample and reagent
2	2	1	3.0	0.77	0.28	0.038	0.015	Aspirate buffer and reagent
3	8	6	50	3.81	1.16	3.180	0.97	Propel toward detector and signal measurement

To promote better mixing inside the SIA manifold between sample/standard and the colour reagent, the merging zones technique was adopted (Morais et al. 2005). In the proposed manifold, two selection valves were used and they were connected by a confluence placed before the reaction coil. In one side, standard/sample and buffer were

drawn up and in the other side, the colour reagent was aspirated. Then they were mixed in the confluence point (c) and the reaction product was propelled to the detector. This manifold configuration is based on a published work (Morais et al. 2005).

3.3. Results and discussion

The optimisation studies included not only physical parameters such as flow rates, tube lengths, plug sizes, sample and reagent volumes, but also chemical parameters such as reagent concentrations. The values used are summarised on Fig. 3.1 and Tables 3.1 and 3.3.

3.3.1. Study of physical and chemical parameters

Initial studies for setting up the physical parameters of the system were carried out using the ferrozine reagent. Initially, total plug size was studied in the range 150-400 µL, but the proportion between sample/standard and colour reagent was maintained (2.5:1). The best sensitivity was obtained for 350 µL total plug volume, with the volume of sample and reagent of 250 and 100 µL, respectively. The length of the reaction coil was studied in the range of 60-160 cm; 85 cm allowed higher sensitivity and therefore was adopted for further studies. Propelling flow rate was also studied in the range of 1.66-3.81 mL min^{-1} for pump 1 (Fig. 3.1) and in the range of 0.56-1.16 mL min^{-1} for pump 2 (Fig. 3.1). Flow rates of 3.81 and 1.16 mL min^{-1} for pumps 1 and 2, respectively, were used. Although sensitivity was similar in all cases, the flow rates chosen contributed to a shorter cycle time.

We also studied the influence of some chemical variables in the performance of the system.

In the case of pH, a good compromise between total reduction of iron(III) to iron(II) and complex formation between iron(II) and colour reagent must be achieved. For the complex formation with iron(II), the best sensitivity was obtained at pH 5.1 for the acetate buffer. The range studied was between 4 and 6 pH units and for complete reduction of iron(III) to iron(II) the pH of the acetate buffer must be below 4.2 (Fernandes et al. 1995). To achieve these conditions, a preliminary study was carried out off-line by mixing equivalent proportions of the sample and buffer solution in a beaker. The pH of the acetate buffer was set to 4.2 to maintain the pH at around pH 4.0 during the reduction process (sample/standards were adjusted to pH 2.0 before analysis). After the study of pH buffer, the concentration of the acetate buffer was also studied over the range of 0.01-2.5 mol L^{-1}. Only at the concentration of 2.0 mol L^{-1} was it possible to maintain pH 4.0 at the reduction process.

The ascorbic acid concentration was studied in order to assure the complete reduction of iron(III) to iron(II) in the range 1.0-4.0% (w/v). The minimum concentration of ascorbic acid that ensured the complete reduction of iron was 2% (w/v). This was proved by the similarity between the calibration curves of iron(III) and iron(II) standard solutions.

Finally, the concentrations of both colour reagents were studied. In the case of 1,10-phenanthroline, concentration values between 0.013 and 0.10 mol L^{-1} were tested and the sensitivity was similar in all cases. The concentration used was 0.013 mol L^{-1} with the aim of reducing reagent consumption. The [1,10-phenanthroline-iron] complex forms almost instantly with maximum absorption at 512 nm and produces an orange-red colour. The concentration of ferrozine was studied in the range of 0.25-5 mmol L^{-1}. The

sensitivity increased up to 2.5 mmol L^{-1}. For higher levels of ferrozine concentration, the sensitivity maintained constant, so the concentration selected was 2.5 mmol L^{-1}. The [ferrozine-iron] complex also forms instantly with maximum absorption at 562 nm and produces magenta colour.

The initial practical difficulties of bubble formation were overcome by filling the unused ports of the selection valve with the water carrier. Schlieren effects were observed in the signals obtained for the standard solution of 2 µg L^{-1} iron; however, they did not influence the peak height reading and can be minimized by using a reference wavelength for monitoring the refractive index changes during measurement. Best day-to-day performance of the LWCC was found when the flow cell was washed daily in counter current with a diluted HCl (0.05 M) solution.

3.3.2. Interference studies for both reagents

The interference of several ions on the determination of total Fe was tested (Table 3.2).

The tolerance level of error accepted was ±5% of the absorbance value of the standard (5.0 µg L^{-1}) used in the study. The ions tested were Cd^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+} and Al^{3+} in the levels of 5, 10, 20, 50, 500 and 1000 µg L^{-1}.

Table 3.2. Study of interfering species expressed as relative deviation from the absorbance value obtained for the standard solution of 5 µg L^{-1} iron for ferrozine reagent and 1,10-phenanthroline reagent.

Species tested	Ferrozine reagent		1,10-phenanthroline reagent	
	Concentration (µg L^{-1})	Difference (%)	Concentration (µg L^{-1})	Difference (%)
Zinc	1000	+5.0	1000	+3.4
Aluminium	1000	+3.7	1000	+12.5
Cadmium	50	+1.8	500	+10.6
Manganese	1000	+11.7	50	+14.8
Copper	10	+7.6	500	+14.2

For ferrozine reagent, the only major interference was from copper(II) at a level two times higher than iron. The copper(II) level in seawater is lower than Fe levels (Lide 1993-1994), so it is unlikely to interfere in the analysis of real samples. In the case of higher Cu levels, the addition of semicarbazide to the samples can remove this interference (Pascual-Reguera et al. 1997). For 1,10-phenanthroline reagent, the major interferences found were from Mn, Cu and Cd ions at levels ten (manganese) and one hundred times (copper and cadmium) higher than iron. Both Mn and Cd levels in seawater are lower than Fe levels (Lide 1993-1994).

The interference of NaCl in the determination of iron was also studied in the range of 0-35 g L^{-1} for both reagents. The deviations obtained for all concentrations studied and for both reagents were less than 5%, indicating that the salt present in seawater does not interfere with the determination and that the method is applicable to estuarine samples with variable salt concentrations.

Oxalate, cyanide and nitrite also cause interference at high concentrations (Stookey 1970). However, these ions are usually present in natural waters at trace levels and therefore their interferences are negligible (Zhang et al. 2001).

3.3.3. Analytical figures of merit

The overall features obtained for both colour reagents are summarized in Table 3.3. The limits of detection (LOD) and quantification (LOQ) were calculated as the concentration corresponding to three and ten times the standard deviation of the blank, respectively, of ten consecutive blank injections (IUPAC 1976).

The linear ranges obtained for ferrozine and for 1,10-phenanthroline reagents were 0.15-20 and 0.35-20 µg L^{-1}, respectively. The LOD and LOQ were lower for ferrozine reagent than for 1,10-phenanthroline reagent. This was expected because of the higher sensitivity of ferrozine reagent for iron determination.

Table 3.3. Figures of merit of the developed method.

Parameters	Values	
	Ferrozine	1,10-phenathroline
Detection limit (µg L^{-1})	0.15	0.35
Quantification limit (µg L^{-1})	0.49	1.21
Working range (µg L^{-1})	0.15 – 20	0.35 – 20
Determination rate (h^{-1})	41	41
Reagent consumption per assay (µmol)		
Colourimetric reagent	0.25	1.3
Ammonium acetate	80	80
Acetic acid	80	80
Ascorbic acid	4.5	4.5
Waste produced per assay (mL)	4.14	4.14

3.3.4. Application to water samples

The proposed method was applied to different types of water samples: seawater, well water, groundwater and river water.

Table 3.4 summarises the results obtained in iron recovery tests with both reagents in groundwater, seawater and well water. Additions of 2, 4, 10 and 20 µg L^{-1} of Fe(III) were made.

Table 3.4. Results obtained for recovery tests with both colour reagents in different types of water.

Colour reagent	Sample number	Recovery (%)[a]			
		Concentration added			
		2 µg L^{-1}	4 µg L^{-1}	10 µg L^{-1}	20 µg L^{-1}
Ferrozine	1	99 ± 9	99 ± 5	105 ± 2	104 ± 5
	2	93 ± 2	92 ± 5	95 ± 1	93 ± 1
	3	106 ± 30	79 ± 7	84 ± 1	83 ± 1
	4	106 ± 14	95 ± 3	99 ± 1	99 ± 1
1,10-phenanthroline	5	92 ± 9	89 ± 3	95 ± 1	91 ± 2
	2	105 ± 2	104 ± 1	103 ± 2	100 ± 1
	3	94 ± 6	95 ± 2	100 ± 2	98 ± 1
	4	96 ± 23	97 ± 4	97 ± 2	98 ± 1

[a] Mean and standard deviation of 5 replicates; Sample types: 1 -Groundwater; 2 to 4 - Seawater; 5 -Well water.

The recovery test results are good except those obtained for the addition of 2 µg L^{-1} iron, where the standards deviations are high. In the recovery tests of seawater samples, the results obtained show that seawater matrix does not seem to interfere with the determination of iron for either of the reagents.

The proposed SIA method with both colour reagents was applied to several water samples. To assess the quality of the results, they were compared with the reference method (AAS). Table 3.5 summarizes the results obtained.

Table 3.5. Comparison of the result obtained in the determination of iron in several samples of water using both SIA methods and the reference procedure.

Water source	Reference procedure ($\mu g\ L^{-1}$)	SIA ferrozine method ($\mu g\ L^{-1}$)	Relative deviation (%)	SIA 1,10-phenanthroline method ($\mu g\ L^{-1}$)	Relative deviation (%)
River	69.6 ± 6.7	70.5 ± 1.1	1.3	65.5 ± 1.2	-5.9
Ground	1.75 ± 0.00	2.02 ± 0.03	15.4	1.69 ± 0.09	-3.4
Potable	13.0 ± 0.6	12.8 ± 0.1	-1.5	13.5 ± 0.1	3.8
	1.0 ± 0.8	1.74 ± 0.02	74.0	1.67 ± 0.06	59.2
	0.75 ± 0.00	1.92 ± 0.05	1.56×10^2	1.1 ± 0.2	46.7
Well	1.69 ± 0.07	23.3 ± 0.1	1.28×10^3	1.59 ± 0.05	-5.9
	7.0 ± 0.4	5.73 ± 0.05	-18.4	6.9 ± 0.1	-1.4
	11.2 ± 1.0	30.4 ± 0.2	1.71×10^2	11.6 ± 0.2	3.6
Sea	14.1 ± 0.3	14.5 ± 0.1	2.8	16.2 ± 0.6	14.9
	7.0 ± 0.5	5.8 ± 0.1	-15.9	7.0 ± 0.2	0.0
	6.1 ± 0.3	4.9 ± 0.3	-17.1	5.0 ± 0.5	-18.0
	11.1 ± 0.0	9.6 ± 0.2	-13.5	10.7 ± 0.4	-3.6
	11.8 ± 0.0	10.1 ± 0.1	-14.4	12.2 ± 0.2	3.4

At low levels of iron, small absolute differences resulted in large relative errors between methods. The SIA ferrozine method is more sensitive and presented a lower standard deviation but also a lower tolerance to interference, as can also be verified in the Table 3.3. The results obtained by the SIA 1,10-phenanthroline method were very similar to the results obtained by reference methods.

For comparison purposes, a linear relationship ($C_{SIA}= C_0 +SC_{RF}$) was established for both colour reagents. The regression equation found for the determination of iron by ferrozine was $C_{SIAferrozine}= 1.01 (\pm0.04)\bullet C_{RF} – 0.511 (\pm0.910)$ (n=11, excluding the two well water samples with higher relative deviation). For the determination of iron by 1,10-phenanthroline the equation can be written as $C_{SIA1,10\text{-}phenanthroline}= 0.95 (\pm0.03) \bullet C_{RF} + 0.615 (\pm0.689)$ (n=13). For both equations, the values in parenthesis are the limits of the 95% confidence intervals and R^2=0.997. From these values, it is clear that the calculated slope and intercept lie close to the values of 1 and 0, respectively. Therefore, it can be concluded that the two sets of results (Miller and Miller, 1993) obtained by the proposed methodologies and reference procedure are in a good agreement.

The proposed SIA method with both colour reagents was also applied to certified reference standard SLRS-4 with certified value of 103 ± 5 µg L^{-1}; the results obtained were 98.9 ± 0.7 µg L^{-1} and 104.7 ± 0.7 µg L^{-1} for the ferrozine and for the 1,10-phenanthroline method, respectively. The results correspond to the average (n=10) and the half width of the 95% confidence interval. The certified sample was diluted 10 times in order to fit in the linear range of both methods. The results obtained showed a good accuracy for both reagents.

3.4. Conclusion

A comparison of figures of merit with different methods for the determination of iron is shown in Table 3.6. The developed work offers some improvements such as low detection limits without any preconcentration step, thus providing high determination throughputs, low reagent consumption and low effluent production. The LWCC flow

cells present a reliable alternative for increasing the sensitivity of the spectrophotometric analytical procedures. Although the achieved detection limits are higher than those required for open ocean iron monitoring, the scope of this work was on estuarine water monitoring, where the expected iron levels are higher.

Another objective of the work was to compare 1,10-phenanthroline and ferrozine reagents in the determination of iron in different sources of water, using this automatic system. It should be emphasized that the sensitivity and extent of interferences depend on the flow systems, as it is in fact a kinetic determination. In these conditions, ferrozine reagent presents a higher sensitivity for iron as well as for copper as an interferent. The consumption per assay of ferrozine reagent is less than 1,10-phenanthroline reagent, although it is significantly more expensive. The two reagents are in the same safety class (WGK=3) in the material safety data sheet. The existing alternative spectrophotometric batch method for iron (Clesceri et al. 1998) is based on the 1,10-phenanthroline reagent; this method is not directly comparable to our method, as it is applicable only to samples with at least 10 $\mu g\ L^{-1}$ iron, where the reagent consumption is typically 1000 times higher than that of our method.

Table 3.6. Comparison of analytical characteristics of flow methods with low detection limits for the determination of iron (continuing).

System	Detection mode	Range ($\mu g\ L^{-1}$)	Detection limit ($\mu g\ L^{-1}$)	Determination rate (h^{-1})	Reagent consumption (mmol $assay^{-1}$)	Matrix	Preconcentration	Reference
Continuous flow	UV-Vis (LWCC)	Up to 0.56	0.011	n.g.	0.002 FZ	Aqueous standards	n.a.	Waterbury et al. 1997
Gas-segmented continuous flow	UV-Vis (LWCC)	Up to 2.8	0.006	40	0.0015 FZ	Aqueous standards	n.a.	Zhang et al. 2001
FIA	CL	Up to 2	0.02	20	0.022 DPD	River and tap water	n.a.	Lunvongsa et al. 2006
FIA	UV-Vis.	Up to 4.2 Fe(II)[a] Up to 0.6 Fe(II)[b] Up to 11 Fe(III)[a] Up to 0.2 Fe(III)[c]	0.006 Fe(II), 0.017 Fe(III)	10	0.001 FZ	Seawater	C18	Blain and Treguer 1995
Continuous flow	CL	Up to 0.1	0.003	5	0.003 luminol 2.7 H_2O_2	Seawaters	MAF-8HQ (8-quinolinol-immobilised fluoride containing metal alkoxide glass)	Obata et al.1993
FIA	CL	Up to 11	0.045 Fe(II), 0.020 total Fe	9	0.002 BSF H_2O_2 NG	River, rain and tap water and coastal seawater	Amberlite XAD-4 with HEED groups	Hirata et al. 1999

Table 3.6. Comparison of analytical characteristics of flow methods with low detection limits for the determination of iron (continuation).

System	Detection mode	Range ($\mu g\ L^{-1}$)	Detection limit ($\mu g\ L^{-1}$)	Determination rate (h^{-1})	Reagent consumption (mmol $assay^{-1}$)	Matrix	Preconcentration	Reference
FIA	CL	Up to 11	0.045 Fe(II), 0.020 total Fe	9	0.002 BSF H_2O_2 NG	River, rain and tap water and coastal seawater	Amberlite XAD-4 with HEED groups	Hirata et al. 1999
FIA	CL	Up to 0.5	0.001	~12	~0.001 DPD	Seawater	Vinyl polymer-8HQ	Measures et al. 1995
SIA	UV-Vis (LWCC)	Up to 20 FZ Up to 20 PHE	0.15 FZ, 0.35 PHE	41 for both reagents	0.0013 FZ 0.00025 PHE	Well, river, ground, potable and seawaters	-	Developed method

n.a.-not applied; n.g.-not given; UV-Vis: spectrophotometric; NG:not given; FZ: ferrozine; CL: Chemiluminometric; DPD: N,N-dimethyl-p-pheny-lenediamine; FL: Fluorimetric; BSF: Brillt sulfoflavine; Amberlite XAD-4: polysterene-divinylbenzene macroporous resin; HEED: N-hydroxyethyl-ethylenediamine; PHE: 1,10-Phenanthroline; Loading time: [a]2 min, [b]6min, [c]10min.

Chapter 4

A multi-syringe flow injection system for the spectrophotometric determination of trace levels of iron in waters using a liquid waveguide capillary cell and different chelating resins and reaction chemistries

The second work of this PhD program was the development of a method able to reach lower levels of iron than previous work. Thus, a long waveguide capillary cell with a preconcentration resin was coupled to a multi-syringe flow injection analysis system to attain this objective. A liquid waveguide capillary cell was used. Two different preconcentration resins were tested, Chelex 100 and NTA Superflow. The determination of iron in this work was also based on a colorimetric reaction and two reagents were also tested, ferrozine and ammonium thiocyanate. The developed method employing the NTA Superflow with ferrozine colorimetric reagent provided a detection limit of 0.05 µg L^{-1} with a linear response up to 8 µg L^{-1} and a sample throughput of 12 per hour. The developed system presents low reagents/sample consumptions. The accuracy was assessed using a certified reference water sample.

4.1. Introduction

The importance of the determination of iron was already discussed in the previous chapter.

There is a wide variety of possible instrumental methods for the determination of iron, spectrophotometry (Gomes et al. 2005; Morais et al. 2005; Pons et al. 2005), chemiluminescence (Elrod et al. 1991), HPLC (Matsumiya et al. 2004), adsorptive stripping voltammetry (Segura et al. 2008), fluorescence (Chen and Chang-Qing 2007), inductively coupled mass spectrometry (ICP-MS) (Yan et al. 2000) and atomic absorption spectrometry (AAS) (Costa and Araújo 2001). However when trace levels of the analyte is concerned, the applicable detection methods are reduced.

Flow analysis systems provide several advantages for sample manipulation including simplicity, equipment cost, accuracy, good reproducibility, elevated sample throughput, in-line sample manipulation, high degree of automation and reduction in the consumption of samples/reagents and in effluent production (Cerdà et al. 2007; Morais et al. 2005; Segundo and Rangel 2002).

There are several spectrophotometric systems for iron determination using different flow strategies. Some of these applications use this determination as a case study to demonstrate the capabilities of some newly developed flow handling techniques. In this context, Pons et al. summarizes the development and improvement of flow procedures based on using the same reaction chemistry for the determination of iron in different flow strategies (Pons et al. 2006). Within the published spectrophotometric flow methods there are several that use preconcentration/separation approaches incorporated in FIA (Blain and Treguer 1995), SIA (Rubí et al. 1997), MSFIA (Gomes et al. 2005) and multi-pumping (Pons et al. 2005) manifolds.

MSFIA is one of the most recent flow analysis techniques and it was first proposed by Cerdà and co-workers (Cerdà et al. 1999). Among with other flow analysis techniques, it offers high sampling rate, robustness, versatility and low consumption of reagents and samples (Miró et al. 2002). MSFIA combines the multi-channel operation of flow injection analysis with the ability to select the exact volumes of sample and reagents needed for analysis, as it is carried out in sequential injection analysis (programmable flow) mode. One not frequently mentioned, but not less important characteristics of the MSFIA system is that it is also able to work under moderate backpressure, thus is very suitable when using intercalated resins (Cerdà et al. 2007; Pons et al. 2006) or even monolithic columns (González-San Miguel 2009).

Various resins are described in literature as C18 (Blain and Treguer 1995), Amberlite XAD-4 (Hirata et al. 1999), MAF-8HQ (Obata et al. 1993), 8HQ (Measures et al. 1995), Chelex 100 (Pons et al. 2006; Rubí et al. 1997) and NTA Superflow (Lohan et al. 2005; de Jong et al. 2008) for iron preconcentration. The latter NTA Superflow resin was originally designed for high throughput sample clean-up procedures based on the affinity chromatography concept. It presents several advantages as, the analyte recovery at low pH and the supported high flow rates which is very interesting for using in applications where high sampling rate is needed. Recently, this commercial resin was applied for the preconcentration of total iron in low pH open sea water samples (Lohan et al. 2005). The developed work presented remarkable analytical characteristics; those in one part can be attributed to the new resin and in other part to the applied ICP-MS detector.

One objective of this work was to apply and compare the characteristics of the NTA resin to the well established Chelex 100 cationic exchange resin (operating at a higher ≈4 pH range), under flow analysis conditions and using a spectrophotometric

detection system with a liquid waveguide capillary cell. In the LWCC, the spectrophotometric pathlength is increased without considerable light attenuation (Fuwa et al. 1984). The light is carried by means of an optical fiber to the LWCC where it undergoes total internal reflection on the cell walls. The potential to exploit this equipment was only possible since 1993 with Teflon AF-2400 (DuPont Fluroproducts, DE, USA). This polymer is chemically stable, inert and is mainly transparent through UV and visible range with refractive index (1.29) lower than water (1.33) (Li et al. 2003).

The ultimate objective of the work was to develop an automated method capable to detect and quantify low levels of iron in waters, with minimized reagent consumption. For this purpose the LWCC and a preconcentration mini-column were coupled to a MSFIA system. Two different chemistries were applied for downstream spectrophotometric detection of iron in the different oxidation states of Fe^{2+} and Fe^{3+}, involving the colorimetric reagents ferrozine and ammonium thiocyanate, respectively.

4.2. Material and methods

4.2.1. Reagents and solutions

River water certified reference material (NCR-SLRS-4) was also analysed for the evaluation of the accuracy of the developed method as recommended by the National Council of Canada.

Ferrozine solution of 2.5 mmol L^{-1} was prepared daily by dissolving 0.0185 g of ferrozine reagent ($C_{20}H_{14}N_4O_6S_2$) in a 2% (w/v) ascorbic acid solution prepared in a 2 mol L^{-1} acetic acid ammonium acetate solution where the final pH was adjusted with

acetic acid to 4.5.

The ammonium thiocyanate solution of 1.5 mol L^{-1} was prepared by dissolving 11.4 g of the solid (NH_4SCN) in a 100 mL of MilliQ water.

The Chelex 100 resin (Bio-Rad, 200-400 mesh, sodium form) was suspended in a conditioning buffer obtained from 6.56 g of sodium acetate and 16 mL of concentrated acetic acid solution in 200 mL of water and with final pH adjusted with acetic acid to 4. The same procedure was adopted to prepare the conditioning buffer/complexing agent solution, with water being replaced by a 1 mol L^{-1} sodium chloride solution. The 1 mol L^{-1} sodium chloride solution was prepared by dissolving 5.84 g of sodium chloride in 100 mL of water.

The resin NTA Superflow resin (Qiagen Inc., Valencia, Spain) is available in a form of suspension therefore it is ready to use.

Before application to iron determination the prepared resin columns were washed with 1.0 mol L^{-1} HCl solution, until stable (RSD< 3%) blank reading was obtained.

4.2.2. MSFIA procedure and system configuration

The manifold configuration used is shown in Fig. 4.1 and the protocol sequence is listed in Table 4.1.

For all solenoid valves, the exchange options were classified in on/off lines. The “on” line was assigned to the flow network and the “off” line to the solution flasks (represented with a solid line and dotted line, respectively on Fig. 4.1).

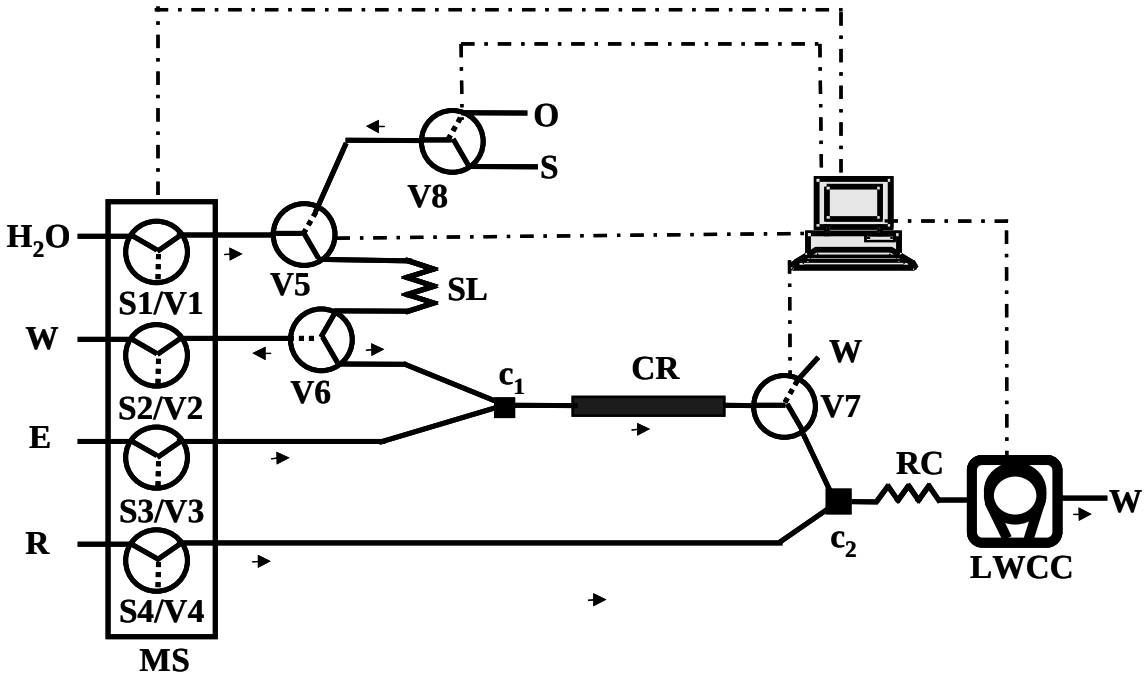

Figure 4.1. Multi-syringe flow injection analysis manifold for the determination of iron in waters. S_i: syringes; V_i: solenoid valves in position "on" (discontinuous line) or "off" (continuous line); SL: sample loop (4 mL); RC: reaction coil (100 cm); c_i: confluence; LWCC: detector (100 cm optical path, 480 and 562 nm for ammonium thiocyanate and ferrozine, respectively); MS: multi-syringe module; CR: chelating resin (NTA Superflow or Chelex 100); W: waste; S: sample or standard; O: oxidant (and conditioning agent for Chelex 100 resin); E: eluent; R: color reagent (ammonium thiocyanate or ferrozine).

The first step consists in washing and conditioning the chelating resin. Afterwards, a total volume of 3.6 and 0.4 mL of sample and oxidant, respectively, are aspirated to the sample loop via syringe 2 in a binary sampling mode. The aspirated solution sequence was made up as 0.2/1.8/0.1/1.8/0.1 mL of alternating oxidant and sample plugs. Then, the mixture contained in the sample loop (SL) is propelled through the chelating resin and solenoid valve (V7) to waste. The elution of the retained analyte is carried out in two steps. First, 0.25 mL of the eluent is propelled through the resin. At the second phase, a portion of the colour reagent (0.25 mL) is introduced at confluence 2 (c_2), downstream and simultaneously with the second part (0.25 mL) of the eluent. In

the final step the resulting mixture is transported to the detector and the analytical signal is registered. The spectrophotometric measurements were carried out at the wavelength of 480 and 562 nm for the detection of iron-ammonium thiocyanate and iron-ferrozine complex, respectively. Reference wavelength for minimizing the schlieren effect was set at 700 nm.

Table 4.1. MSFIA protocol sequence for the determination of iron in waters.

Step	Piston movement	Position of solenoid valves								Volume (mL)	Flow rate (mL min^{-1})	Description
		V1	V2	V3	V4	V5	V6	V7	V8			
1	Dispense	0	0	1	0	0	0	0	0	1.3	4	Cleaning the chelating resin
2	Pick up	0	1	0	0	0	0	0	0/1	3.6 [s]; 0.9 [o]	5	Aspirate sample and oxidant
3	Dispense	1	0	0	0	1	1	0	0	4.5	2.5	Propel sample through the resin
4	Dispense	0	0	1	0	0	0	1	0	0.25 [e]	2	Propel eluent through the resin
5	Dispense	0	0	1	1	0	0	1	0	0.25 [e]; 0.25 [r]	2	Propel eluent and colour reagent to the detector
6	Dispense	1	0	0	0	0	0	1	0	2.5	3	Propel reaction mixture to the detector and signal registration

[s] sample; [o] oxidant; [e] eluent; [r] colour reagent; 0 -off; 1 -on.

The MSFIA system was designed to permit the determination and monitoring of iron in estuarine waters at low concentrations. To attain this objective, the advantages of the increased sensitivity of LWCC and the preconcentration chelating resin were coupled in this MSFIA manifold. Four solenoid valves were included (Fig. 4.1) in the set-up. The sampling step was based on the aspiration of the sample into a fixed volume

sample loop, as introducing the sample to the system from one of the syringes would require cumbersome washing steps to avoid contamination between consecutive solutions. For that reason, three additional solenoid valves (V5, V6 and V8) were attached to the system. The solenoid valve V7 was connected to minimize the contact between the solutions and the flow cell.

The volume of sample introduced was controlled by the length of the sample loop and the binary sampling technique was used to promote a better mixing between the sample or standard with the oxidant (when using Chelex 100 resin the solution of oxidant was prepared with conditioning buffer/complexing agent solution for resin regeneration). The binary sampling mode was achieved by rapid commutation of valve V8, allowing the consecutive and alternate aspiration of plugs of sample and oxidant, promoting their mutual overlapping before filling the injection loop. If this mixing was achieved with a conventional confluence, the sample would be diluted and the repeatability would be compromised.

4.3. Results and discussion

Several physical and chemical parameters were studied and optimised by the univariate method, where only one parameter was changed while others were kept constant.

4.3.1. Study of physical and chemical parameters

Initial studies for setting up of the physical parameters of the system were carried out using the ammonium thiocyanate reagent with both resins. First, the effect of

the size of the resin column was studied by changing the length (10-50 mm) and the diameter (1.65-2.29 mm) of the PVC tubing. The highest sensitivity was achieved using the dimensions of 25 mm of length and 2.29 mm of diameter, for longer columns the operational stability decreased. In preliminarily studies it was observed that Chelex 100 resin became more compressed than NTA Superflow resin at the same flow rates and this alteration was more evident in longer columns.

The influence of the sample volume was studied using injection volumes in the range of 1-5 mL. Increasing the sample volume increased the sensitivity of the method without evidence on saturating the resin column, however considerable increase in cycle time was registered. A 4 mL sample volume was selected as a compromise between low detection limits and cycle time.

As the elution step was reported to be largely predominant upon the extraction step (Vanloot et al. 2007), the following study consisted in evaluating the effect of the eluent plug size on sensitivity. The range studied for the plug size of the eluent was 0.125-0.5 mL and the best sensitivity was obtained with 0.25 mL (for both steps 4 and 5, Table 4.1). The influence of plug size of the colour reagent on the sensitivity was also studied between 0.0625-0.5 mL and 0.25 mL was chosen because it presented the highest sensitivity.

Following this, the effect of the length of the reaction coil (RC) on the sensitivity of the method was studied for lengths between 25 and 200 cm and the 100 cm was selected as allowed better sensitivity, while in longer reactors the sensitivity decreased probably due to the increased sample dispersion.

At the end of the physical setup, the effect of the flow rate of every step in the analytical cycle was also evaluated and the results are summarised in Table 4.2. The flow rate for each step was selected as a compromise between sensitivity, shorter cycle

time and low pressure for the resins columns.

Table 4.2. Study of the flow rates for all the steps.

Step	Range studied (mL min^{-1})	Selected value (mL min^{-1})
1	1-5	4
2	1-10	5
3	1-5	2.5
4	1-5	2
5	1-5	2
6	1-5	3

The chemical variables studied included the eluent concentration, the pH and the concentration of ascorbic acid.

Firstly, the effect of the HCl eluent concentration was studied in the range of 0.25-1.5 mol L^{-1} and 1.0 mol L^{-1} was chosen because it presented the highest sensitivity and same as the 1.5 mol L^{-1} solution. The elution step is particularly studied in the literature and it is an important parameter. It is generally accepted that the most appropriate acids to elute is HCl (Measures et al. 1995; Vanloot et al. 2007) and HNO_3 (Rubí et al. 1997). Lohan et al. reported no difference between the two acids when applied as elution solutions, and the manufacturer of the LWCC recommended the use of hydrochloric acid in order to prevent flow cell damage, therefore this acid was applied.

The effect of the ammonium thiocyanate colour reagent concentration was studied in the range of 0.5 - 3 mol L^{-1} and the best sensitivity was obtained for 1.5 mol

L^{-1}.

Similarly, the effect of the ferrozine colour reagent concentration was tested in the range of 0.62-5.0 mmol L^{-1} and the concentration of 2.5 mmol L^{-1} allowed the highest sensitivity. When using ferrozine colour reagent, the effect of pH was studied in order to achieve a good compromise between total reduction of iron(III) to iron(II) and complex formation between iron(II) and ferrozine. For complete iron(III) reduction, pH must be below 4.2 (Fernandes et al. 1995). An off-line study was performed in order to maintain the pH at the reduction step around pH 4.0 (the eluent had a concentration of 1.0 mol L^{-1} HCl). Therefore, the range of the concentration and the pH of the ammonium acetate buffer studied were 0.01-2.5 mol L^{-1} and 4-5 pH units, respectively. Only a solution with 2.0 mol L^{-1} and 4.5 pH units was capable to achieve the objective above. The concentration of ascorbic acid solution was varied between 1-4% (w/v) and the minimum concentration to assure complete iron reduction was 2% (w/v).

Finally, the effect of H_2O_2 oxidant concentration was studied using the NTA Superflow resin. For this study solutions of iron(II) and iron(III) were prepared from ammonium iron(II) sulphate hexahydrate and for iron(III) solution potassium permanganate was added to the standard solution as an oxidizing agent. The H_2O_2 concentration was varied between 0.002-0.2 mol $L^{-1}$and the minimum concentration necessary to oxide iron(II) to iron(III) in the samples/standards was 0.2 mol L^{-1}.

Schlieren effect in the detection cell was noticed although it could be minimised by using a reference wavelength for monitoring the refractive index changes during measurement.

At the end of a working day, the LWCC was washed consecutively with HCl (0.05 mol L^{-1}) and NaOH (0.05 mol L^{-1}) solutions in counter current.

4.3.2. Comparison of the resins

It should be emphasized that the incorporated resin columns not only act as preconcentration units but as well have the objective of matrix removal. Both resins have been applied to the determination of trace elements in natural waters and efficient matrix removal was reported for NTA by Lohan and for Chelex 100 by Jiménez et al. in the case of application to sea water samples. However pH dependent swelling is frequently reported for Chelex and ammonium acetate washing of this column is necessary to remove alkaline and alkaline-earth metals in excess.

These two resins were compared in several features and the calibration curves obtained for both resins are presented in Fig. 4.2.

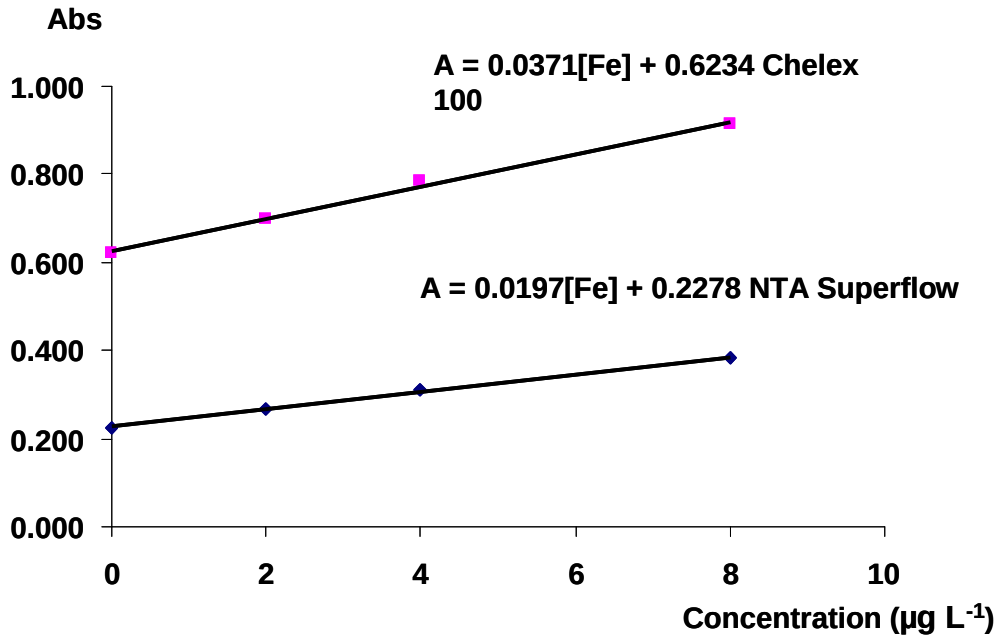

Figure 4.2. Calibration curves using the same manifold configuration and concentration of reagents for the different resins applied (n=3, for all standard solutions injected)

The use of Chelex 100 resulted in a higher sensitivity for iron(III) by comparison of the slopes of the equations of the calibration curves (same system set-up and the

same ammonium thiocyanate reagent for both resins). The equations of the calibration curves for Chelex 100 and NTA Superflow resins were, Abs=0.0371[Fe] + 0.6234 and Abs=0.0197[Fe] + 0.2278, respectively (Fig. 4.2). Iron concentration expressed as µg L^{-1}.

However, with Chelex 100 resin, the use of a regeneration solution was imperative and this solution contributed to higher blank signals. The standard deviation from the ten consecutive injections of the blank was always higher. Consequently, both detection and quantification limits obtained were higher too. Additionally, at higher flow rates the compression of the column occurred and leak of the solutions passing through the column was noticed due to the increased pressure inside the system. In all these operational characteristics considered the NTA Superflow resin showed superior stability.

4.3.3. Comparison of the reaction chemistries

In this study, the two colour reactions were compared. The ferrozine reagent forms a complex with iron(II), therefore the solution containing ferrozine must have ascorbic acid in order to reduce all of the iron(III) to iron(II). The ammonium thiocyanate reagent forms a complex with iron(III), hence there is no need of iron(III) reduction. The equations of the calibration curves using ferrozine and ammonium thiocyanate with Chelex 100 resin are, Abs=0.087[Fe] + 0.7806 and Abs=0.0371[Fe] + 0.6234, respectively (Fig. 4.3).

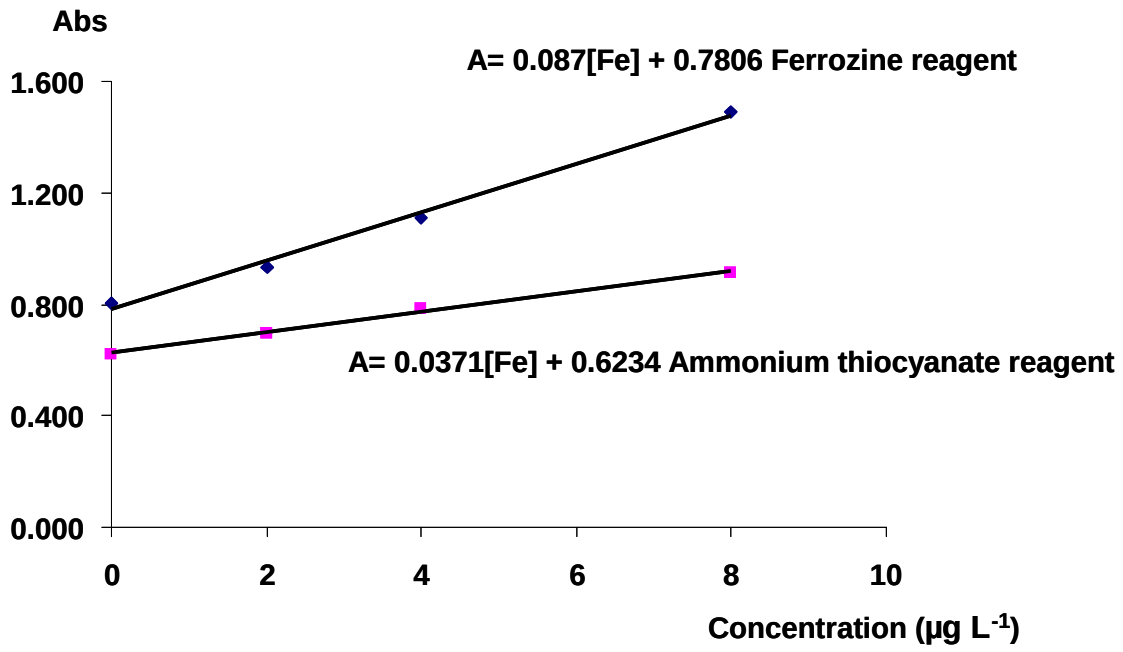

Figure 4.3. Calibration curves using the same system configuration and the Chelex 100 resin for the two tested colour reagents

From the comparison of the slopes, it can be concluded that ferrozine reagent presents a higher sensitivity. The blank signals are quiet high for ferrozine reagent even though the detection and quantification limits are lower. This can only be attributed to the higher sensitivity. Hence, the ferrozine colour reagent was the best option.

4.3.4. Analytical figures of merit

All the analytical characteristics achieved using the two resins and colour reagents are summarized in Table 4.3. Both detection (LOD) and quantification (LOQ) limits were calculated as the concentration corresponding to the blank signal three and ten times the standard deviation, respectively, of ten consecutive blank injections (IUPAC 1976).

Table 4.3. Figures of merit of developed methods.

Resin	Chelex 100		NTA Superflow	
Reagents	Thiocyanate	Ferrozine	Thiocyanate	Ferrozine
Detection limit ($\mu g\ L^{-1}$)[a]	0.56	0.20	0.52	0.05
Quantification limit ($\mu g\ L^{-1}$)	1.15	0.83	1.16	0.08
Working range ($\mu g\ L^{-1}$)	0-20	0-15	0-50	0-8
Determination rate (h^{-1})	12	12	12	12
Reagent consumption per assay (mmol)				
HCl	0.5	0.5	0.5	0.5
Ammonium thiocyanate	0.38	-----	0.38	-----
Ferrozine	-----	0.00062	-----	0.00062
Hydrogen peroxide	0.18	0.18	0.18	0.18
Acetic acid	1.26	-----	1.26	-----
Sodium acetate	0.36	-----	0.36	-----
Ascorbic acid	-----	0.028	-----	0.028
Ammonium acetate/acetic acid	-----	0.5	-----	0.5

[a] assessed from the standard deviation from blank signal (n=10) (IUPAC 1976); ----- - not applied.

The detection and quantification limits were always lower when using ferrozine colour reagent because of the higher sensitivity for iron. Although Chelex 100 resin presents a higher sensitivity for iron(III), as it was said above, it is the combination of NTA Superflow with ferrozine reagent that presents the lower detection and

quantification limits. The innovation in this strategy lay on using a resin column that acts as a preconcentration and interference elimination unit and combine it with a LWCC in a MSFIA manifold. Nonetheless, with the increase of the light pathlength, all absorbance signals are amplified including the blank and in this case it can be a limitation for LWCC use, as high blank signals normally predict high detection and quantification values.

4.3.5. Application to water samples

Concluded the comparative studies, the method using NTA Superflow resin and ferrozine colour reagent was applied to a certified reference sample. The sample SLRS-4 with certified value of 103 ± 5 µg L^{-1} was analyzed and the result obtained was 102 ± 3 µg L^{-1}. The result corresponded to the average (n=11) assays and the half width of 95% confidence interval. The certified sample was diluted fifty times in order to fit in the linear range. The result obtained for the sample shows a good accuracy for the developed method.

4.4. Conclusion

In this manuscript, a systematic study on the comparison of the preconcentration resins of Chelex 100 with NTA Superflow resin and ammonium thiocyanate with ferrozine is demonstrated in the determination of iron.

From the developed work, NTA Superflow resin clearly present better operational stability moreover no additional conditioning solution is necessary. The

developed method reached low detection limits for iron in waters with a good sampling rate. It should be emphasized that if sampling rate is not a crucial parameter, even lower detection limits can be reached by increasing the sample load.

In Table 4.4 there is a comparison of several features of different flow methods for the determination of iron. If compared to other existing spectrophotometric alternatives, the present work shows low reagent consumption and effluent production.

The developed method is the first to combine a liquid waveguide capillary cell with a preconcentration resin in a MSFIA system. It can be also confirmed that the LWCC equipment provides a consistent and reliable option for increasing the sensitivity of spectrophotometric analytical procedures.

Table 4.4. Comparison of analytical figures of different flow methods for the determination of iron (continuing).

System	Detection mode	Range (μg L^{-1})	Detection limit (μg L^{-1})	Determination rate (h^{-1})	Sample volume (mL)	Reagent consumption (mmol/assay	Matrix	Preconcentration	Reference
FIA	UV-Vis.	Up to 4.2 Fe(II)[a] Up to 0.6 Fe(II)[b] Up to 11 Fe(III)[a] Up to 0.2 Fe(III)[c]	0.006 Fe(II) 0.017 Fe(III)	3	40	0.001 FZ	Seawater	C18	Blain and Treguer 1995
FIA	CL	Up to 11	0.045 Fe(II) 0.020 total Fe	9	5.6	0.002 BSF H_2O_2 n.g.	River, rain, tap waters and coastal seawater	Amberlite XAD-4 with HEED groups	Hirata et al. 1999
Continuous flow	CL	Up to 0.1	0.003	5	18	0.003 luminol 2.7 H_2O_2	Seawater	MAF-8HQ	Obata et al. 1995

Table 4.4. Comparison of analytical figures of different flow methods for the determination of iron (continuation).

System	Detection mode	Range (μg L^{-1})	Detection limit (μg L^{-1})	Determination rate (h^{-1})	Sample volume (mL)	Reagent consumption (mmol/assay	Matrix	Preconcentration	Reference
FIA	UV-Vis.	Up to 0.5	0.001	~12	n.g	~0.001 DPD	Seawater	Vinyl polymer-8HQ	Measures et al. 1995
SIA	AAS	Up to 1200[d] Up to 400[e]	12[d] 6[e]	n.g.	9 27	n.n.	Water samples	Chelex 100	Rubí et al. 1997
MSFIA	UV-Vis.	Up to 8	0.05	12	4	0.0006 FZ	River water	NTA Superflow	Developed method

n.g.: not given; n.n.: not necessary; UV-Vis: Spectrophotometric; CL: Chemiluminometric; AAS: Atomic Absorption Spectrometry; FZ: Ferrozine; DPD- N,N: dimethyl-p-phenylenediamine; BSF: Brilliant sulfoflavine; LWCC: Liquid waveguide capillary cell; HEED: *N*-hydroxyethylethylenediamine; MAF-8HQ: 8-quinolinol-immobilised fluoride containing metal alkoxide glass; [a]- 2 min of loading time; [b]- 6 min of loading time; [c]- 10 min of loading time; [d]- 9 mL of sample; [e]- 27 mL of sample.

Chapter 5

Spectrophotometric determination of zinc and copper in a multi-syringe flow injection analysis system using a liquid waveguide capillary cell

The third work of this PhD program was the development of a multi-syringe injection analysis system coupled with a liquid waveguide capillary cell for the spectrophotometric determination of zinc and copper at low levels in waters. The developed methodology for both ions was based on a colorimetric reaction with zincon reagent at different pH values. A liquid waveguide capillary cell was used to enhance the sensitivity of the detection. The detection limit for copper and zinc was 0.1 and 2 µg L^{-1}, respectively, without the need for any preconcentration step. The system also provided a linear response up to 100 µg L^{-1} with a high throughput (43 h^{-1}) and low reagent consumption and effluent production for both ions. The developed work was applied to natural waters and three certified reference water samples.

5.1. Introduction

In recent years, due to the increase of pollution strictly connected with human activity, quantitative routine analysis has been in focus. Flow analysis systems, especially in water analysis, are very suitable for this purpose because of increased accuracy, good reproducibility, precision, equipment cost, elevated throughput, simplified sample handling, reduced contamination risks, high degree of automation and reduction in the consumption of samples/reagents and in effluent production (Cerdà et al. 2007; Segundo and Rangel 2002). Within the various flow methods, one of the most recent is MSFIA. It was first proposed by Cerdà et al. 1999 and along with other flow analysis techniques, it presents versatility, robustness, high sample throughput and low consumption of reagents and samples (Miró et al. 2002). MSFIA combines the multi-channel operation of flow injection analysis with the ability to select the required volume of sample and reagents for analysis, a characteristic feature of sequential analysis (programmable flow) mode. Therefore, MSFIA can be an advantageous alternative to downscale environmental monitoring assays.

Zinc and copper ions are essential for normal physiological processes of living organisms (Burguera-Pascu et al. 2007). In humans, zinc is the second most abundant transition metal ion, acting in several biological systems and is also a cofactor in diverse biochemical processes of bacteria and plants (Yang et al. 2004). It is an essential nanonutrient in ocean surface waters and can be present in organically complexed and in phytoplankton integrated forms (Nowicki et al. 1994). Copper also plays an important role as a component of some oxidoreductases in the growth of phytoplankton (Morel et al. 1991) as well as in most living organisms (Leelasattarathkul et al. 2006). Excessive amounts or defects in intake of both ions cause several possible alterations to

physiological processes (Burguera-Pascu et al. 2007; Shpigun et al. 2007). In humans, the maximum daily intake of 0.5 and 1.0 mg kg^{-1} was established, for copper and zinc, respectively (Shams et al. 2004). Excessive amounts of zinc found in the environment can have diverse origins: domestic, metallurgy galvanising, alloy manufacturing, agricultural, clinical, geological, pharmaceutical products (Aggarwal and Patel 1998; van Staden and Tlowana 2002) and copper is often connected to effluents from septic tanks and municipal wastewaters, discharges from power plants as well as leaching from antifouling paints and pressure-treated docks pilings (Callahan et al. 2004; Croot et al. 2000; Moffett et al. 1997). Moreover, both ions are often found together in many samples of distinct nature (Richter et al. 1997; Shpigun et al. 2006) and this reason justify the development of a low cost method able to determine both ions at low levels.

There are several methods for the determination of both analytes or just a single one. Within the methods used for the determination of both analytes in waters, the majority are based on spectrophometry (Liu et al. 1995; Oliveira et al. 1996; Richter et al. 1997; Ruedas-Rama et al. 2005; Shams et al. 2004; Shpigun et al. 2006; Shpigun et al. 2007; Teshima et al. 2006), but voltammetric detection was also employed by Shams et al. 2004 and Suteerapataranon et al. 2002; this technique can have limitations in the zinc determination due to hydrogen wave interference in acidified samples along with the incapacity to detect Zn at natural pH (near 8) at which Zn is strongly connected to organic ligands (Nowicki et al. 1994). There are also several ICP-MS methods for the analysis of both analytes at low levels in blood plasma and urine (Szpunar et al. 1997) and in seawater (Otero-Romaní et al 2009), with the associated high maintenance costs of this detection system.

For zinc alone a variety of methods using different detection approaches like electrothermal atomic absorption spectrometry (Burguera-Pascu et al. 2007), flame

atomic absorption spectrometry (Dutra et al. 2006), fluorimetry (Nowicki et al. 1994; Yang et al. 2004), ICP-MS (Otero-Romaní et al 2009; Szpunar et al. 1997), chemiluminometry (Watanebe et al. 1999), and voltammetry (Shams et al. 2004; Suteerapataranon et al. 2002) were proposed.

There are also several methods for copper determination using different detection techniques as fluorescence (Luo et al. 2009), chemiluminescence (Lunvongsa et al. 2006), spectrophometry (Leelasattarathkul et al. 2007), ICP-MS (Otero-Romaní et al 2009; Szpunar et al. 1997; Wu and Boyle 1997), voltammetry (Collado-Sánchez et al. 1996), atomic absorption spectroscopy (Chan and Huang 2000), and flame atomic absorption (Anthemidis and Ioannou 2009).

Regarding flow analysis techniques, most systems use spectrophotometric detection since the colorimetric procedures are simple, fast and robust; however, to reach the trace levels of elements targeted in this case, a preconcentration step is commonly necessary (Richter et al. 1997; Ruedas-Rama et al. 2005).

To avoid the use of a more complex experimental set-up, in this work we propose to use a liquid waveguide capillary cell, where the optical pathlength is increased without light attenuation (Fuwa et al. 1984). The light is carried in and out of this detection cell by means of optical fibers. Inside the LWCC light undergoes total internal reflection on the walls as the light conducting path is transparent in the wavelength of interest and has a refractive index higher than that of the wall materials, as a result light is kept in the optically denser core. The potential to exploit this equipment was only possible since 1993 with Teflon AF-2400 (DuPont Fluoroproducts, DE, USA). This polymer is mostly transparent throughout the UV and visible range with refractive index (1.29) lower than water (1.33), chemically stable and inert (Li et al. 2003).

To sequentially quantify copper and zinc, an option was made to use the colorimetric reagent Zincon that reacts with both analytes at different pH values. The values of the equilibrium constants for the Zn-zincon and Cu-zincon complex are highly pH dependent. The pK values for the Zn-zincon complex are 7.9 and 0.6 at pH 9 and 5, respectively, showing that complexation of Zn at pH 5.0 is insignificant; whereas the formation of Cu-zincon complex is favoured at pH 5.0 (Richter et al. 1997). With this work, we also attempted to reach low levels of determination for both analytes in natural waters with low reagent consumption in a low cost system with elevated throughput. With all the apprehension and information about environmental problems, "Green Chemistry" aproaches should be in focus. With this aim, an MSFIA system was used to automate sample handling and transport to LWCC in order to detect and quantify low levels of zinc and copper in waters. The methodology is based on the sequential determination of the two analytes based on their complexation with a common reagent at different pH.

5.2. Material and methods

5.2.1. Reagents and solutions

Three certified reference water samples (NWRI-TM-24.2, NIST-SRM 1640 and ERM-CA021a) were analysed in order to evaluate the accuracy of the developed method.

All solutions used in interference studies (Fe, Mn, Cd, Al, Pb) were prepared by diluting commercial atomic absorption standards (Spectrosol).

Zincon (2-Carboxy-2′-hydroxy-5′-sulfoformazyl-benzene monosodium salt, $C_{20}H_{15}N_4NaO_6S \cdot H_2O$) 4.6 mmol L^{-1} reagent solution was prepared by dissolving 0.22 g of the solid in 0.02 mol L^{-1} NaOH solution. A daily 4.6 x 10^{-2} mmol L^{-1} zincon solution was prepared by diluting the reagent solution prepared above in 0.02 mol L^{-1} NaOH solution.

A 0.2 mol L^{-1} sodium acetate buffer for copper determination was prepared by dissolution of the corresponding quantity of solid and the final pH was adjusted with acetic acid to 5.0.

A 0.25 mol L^{-1} boric acid solution was prepared by dissolution of the solid in a solution containing 0.05 mol L^{-1} potassium chloride and 0.5 mol L^{-1} NaOH with the final pH adjusted with sodium hydroxide to 9.0.

5.2.2. MSFIA procedure and system configuration

The MSFIA system (Fig. 5.1) was designed to allow the determination of copper and zinc in waters at low levels. In order to attain this objective, a LWCC was coupled to the MSFIA manifold.

For all solenoid valves, the exchange options were classified in on/off lines. The “on” line was assigned to the flow network and the “off” line to the solution flasks (represented with a solid line and dotted line, respectively on Fig. 5.1).

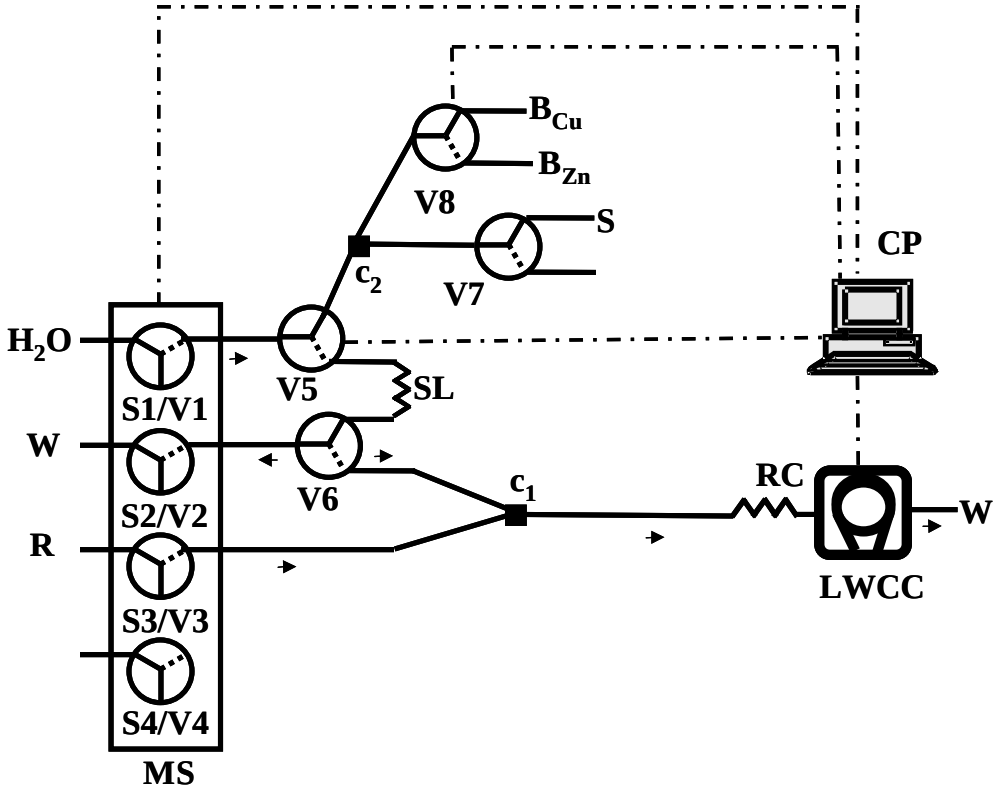

Figure 5.1. Multi-syringe flow injection analysis manifold for the determination of zinc and copper in waters. S_i: syringes; V_i: solenoid valves in position "on" (discontinuous line) or "off" (continuous line); SL: sample loop (400 µL); RC: reaction coil (200 cm); c_i: confluences; LWCC: detector (100 cm of optical path, 620 nm); MS: multi-syringe module; CP: computer; W: waste; S: sample or standard; B_{Cu}: copper buffer solution (sodium acetate); B_{Zn}: zinc buffer solution (boric acid); R: colour reagent (Zincon).

Four solenoid valves were included in the set-up. The sample introduction from one of the syringes by impulsion would require cumbersome washing steps of the syringe itself to avoid contamination between consecutive solutions. For that reason, additional solenoid valves (V5, V6) were attached to the system to accommodate the aspiration based sampling. The volume of the sample introduced was controlled by the length of the sample loop placed between V5 and V6. Confluence (c_2) was added to promote mixing between the sample and the buffer solution. The solenoid valve V7 was used for the introduction of the sample or standard solution and V8 for the selection of the buffer solutions.

The protocol sequence is listed in Table 5.1.

Table 5.1. MSFIA protocol sequence for the determination of zinc and copper in waters.

Step	Piston movement	Position of solenoid valves							Volume (mL)	Flow rate (mL min^{-1})	Action
		V1	V2	V3	V5	V6	V7	V8			
1	Pick up	0	1	0	1	1	1	1[a]/ 0[b]	1.0	5	Aspirate sample and buffer solution
2	Dispense	1	0	1	0	0	0	0	0.5 C 0.25 R	1.5 C 0.75 R	Propel carrier and colour reagent to the detector
3	Dispense	1	0	0	0	0	0	0	2.0	4	Propel the mixture to the detector and signal registration

C -carrier; R -colour reagent; 0 -off; 1 -on; [a] Copper buffer solution; [b] Zinc buffer solution.

The first step consists of aspirating 1.0 mL of the sample/standard and respective buffer solution to the sample loop. Afterwards, the mixture contained in the sample loop (SL) was propelled with the carrier to the confluence (c_1) where a portion of the colour reagent (0.25 mL) was introduced downstream. In the final step, the resulting mixture was transported to the detector. The spectrophotometric measurements were carried out at the wavelength of 620 nm.

The calculation of the analytes concentrations was based on the following procedure. Calibrations curves were traced at pH values of 5 and 9. At pH 5, copper standards in the range 10-100 µg L^{-1} were introduced in the flow system and the copper sample concentration was estimated by interpolation. Then, at pH 9 (equivalent sensitivity for copper and zinc), a calibration curve was established with zinc standards

in the same working range and, by interpolation, the sum of the molar concentrations of both ions was obtained. Therefore, the zinc sample concentration can be assessed by the difference.

At the end of a working day, the LWCC was washed consecutively with HCl (0.05 mol L^{-1}) and NaOH (0.05 mol L^{-1}) solutions in counter current.

5.3. Results and discussion

5.3.1. Study of physical and chemical parameters

Several physical parameters such as flow rate, sample and reagent plug volumes along with chemical parameters, for instance reagent concentrations, were studied in order to optimise the system.

Initial studies were carried out to optimise the physical parameters for the zinc determination since the objective was to use the same system (manifold) for both determinations and this assay has lower sensitivity. Previous experiments with LWCC equipment allowed to conclude that its applicability is limited by the blank absorbance values of the solutions (Infante and Rocha 2008). For this reason, this study was focused on increasing sensitivity while maintaining low blank absorbance values. The sample loop (SL) volume was varied within 250-650 µL. It was noticed that sensitivity and the blank values were higher with the increase of the volume. A good compromise between the sensitivity and the blank values was achieved at 400 µL. The effect of reaction coil length was studied over the range of 50-200 cm. The sensitivity increased through the range studied, although between 125 and 200 cm, the blank signal

stabilised; therefore, 200 cm was selected. The influence of the reagent plug size was also studied between 125-500 µL. This study was carried out by changing the time interval that the valve 3 was kept open. Under these conditions, the volume of 250 µL was chosen, since for higher volumes the sample dilution effect became more considerable and sensitivity did not increase any further.

The monitoring wavelength was varied within 615-625 nm and 620 nm allowed the best sensitivity. An attempt to reduce blank values was made by subtracting the absorbance values registered of several wavelengths between 650-800 nm in order to reach lower detection limits. None of them improved sensitivity; therefore no absorbance subtraction was carried out.

With regard to the first analytical step, the aspiration of sample/standard with respective buffer solution, it should be stated that a larger portion (1 mL) than the capacity of SL (400 µL) has to be aspirated in order to promote a better mixture. Mixing was assumed to be satisfactory if repeatability of the signals yielded RSD values lower than 5%. The effect of the aspiration flow rate for the first step (Table 5.1) was tested in the range of 1-15 mL min^{-1}, and 5 mL min^{-1} presented good mixing. The flow rate in the second and third step of the analytical cycle was varied between 1.5-4.5 and 2-5 mL min^{-1}, obtaining the best sensitivity at 2.25 and 4 ml min^{-1}, respectively.

The influence of chemical variables was also studied in order to improve the system performance. Firstly, the reagent concentration was tested over the range of 4.625×10^{-7}-4.625×10^{-4} mol L^{-1} and the best sensitivity was obtained with a 4.625×10^{-5} mol L^{-1}.

The concentration and the pH of the borate buffer were also studied for the zinc determination. The concentration was studied within 0.04-0.5 mol L^{-1} and 0.25 mol L^{-1} was chosen, since for higher levels, the sensitivity remained constant. In literature,

different pH values were referred for the zinc determination. This colorimetric reaction can be carried out at pH values between 8 and 10. In the present study the best sensitivity was obtained at pH 9.0. A batch study was performed by mixing the same sample/buffer proportions (1:1) in a scaled up volume, in order to prove that the concentration chosen was high enough to ensure a final sample solution pH around 9.0 units.

Regarding the chemical variables for the copper determination, only the concentration of the buffer solution needed to be tested. Thus, the effect of the concentration of sodium acetate solution was studied within 0.05-0.5 mol L^{-1}. The best sensitivity was obtained for a concentration of 0.25 mol L^{-1}. At higher concentrations the sensitivity of the system kept constant. The pH of this buffer solution was set to 5.0 units to minimise the formation of the Zn-zincon complex.

5.3.2. Interference studies

Several possible interference ions were tested in the determination of zinc or copper. Deviations higher than ±5% of the absorbance level of the respective standard (20 µg L^{-1}) were considered as interference. The ions studied in this experiment were Fe^{3+}, Cd^{2+}, Pb^{2+}, Al^{3+} and Mn^{2+} at concentrations of 20, 40, 200, 1000, 2000 and 20000 µg L^{-1}, respectively (Table 5.2).

Table 5.2. Study of interfering species expressed as relative deviation from the absorbance value obtained for the standard solution of 20 µg L^{-1} of copper or zinc.

Species tested	Copper		Zinc	
	Concentration (µg L^{-1})	Difference (%)	Concentration (µg L^{-1})	Difference (%)
Iron	40	+5.3	200	+3.6
Aluminium	2000	-4.9	200	+4.9
Cadmium	20000	+4.8	40	+5.1
Manganese	200	+4.8	20	+3.3
Lead	20000	+5.1	1000	+5.4

Sodium citrate is referred in the literature as a masking agent used in buffer streams with the aim of avoiding interferences from iron, aluminium and manganese (Richter et al. 1997). Therefore, the use of sodium citrate was tested: it was incorporated in the buffer solution for the zinc determination and the interference of several ions was significantly reduced although the sensitivity dropped a lot. Therefore, it is not an efficient solution to reach low concentration levels and so the use of sodium citrate was discarded. For copper determination, the major interference was from iron at a level two times higher followed by manganese at a level ten times higher. The iron interference can be masked by the addition of ferrozine. Manganese levels in fresh, river and seawater samples are lower than the tolerated concentration for copper determination (Ward 2000). For zinc determination, manganese interferes at the same level of concentration and cadmium at a level two times higher. Aluminium and iron interfere at a level hundred times higher. Copper determination was less susceptible to interference from the species tested than the zinc determination.

5.3.3. Analytical figures of merit

The overall features achieved for both determinations are summarised in Table 5.3. The limit of detection (LOD) and limit of quantification (LOQ) were calculated as the concentration corresponding to three and ten times the standard deviation of the blank, respectively (IUPAC 1976). The linear ranges obtained for both species were similar.

Table 5.3. Figures of merit of the developed method.

Parameters	Values	
	Zinc	Copper
Detection limit ($\mu g\ L^{-1}$)	2	0.1
Quantification limit ($\mu g\ L^{-1}$)	4	0.8
Working range ($\mu g\ L^{-1}$)	Up to 100	Up to 100
Determination rate (h^{-1})	43	43
Reagent consumption per assay (μmol)		
Zincon	0.01	0.01
Sodium acetate	---	140
Boric acid	18	---
Potassium chloride	7	---
Sodium hydroxide	35	---
Waste produced per assay (mL)	3.75	3.75

5.3.4. Application to water samples

The developed system was applied to the determination of zinc and copper in different types of water samples in order to assess its accuracy.

Firstly, recovery tests were prepared using both species in different types of water samples: surface (sea), ground (well and spring) waters. Table 5.4 summarizes the results obtained at three levels of additions (4, 10 and 20 µg L^{-1}) for both species.

Table 5.4. Results obtained for recovery tests with zinc and copper in different types of water.

Analyte	Sample number	Recovery (%)[a]		
		Concentration added		
		4 µg L^{-1}	10 µg L^{-1}	20 µg L^{-1}
Zinc	1	102 ± 6	96 ± 4	103 ± 6
	2	96 ± 6	93 ± 2	104 ± 6
	3	94 ± 7	107 ± 9	104 ± 3
	4	104 ± 4	107 ± 5	105 ± 1
Copper	1	97 ± 2	100 ± 2	102 ± 1
	2	102 ± 1	102 ± 1	103 ± 1
	3	102 ± 3	99 ± 1	104 ± 1
	4	99 ± 5	95 ± 3	94 ± 1

1 -Well water; 2 -Spring water; 3 -Groundwater; 4 -Seawater; [a] Mean and standard deviation of 5 replicates.

The recovery results obtained are very acceptable although for zinc determination the standard deviations are higher than for copper. During the experiments using the proposed method some differences were noticed between the behaviour of the two species. For example, in the zinc calibration curves the absorbance signal obtained for consecutive injections of concentrations higher than 50 µg L^{-1}, was constantly increasing, and when the blank solution was injected once more, the absorbance signal was higher compared to the initial injection. However, after cleaning the LWCC with HCl solution 0.5 mol L^{-1} the blank signal was reduced to the initial value. This effect could be explained by the formation of precipitates in the solutions and their accumulation at the LWCC walls. We must keep in mind that the buffer solution used for zinc determination has pH 9.0 and the internal diameter of LWCC has 0.6 mm. At this reaction pH, in natural water samples, solubility problems may occur due to metallic hydroxide species and the repeatability of the analytical signal can be deteriorated (Richter et al. 1997). Before analysing certified reference water samples, studies were carried out on the reaction response to the presence of both analytes. As above mentioned, at pH 9.0 zincon reacts with both analytes, therefore a comparison study using equal molar concentrations of zinc standards and copper with zinc standards (mixed standards) was performed. The equation obtained with zinc standards and with zinc and copper standards (mixed standards) is Abs_{620nm} = 0.502 (± 0.063) C_{Zn} + 0.272 (± 0.052) and Abs_{620nm} = 0.471 (± 0.049) C_{ZnCu} + 0.286 (± 0.041), respectively. The equation values obtained for the two standard curves show no significant difference, indicating additive behaviour. At pH 9.0 when separated standard solutions of zinc and copper are analysed equivalent sensitivities were obtained. When zinc standard solutions were analysed at pH 5.0 the absorbance values obtained for all the standards

are equal to blank absorbance value, demonstrating that there is no reaction between zinc and zincon at this pH.

Therefore, when the two analytes are simultaneously present in the sample, Cu(II) concentrations can be assessed directly from calibrations performed at pH 5.0, while the quantification of zinc has to be based on the already defined copper concentration and on the calibration curve established for Zn at pH 9.0.

The developed system was applied in the quantification of Zn and Cu in three certified reference water samples: NWRI-TM-24.2, NIST-SRM 1640 and ERM-CA021a. The results obtained are summarised in Table 5.5.

Table 5.5. Results obtained for the determination of copper and zinc in three certified reference water samples.

Sample	Copper ($\mu g\ L^{-1}$)		Zinc ($\mu g\ L^{-1}$)	
	MSFIA-LWCC	Certified value[d]	MSFIA-LWCC	Certified value[d]
ERM-CA021a	2028 ± 50[a]	1975 ± 54	669 ± 231[a]	514 ± 9
NIST-SRM 1640	87 ± 2[b]	85.2 ±1.2	67 ± 6[b]	53.2 ± 1.1
NWRI-TM-24.2	8.2 ± 0.2[c]	7.3 ± 0.1	22.8 ± 2.8[c]	20 ± 0.5

[a] standard deviation (n=20), dilution 50 times; [b] standard deviation (n=10), dilution 5 times; [c] standard deviation (n=10), no dilution; [d] mean and associated uncertainty.

Two out of the three reference materials analysed had certified concentration values higher than the upper limit of application range, therefore dilution of these samples was needed. From the results obtained several conclusions can be pointed out. First, the quality of the results (RSDs) obtained for copper determination is better than the ones obtained for zinc determination and it should be reminded that the errors in the determination of zinc are affected by the deviations obtained in copper determination. Second, the copper determination does not seem to be affected by the different degrees

of dilution while in the zinc determination the standard deviations obtained are increasing with the dilution.

5.4. Conclusions

The proposed work provides a good alternative for the spectrophotometric sequential determination of copper and zinc at low levels in a simple and low cost way with elevated throughput and low reagent consumption ("Green Chemistry"). Comparing to other previous flow methods displaying similar working ranges, the manifold is simpler as it was not necessary to use a preconcentration step to reach such low levels of both metals. The present strategy is the first one in the literature to use the LWCC detection cell for the determination of the two analytes and underlines the usefulness of this detection cell for the simultaneous determination, even at this low concentration levels.

The developed work compared well with other spectrophothometric flow methods using the same reagent as can be concluded from Table 5.6. It presents several advantages as low detection limits achieved without a preconcentration unit, low reagent consumption, high sampling rate and it was successfully applied to different water types. Analytical characteristics of other applications, using different detection modes, like voltammetry (Shams et al. 2004; Suteerapataranon et al. 2002); molecular spectrophotometry (Ayora-Cañada et al. 1998; Callahan et al. 2002; Callahan et al. 2004; Koupparis and Anagnostopoulou 1986; Pinto et al. 2004; Rumori and Cerdà 2003; Teshima et al. 2006; van Staden and Tlowana 2002; Vendramini et al. 2006;) atomic absorption spectrophotometry (Anthemidis and Ioannou 2009; Burguera-Pascu et al. 2007), fluorimetry (Nowicki et al. 1994; Yang et al. 2004) and chemiluminometry

(Zamzow et al. 1998) are presented in a form of a table (Table 5.7). The table allows us to conclude that in those environmental samples where trace levels of these metals have to be assessed only luminometric assays (Nowicki et al. 1994; Yang et al. 2004; Zamzow et al. 1998) give a comparable alternative (in terms of potential portability and detection limits) to the one presented here.

Table 5.6. Comparison of analytical figures of different flow methods using zincon and spectrophotometric determination (continuing).

System	Range (µg L^{-1})	Detection limit (µg L^{-1})	Determination rate (h^{-1})	Reagent consumption (µmol $assay^{-1}$)	Matrix	Preconcentration	Reference
Lab-on-valve	Up to2000 Cu	100 Cu	120	0.03 ZN	Wastewater	n.a.	Leelasattarathkul et al. 2006
Micro flow	Up to 3000 Cu	100 Cu	30	0.0033 ZN	Wastewater	n.a.	Leelasattarathkul et al. 2007
FIA	Up to 10000 Zn	50 Zn	80	0.7 ZN	Alloys, insulin formulations and waters	n.a.	Koupparis and Anagnostopoulou 1986
FIA	Up to 25 Zn Up to 25 Cu	0.35 Zn 0.80 Cu	14	1.2 ZN	Tap waters and digested brass	Chelex 100	Richter et al. 1997
FIA	Up to 9700 Zn Up to 3500 Cu	200 Zn 200 Cu	90	0.08 ZN	Vitamin formulations	n.a.	Shpigun et al. 2006
FIA	Up to 12000 Zn Up to 3000 Cu	120 Zn 20 Cu	20-30	n.g.	Children's hair	n.a.	Liu et al. 1995
FIA	Up to 2300 Zn Up to 1900 Cu	73 Zn 63 Cu	90	1 PAR 0.2 ZN	Pharmaceutical preparations	n.a.	Shpigun et al. 2007

Table 5.6. Comparison of analytical figures of different flow methods using zincon and spectrophotometric determination (continuation).

System	Range ($\mu g\ L^{-1}$)	Detection limit ($\mu g\ L^{-1}$)	Determination rate (h^{-1})	Reagent consumption ($\mu mol\ assay^{-1}$)	Matrix	Preconcentration	Reference
Multicommutation	Up to 1000 Zn Up to 2000 Cu	40 Zn 50 Cu	45	0.56 ZN	Digested plants	n.a.	Oliveria et al. 1996
FIA-bead injection	Up to 1800 Zn Up to 1000 Cu	40 Zn 29 Cu	16	0.016 ZN	Water samples, human hair, soils and pharmaceutical formulations	Sephadex QAE A-25	Ruedas-Rama et al. 2005
MSFIA	Up to 100 Zn Up to 100 Cu	2 Zn 0.1 Cu	43	0.01 ZN	Aqueous (well, river and seawaters and certified river water sample)	n.a.	Developed method

n.g.- not given; n.a.- not applies; ZN-Zincon (2-carboxy-2'-hydroxy-5'-sulfoformazylbenzene); PAR- 4-(2-pyridylazo)-resorcinol; QAE A-25- diethyl-(2-hydroxypropyl)aminoethyl

Table 5.7. Comparison of analytical figures of different methods for zinc and/or copper determination (continuing).

Method	Detection mode	Range (µg L^{-1})	Detection limit (µg L^{-1})	Determination rate (h^{-1})	Reagent consumption (µmol $assay^{-1}$)	Matrix	Preconcentration	Reference
FIA and SIA	Anodic stripping voltammetry	Up to 200 Cu FIA Up to 200 Cu SIA Up to 200 Zn FIA Up to 700 Zn SIA	18 Cu FIA 3 Cu SIA 17 Zn FIA 470 Zn SIA	20	n.a.	Drinking and wastewaters	n.a.	Suteerapataranon et al. 2002
Continuous flow	Spectrophoto-metry	Up to 11 Cu	0.19 Cu	20	0.2 BDS	Estuarine waters	n.a.	Callahan et al. 2004
FIA	Spectrophoto-metry	Up to 800 Cu	10 Cu	20	0.32 mg Br-PADAP	Digested plants	n.a.	Vendramini et al. 2006
Batch	Adsorptive stripping voltammetry	Up to 60 Cu Up to 70 Zn	0.06 Cu 0.06 Zn	15	n.a.	Tap water and alloys	n.a.	Shams et al. 2004
SIA	Spectrophoto-metry	Up to 4000 Cu	4 Cu	48	10 CUP	Mineral and tap waters	n.a.	Rumori and Cerdá 2003
FIA	Chemilumi-nescence	Up to 6.4 Cu	0.006 Cu	45	n.g.	Seawater	n.a.	Zamzow et al. 1998
FIA	Spectrophoto-metry	Up to 1000 for Zn and Cu	4.0 Zn 3.9 Cu	20	n.g.	Patients sera	n.a.	Teshima et al. 2006
FIA	Spectrophoto-metry	Up to 3000 Cu	4.6 Cu	40	3 dPKBH	River and tap waters	n.a.	Pinto et al. 2004

Table 5.7. Comparison of analytical figures of different methods for zinc and/or copper determination (part 1).

Method	Detection mode	Range (µg L^{-1})	Detection limit (µg L^{-1})	Determination rate (h^{-1})	Reagent consumption (µmol $assay^{-1}$)	Matrix	Preconcentration	Reference
SIA	Flame atomic absorption	Up to 12 Cu	0.04 Cu	12	n.a.	Seawater, river and tap waters	Extraction solvent xylene and ammonium diethyldithiophosphate as chelating agent.	Anthemidis and Ioannou 2009
Continuous flow	Spectrophoto-metry	Up to 10 Cu	0.025 Cu	17	9 BDS	Seawater, river and mineral waters	n.a.	Callahan et al. 2002
FIA	Fluorescence	Up to 32 Zn	0.006 Zn	10	0.6 pTAQ	Seawater	8-hydroxyquinoline cation exchanger	Nowicki et al. 1994
Batch	Fluorescence	Up to 317 Zn	1 Zn	60	n.g.	n.a.	n.a.	Yang et al. 2004
FIA	Electrothermal atomic absoprtion	Up to 120 Zn	0.35 Zn	45	n.a.	Human saliva	n.a.	Burgueara-Pascu et al. 2007
SIA	Spectrophoto-metry	Up to 50000 Zn	4750 Zn	10	0.07 XO	Fertilisers	n.a.	van Staden and Tlowana 2002

Table 5.7. Comparison of analytical figures of different methods for zinc and/or copper determination (part 2).

Method	Detection mode	Range (µg L^{-1})	Detection limit (µg L^{-1})	Determination rate (h^{-1})	Reagent consumption (µmol $assay^{-1}$)	Matrix	Preconcentration	Reference
SIA	Spectrophoto-metry	Up to 50000 Zn	4750 Zn	10	0.07 XO	Fertilisers	n.a.	van Staden and Tlowana 2002
FIA	Spectrophoto-metry	Up to 500 Zn	5 Zn	15	0.14 PAN	Water samples, human hair and pharmaceuti cal and cosmetic formulations	Dowes cation exchanger	Ayora-Cañada et al. 1998
MSFIA	Spectrophoto-metry	Up to 100 Zn Up to 100 Cu	2 Zn 0.1 Cu	43	0.01 ZN	Aqueous (well, river and seawaters and certified river water sample)	n.a.	Developd method

n.g.- not given; n.a. - not applies; ZN-Zincon (2-carboxy-2'-hydroxy-5'-sulfoformazylbenzene); PAN- 1-(2-pyridylazo)-2-naphthol; XO- xylenol orange (3,3"-Bis[bis(carboxymethyl)aminoethyl] cresol sulfone phtrane sodium salt); pTAQ- p-tosyl-8-aminoquinoline; BDS- bathocuproine disulfonic acid disodium salt; dPKBH-di-2-pyridyl ketone benzoylhydrazone; CUP-cuprizone (oxalic acid bis(cyclohexylidene hydrazida); Br-PADAP- 2-(5-brom-2-pyridylazo)-5-(diethylamino)-phenol.

Chapter 6

Spectrophotometric system based on a liquid waveguide capillary cell for the determination of titanium: application to waters, sunscreens and a lake sediment

The fourth work of this PhD program was the development of an analytical procedure for the spectrophotometric determination of titanium at trace levels. The procedure used a multi-pumping flow system coupled with a liquid waveguide capillary cell, which enabled to enhance the sensitivity of the determination and thus avoid complex and time-consuming preconcentration steps. The determination was based on the colorimetric reaction of titanium with chromotropic acid. The detection limit was 0.4 µg L^{-1}, linear up to 100 µg L^{-1}, with a sample throughput of 46 per hour and a low reagent consumption/effluent production. The developed procedure was applied to natural waters, sunscreen formulations and one certified lake sediment sample.

6.1. Introduction

Titanium is the ninth most abundant element in earth's crust, a natural constituent of rocks, soils and sediments (Skrabal and Terry 2002). The levels of Ti in rocks are lower than 2% (w/w) (Santelli and Araujo 1992). The Ti concentrations found in riverine, estuarine and coastal waters range from 0.005 to more than 4.8 μg L^{-1} (Skrabal 1995) and from 0.2 to 17 ng L^{-1} in ocean waters (Skrabal and Terry 2002). The Ti minerals are very resistant to chemical weathering in soil and sedimentary environments, allowing the common use of Ti as a guide element to compare the mobility of the different elements (Skrabal and Terry 2002).

In recent years, Ti has acquired growing importance in several industrial fields because of its particular physical and chemical characteristics. The main commercially available compound, titanium dioxide, is used in solar energy cells (Shipway et al. 2000), as a photocatalyst in sterilization, air cleaning and water purification processes (Hund-Rinke and Simon 2006; Lovern and Klaper 2006), as an ingredient of sunscreens, cosmetics, toothpastes, paints and plastics, and in the manufacture of building materials, aircrafts and missiles (Federici et al. 2007; Kika and Themelis 2007). It also shows great promise in the development of antitumor agents (Cai et al. 1992), and for drug delivery, environmental cleanup and computer manufacture (Masciongioli and Zhang 2003). Titanium dioxide nanoparticles also shows durable photocatalytic activity, induced by UV-light, causing photochemical degradation of organic compounds (Caruso et al. 2001; Hund-Rinke and Simon 2006), suggesting a potential use in wastewater treatment plants (Kika and Themelis 2007).

TiO_2 in the form of nanoparticles is not considered as new materials, as bulk TiO_2 has been incorporated into various products as a white pigment for decades; it is

therefore categorized as a new form of an existing substance. Nanoparticles are ultrafine particles with length in two or three dimensions greater than 1 nm and smaller than 100 nm (Lovern et al. 2007; Masciongioli and Zhang 2003). Despite the large scale and increasing production of these materials, few studies have addressed the possible environmental threat posed by nanoparticles.

The nanoparticles toxicity differs with particle type, size, surface area and functional groups attached (Hund-Rinke and Simon 2006; Lovern et al. 2007). Oberdörster et al. revealed that the smaller the size of the nanoparticles, stronger the exerted toxicity is (Oberdörster et al 2005), although the relation between the physicochemical properties of nanoparticles and their toxicity appears to be more complex (Lee et al. 2009).

In a recent study (Kahru and Dubourguier 2010), based on the already existing quantitative toxicity data (ex. LC_{50} or EC_{50}) for the evaluation of the potential hazardous effects of nanoparticles (EC 2003), TiO_2 was classified as “harmful”. This classification is based on studies that involved different groups of organisms (crustaceans, bacteria, algae, fish, nematodes and yeasts) and concluded that algae are the most sensitive ones. In the case of bulk TiO_2 the toxicity falls into the same classification, showing a lowest LC_{50} value for algae, similar to the value found for the nanoparticles formulation.

The aquifers may be the principal receiver of nanotechnology industry discharges. Therefore, ecotoxicology studies on water column organisms and across several taxonomic groups are of great interest for the comprehensive effect assessment of the nanoparticles in the aquatic environment (Federici et al. 2007; Lee et al. 2009; Lovern and Klaper 2006; Ramsden et al. 2009; Zhang et al. 2007).

The development of analytical methods focused on understanding the fate of nanoparticles in the aquatic environment is important, as the lack of detection/quantification tools hampers the advancements on other areas related to its toxicity.

In general, flow systems are very suitable for water analysis because of increased accuracy, good repeatability, low equipment cost, high sample throughput, simplified sample handling, reduced contamination risks, high degree of automation and reduction in sample/reagents consumption and effluent production (Cerda et al. 2007; Segundo and Rangel 2002).

Regarding flow methodologies for the determination of Ti, the majority are based on molecular absorption spectrophotometry (Kika and Themelis 2007; Kozuka et al. 1990; Munoz et al. 1990; Santelli and Araujo 1992), although there are some works exploiting other detection techniques such as chemiluminescence (Alwarthan and Townshend 1987), ICP-AES (Hirata et al. 1986) and ICP-MS (Yang et al. 1996).

The spectrophotometric procedures are simple, fast and robust and there are some colorimetric reagents used for titanium determination as chromotropic acid, tiron, sulfosalicylic acid (Santelli and Araujo 1992) and 4-4'-diantipyrylmethane (Munoz et al. 1990). However, to reach the determination of titanium at trace levels with spectrophtotometric procedures, a preconcentration step is necessary (Ruedas-Rama et al. 2005), significantly increasing the complexity of the flow systems manifold and decreasing the determination rate.

To overcome these difficulties, in this work we propose the use of a liquid waveguide capillary cell, a sample cell where the optical path length is increased without light attenuation (Fuwa et al. 1984), which enables to significantly increase sensitivity, in order to attain the direct determination (i.e., without a preconcentration

step) of trace levels of titanium in samples of different types (as natural waters, sunscreens and lake sediments). This approach was already successfully used in the determination of iron at low concentration levels (Pascoa et al. 2009) as it is described in Chapter 5. The characteristics of this device permit total internal reflection of the light on the walls as the light conducting path is transparent to the wavelength of interest and has a refractive index higher than the walls material. Thus, light is kept in the optically denser core.

To carry out the in-line sample reaction needed for the spectrophotometric determination of Ti, an option was made for the utilization of a manifold based on the recently proposed multi-pumping flow technique. This is characterised by a pulsed flow capable of producing an improved sample/reagent mixing and reaction zone homogenisation (Lapa et al. 2002). Multi-pumping flow systems use multiple low-cost solenoid micro-pumps strategically positioned in the manifold, which, when controlled by computer software, provide easy and versatile automated fluid handling operations. The solenoid micro-pumps require low-power supply voltage to work and have a small size when compared to peristaltic pumps, making it an advantageous alternative for portable equipment/in-situ analysis.

6.2. Materials and methods

6.2.1. Reagents and solutions

A certified reference material (CRM), a lake sediment sample ref.[a] IAEA-SL-1, was analysed in order to evaluate the accuracy of the developed analytical procedure.

Five sunscreen samples were also analysed by the developed method and the results obtained were compared with the alternative ICP-MS procedure.

The TiO_2 nanoparticles (< 100 nm) used in the recovery tests performed with the CRM sample were from Sigma-Aldrich (Ref. 634662).

The solutions used in interference studies were prepared by diluting commercial atomic absorption standard solutions (Spectrosol, BDH) of Fe, Al, Cu or Pb by dissolving the respective salts NH_4VO_3, $K_2Cr_2O_7$ and NaF (Merck), in the case of V, Cr and F.

Chromotropic acid (1,8-Dihydroxynaphthalene-3,6-disulfonic acid disodium salt, $C_{10}H_6Na_2O_8S_2 \cdot 2\ H_2O$) colour reagent was daily prepared by dissolving 0.025 g of the solid (Sigma-Aldrich) and 1.0 g of ascorbic acid (VWR International) in 100 mL of a 0.2 mol L^{-1} acetic acid-sodium acetate solution, resulting in a solution with 1.25 mmol L^{-1} and 0.113 mol L^{-1} of chromotropic acid and ascorbic acid, respectively.

6.2.2. Apparatus

An Anton Paar (Graz, Austria) Multiwave 3000 microwave oven, with a rotating turntable (a 16MF100/HF100 rotor with sixteen PTFE vessels of 50 mL maximum capacity), a 2455 MHz magnetron and a nominal exit power of 1400 W was employed for acid digestion of the samples.

A VG Elemental (Winsford, UK), PlasmaQuad 3 ICP-MS instrument, equipped with a Meinhard type A pneumatic concentric nebuliser, a quartz water cooled impact-bead spray chamber, a standard quartz tube torch and nickel sample and skimmer cones, was used in the comparison method. Both the spray chamber and sampling interface

were cooled to 10 ºC by circulating water. Argon of 99.9999% purity (Alphagaz 2, supplied by Air Liquide, Maia, Portugal) was used as plasmogenic gas. For ICP-MS sample introduction and waste draining, a Gilson Minipuls 3 peristaltic pump was used. The main operating conditions for the ICP-MS determination of Ti are indicated in Table 6.1. The isotopes (m/z ratios) ^{47}Ti (as analyte) and ^{45}Sc (as internal standard) were monitored. Both the ICP-MS instrument control and data acquisition were accomplished by using the VG Elemental PlasmaLab software.

Table 6.1. Main instrument and operating conditions for ICP-MS equipment.

Instrument parameter	Condition
Detector	Sequential
Detection mode	Pulse counting
Acquisition mode	Continuous
Pre-scan	No
Setup for ICP	
Rf power (W)	1350
Nebulizer gas flow (L/min)	0.91
Auxiliary gas flow rate (L/min)	0.96
Cool gas flow (L/min)	12.9
Setup for Main run	
Acquisition mode	Peak jumping
Sweeps	100
Dwell time (ms)	10
Channels per mass	3
Channel spacing	0.02
Replicates	2

6.2.3. *MPFS configuration and procedure*

The MPFS (Fig. 6.1) was designed to allow the determination of titanium in waters, cosmetic and other environmental samples digests at very low levels.

For the solenoid valves, the exchange options were classified in on/off lines. The "on" line was assigned to the flow manifold and the "off" line to the solutions flasks (represented with a solid line and dotted line, respectively, on Fig. 6.1).

The spectrophotometric measurements were carried out at 425 nm. Reference wavelength for minimizing the schlieren effect was set at 800 nm (Zagatto et al. 1990).

The set-up included three solenoid pumps and two commutation valves. The volume of the sample introduced into the MPFS can be set by controlling the volume dispensed in each stroke and/or the frequency of the strokes, however, the analytical repeatability is affected by the variation of the dispensed volume. Therefore, an option for a volume-based sample injection was taken, instead of a time-based injection approach. To implement this strategy, two commutation valves were used and the sample volume was controlled by the length of the sample loop placed between them. A confluence point was added downstream to promote mixing between sample/standard and colour reagent.

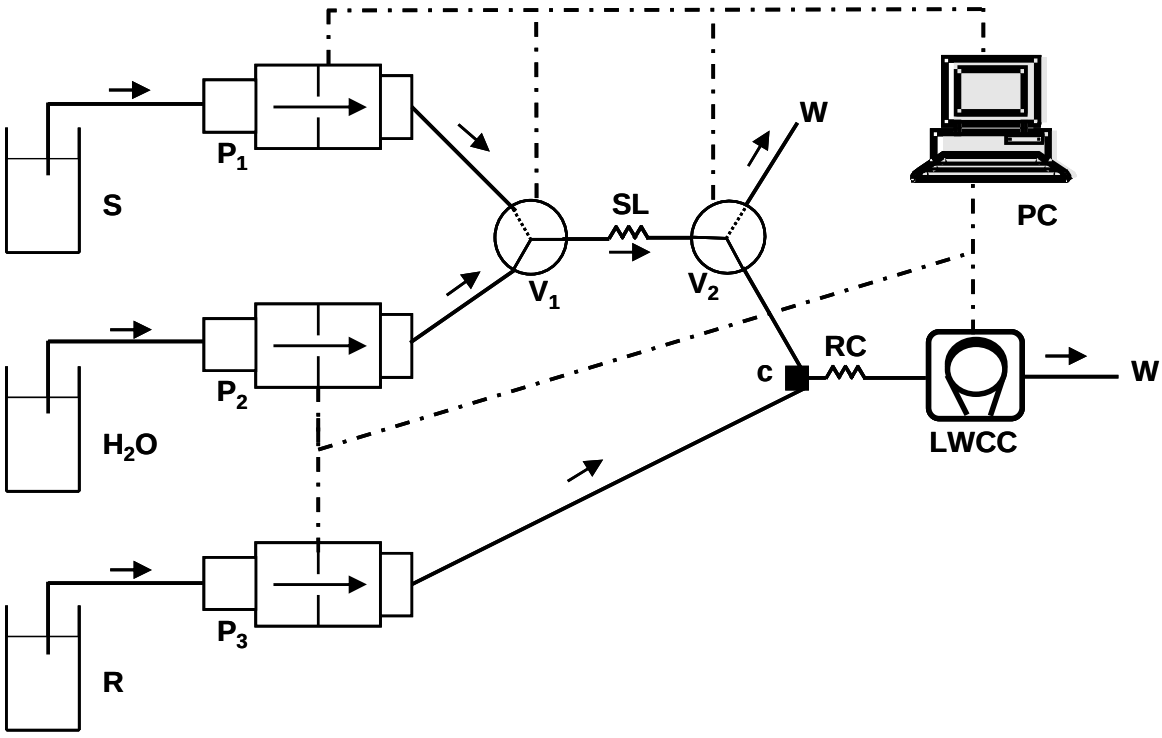

Figure 6.1. Multi-pumping flow system for the determination of titanium. P_i: pumps; V_i: solenoid valves; SL: sample loop (200 µL); RC: reaction coil (50 cm); c: confluence; LWCC: detector (100 cm optical path, 425 nm); PC: computer; W: waste; S: sample or standard; R: colour reagent (chromotropic acid) and buffer solution (acetate buffer with ascorbic acid).

The volumes dispensed by the micro-pumps in each stroke were verified and are shown in Table 6.2.

The operation sequence of the MPFS is listed in Table 6.3. The first step consisted of the introduction of 500 µL of sample/standard in the SL by pump 2. Subsequently, the sample/standard and colour reagent (100 µL) were propelled to the confluence by pump 2 and 3, respectively. In the final step, the resulting mixture was propelled to the detector and the analytical signal was registered.

Table 6.2. Volumes actually propelled by the MPFS micro-pumps.

Micro-pump	Nominal volume per stroke (µL)	Actual volume per stroke (µL)[a]
P_1	20	14.0 ± 0.1
P_2	20	17.2 ± 0.1
P_3	30	20.4 ± 0.4

[a]-Mean and standard deviation of 20 replicates.

Table 6.3. MPFS sequence of operations for the determination of titanium.

Step	Pump	Volume (mL)	Flow rate (mL min^{-1})	Valve position V1	Valve position V2	Action
1	P_2	0.5	5	1	1	Sampling
2	P_1	0.1	1	0	0	Propel sample with carrier to the confluence
3	P_1 and P_3	0.1 C 0.1 R	1	0	0	Propel sample with carrier and colour reagent to the detector
4	P_1	5	5	0	0	Propel the mixture to the detector and signal registration

C -carrier; R -colour reagent; 0 -off; 1 -on.

At the end of a working day, the LWCC was sequentially washed in counter current with HCl (0.05 mol L^{-1}) and NaOH (0.05 mol L^{-1}) solutions, and finally with water.

6.2.4. Microwave digestion procedure

The microwave digestion procedures used for each type of sample are summarized in Table 6.4. Samples were directly weighed into the PTFE reactors of the microwave digestion unit and, after addition of the digestion reagents, the mixture was subjected to the following program: a pre-set time period to attain the maximum power of 800 W, followed by a holding period at this power and a 15 min cooling period at 0 W. After that, and to complex free fluoride, saturated boric acid solution was added at a ratio of 6 ml per each mL of HF used in the digestion. The resulting clear solutions were quantitatively transferred to volumetric flasks and, after appropriate dilution with deionized water, they were analysed both by the developed and the reference procedure.

Table 6.4. Microwave digestion procedures used for the different kind of samples.

	TiO_2 powder	Sunscreens	Lake sediment
Sample weight (g)	0.5	0.3	0.25
Digestion reagent (mL)			
H_2SO_4	-	2	-
HNO_3	-	3	1
HCl	-	-	2
HF	3	2	3
H_2O	3	-	-
Digestion program			
Ramp time (min)	10	10	5
Power (W)	800	800	800
Hold time (min)	20	30	30
Cool time (min)	15	15	15
Boric acid added (mL)	18	12	18

6.3. Results and discussion

6.3.1. Study of physical and chemical parameters of the MPFS

The optimization studies included both physical parameters (flow rate, plug size, reaction coil length, sample and reagent volumes) and chemical parameters (reagents concentration and pH) of the whole analytical procedure. The results are summarized in Fig. 6.1 and in Table 6.3.

Initially, the volumes actually dispensed by the micro-pumps were evaluated. Two different flow rates (1 and 5 mL min^{-1}) and two different volumes (1 and 5 mL) were selected to perform this evaluation in each micro-pump. The results obtained are summarized in Table 2 and represent the mean of twenty replicates (five replicates for each flow rate and volume).

As referred above, the sample volume introduced in the system was controlled by the length of the tube placed between the two commutation valves (the "sample loop"–SL), since in preliminary experiments an improvement in the repeatability was verified using this sampling mode.

The SL volume was varied within 100 and 400 µL and 200 µL was the selected value. The reaction coil length was studied in the range 10-150 cm and 50 cm was the length that allowed better sensitivity. The flow rate was varied within 0.5 and 5 mL min^{-1} in all the steps of the analytical cycle (Table 6.3). In the first and fourth steps, the sensitivity and the blank values were independent of the flow rate, therefore 5 mL min^{-1} was used in order to maximize the sampling rate. In the second and third steps, the sensitivity and the blank values increased for lower flow rates. The best compromise between sensitivity and blank values was achieved at 1 mL min^{-1}.

The analytical wavelength was evaluated in the range from 415 to 445 nm and 425 nm allowed the best compromise regarding sensitivity and low blank values.

Following this study, reagent volumes from 60 to 200 µL were tested and 100 µL (corresponding to 5 micro-pump pulses) were chosen as this volume provided a better repeatability (RSD <3%). The chemical parameters evaluated in the optimization of the methodology included the concentration of the colour reagent (chromotropic acid) and reducing agent (ascorbic acid), and the concentration and pH of the buffer solution (sodium acetate/acetic acid). Firstly, the reagent concentration was studied in

the range of 1.25×10^{-4} to 2.5×10^{-3} mol L^{-1} and the best sensitivity with a relatively low blank value (0.030) was obtained for 1.25×10^{-3} mol L^{-1}. High blank values shorten the application range of the methodology since CCD spectrophotometers performance deteriorates for absorbance values over 1.8 (Galbam et al. 2010).

The concentration (in the range 0.05-1 mol L^{-1}) and the pH (in the range 4.2-5.0) of the acetate buffer were then studied. The best sensitivity was obtained for a concentration of 0.2 mol L^{-1} and a pH of 4.6, and these values were used in subsequent experiments.

Finally, the ascorbic acid concentration was evaluated. Ascorbic acid was necessary to assure the complete reduction of iron(III) to iron(II), since iron(III) interferes with titanium determination (Table 6.5). The ascorbic acid concentration was varied between 1 and 4% (w/v) and a concentration of 2% showed to guarantee the total iron(III) reduction at concentrations up to 2 mg L^{-1}.

6.3.2. Interference studies

The interference of several ions on the determination of titanium was tested. Solutions with a fixed concentration of titanium (20 µg L^{-1}) and increasing concentrations of the tested ions were prepared. Ions causing deviations higher than 5% in the absorbance value of the pure 20 µg L^{-1} titanium standard solution, were considered as interferents. The ions studied were Fe^{3+}, Pb^{2+}, Al^{3+}, F^{-}, Cu^{2+}, V^{5+} and Cr^{6+} at concentrations of 20, 40, 100, 200, 500, 1000 and 2000 µg L^{-1}, respectively (Table 6.5).

Table 6.5. Interferences study. Results expressed as the relative deviation from the absorbance value of a 20 µg L^{-1} titanium standard solution.

Species tested	Concentration (µg L^{-1})	Difference (%)
Iron[a]	20	+5.4
Iron[b]	2000	-2.4
Aluminium	100	+4.5
Copper	500	+5.6
Lead	1000	+4.9
Vanadium	500	+5.6
Chromium	200	+2.9
Fluoride	200	+3.9

[a] Without ascorbic acid; [b] With ascorbic acid.

The major interference was from Fe(III) at the same level of titanium. This interference was minimised by the addition of ascorbic acid to the colour reagent as can be seen in Table 6.5. The other main interferences were from aluminium at a concentration five times higher than titanium and fluorine and chromium at a concentration ten times higher than titanium.

6.3.3. Analytical figures of merit

All the analytical performance characteristics obtained for titanium determination are summarised in Table 6.6. The limit of detection (LOD) and limit of

quantification (LOQ) were calculated as the concentration corresponding to three and ten times the standard deviation of the blank, respectively (IUPAC 1976).

Table 6.6. Figures of merit of the developed methodology.

Parameters	Values
Detection limit(µg L^{-1})	0.4
Quantification limit (µg L^{-1})	0.8
Working range (µg L^{-1})	Up to 100
Determination rate (h^{-1})	46
Reagent consumption per assay (µmol)	
Chromotropic acid	0.125
Ascorbic acid	5.68
Sodium acetate	20
Acetic acid	20
Waste produced per assay (mL)	5.8

6.3.4. Application to water samples

Concluded the optimisation of all of the physical and chemical parameters, the proposed analytical procedure was applied to different types of water in order to assess its accuracy.

Initially, recovery tests were carried out on different types of water samples: surface (sea and river) and ground (well and mine) waters. Table 6.7 summarises the results obtained for three levels of analyte addition (4, 10 and 20 µg L^{-1}).

Table 6.7. Recovery tests. Results (%) obtained in different types of water for three different addition levels.

Water types	Concentration added		
	4 µg L^{-1}	10 µg L^{-1}	20 µg L^{-1}
Well	95 ± 3	106 ± 1	105 ± 1
Polluted river not filtered	89 ± 2	94 ± 1	98 ± 2
Sea	92 ± 1	106 ± 3	106 ± 2
Sea	96 ± 8	102 ± 6	96 ± 4
Estuarine	88 ± 3	102 ± 1	107 ± 1
Mine	96 ± 3	94 ± 1	99 ± 1
Polluted river	90 ± 4	96 ± 1	98 ± 1
Polluted river	91 ± 2	93 ± 2	97 ± 1

[a] Mean and standard deviation of 5 replicates.

They suggest that the matrix of water samples does not appreciably interfere in the determination of titanium.

Chromotropic acid reacts with Ti(IV). However, TiO_2 is the most frequent form of the analyte present in environmental and cosmetic samples. Therefore, a comparison study using similar concentrations of standards of Ti(IV) (prepared both from the commercial standard solution and from the dissolved/digested TiO_2) was performed. The linear relationship between the molar concentration of Ti ($C_{Ti(IV)}$; C_{TiO2}) obtained using Ti(IV) standards or TiO_2 standards was $Abs_{425nm} = 0.0121\ (\pm\ 2 \times 10^{-4})\ C_{Ti(IV)} + 0.0026\ (\pm\ 0.0040)$ and $Abs_{425nm} = 0.0122\ (\pm\ 1 \times 10^{-4})\ C_{TiO2} + 0.0052\ (\pm\ 0.0011)$,

respectively. The equation parameters obtained for the two standard curves show no significant difference (standard errors on the estimated parameters are indicated between brackets). These results demonstrate that the developed method is applicable to the determination of Ti in both chemical forms.

6.3.5. Application to commercial sunscreens

Although the primary objective of the proposed method is to determine Ti(IV) in waters, it was also applied to different commercial sunscreen samples, previously submitted to a microwave acid digestion. To assess the quality of the results, they were compared with those obtained by ICP-MS (Table 6.8).

Table 6.8. Comparison of the results obtained in the determination of TiO_2 in five commercial sunscreens products.

Sample	TiO_2 % (w/w)	
	Developed method[a]	ICP-MS[b]
1	2.33 ± 0.01	2.29 ± 0.13
2	1.77 ± 0.01	1.70 ± 0.11
3	1.66 ± 0.01	1.65 ± 0.10
4	2.29 ± 0.02	2.33 ± 0.07
5	1.40 ± 0.01	1.35 ± 0.12

[a] Mean and standard deviation of 5 replicate analysis of the same digestion solution;

[b] Mean and standard deviation of 2 replicate analysis of the same digestion solution.

The calculated t-values were below the critical t-value (6.31) and the linear regression parameters showed no significant difference between the results obtained by

the developed system (C_{MPFS}) and by the alternative procedure, C_{MPFS} = 0.94 (± 0.15) C_{ICP-MS} + 0.13 (± 0.29), where the values between brackets are the limits of the 95% confidence intervals.

6.3.6. Application to a lake sediment

The developed analytical procedure was also applied to one reference lake sediment material (IAEA-SL-1) with a certified value of 5170 ± 430 mg Ti per kg of sediment. When analysed by the developed procedure the result obtained was 5155 ± 113 mg kg^{-1}. This result corresponds to the average of 5 assays and the half width of the 95% confidence interval. The solution obtained in the sample digestion was diluted 1000 times in order to adjust its concentration to the linear response range of the MPFS analytical procedure. The result shows a good accuracy of the developed procedure.

Additionally, recovery tests were performed on the certified lake sediment sample, to which TiO_2 nanoparticles were added in their solid form. The recovery results, presented in Table 6.9, are relative to the expected concentrations in the digests after spiking the sediment sample.

Table 6.9. Recovery tests. Results obtained with a certified lake sediment sample.

Assay number	Expected Ti concentration ($\mu g\ L^{-1}$)	Recovery (%)[a]
1	41.1	97 ± 1
2	29.7	105 ± 1
3	18.9	97 ± 1
4	26.2	108 ± 1
5	25.5	92 ± 1
6	37.6	93 ± 1

[a] Mean and standard deviation of 5 replicates.

6.4. Conclusions

This work presents a reliable spectrophotometric determination of titanium at low levels applicable to different water samples, digested commercial sunscreens and sediments. The proposed methodology is relatively simple and inexpensive, with a good analytical throughput and low reagent consumption/effluent production ("Green Chemistry" approach). The employment of a LWCC allows the determination of Ti at very low levels without using a preconcentration step.

When compared to previous works (Table 6.10), this analytical procedure allows titanium determinations in different types of samples with a low detection limit, low reagent consumption and high throughput.

Table 6.10. Comparison of analytical figures of different flow methods with spectrophotometric determination of titanium (IV).

Method	Range (µg L^{-1})	Detection limit (µg L^{-1})	Determination rate (h^{-1})	Reagent consumption (µmol $assay^{-1}$)	Matrix	Preconcentration	Reference
SIA	Up to 10000	700	24	52.5 CA	Dental implant and Maroccan rocks	n.a.	Kika and Themelis et al. 2007
FIA	Up to 12400	n.g.	30	64 Tiron	Rock samples	n.a.	Kozuka et al. 1990
FIA	Up to 150000	9000	120	400 H_2O_2	Hydro-metallurgical samples	n.a.	Munoz et al. 1990
FIA	Up to 30000	50	80	16 CA	Rock samples	n.a.	Santelli and Araujo 1992
MPFS	Up to 100	0.4	46	0.125 CA	Well, mine, river and sea waters, commercial sunscreens and lake sediment	n.a.	Developed system

n.g.- not given; n.a. - not applies; CA – chromotropic acid; Tiron – disodium 1,2-dihydroxybenzene-3,5-disulphonate;

Chapter 7

Spectrophotometric determination of bromate: comparison of different reagents and flow techniques

The last work of this PhD program was the development of a method able to determine bromate in waters at trace levels. For this reason, a liquid waveguide capillary cell was coupled to a multi-pumping flow system. The developed methodology was based on a colorimetric reaction and two colour reagents were tested, chlorpromazine and trifluoperazine. The detection limit obtained with chlorpromazine and trifluoperazine in the MPFS approach was 1.5 and 1.2 µg L^{-1}, respectively and the determination was linear up to 100 µg L^{-1} for both reagents. This procedure presented a sample throughout of 23 per hour and low reagent consumption. The developed method was compared with a FIA approach because of the lack of repeatability observed in the MPFS approach.

7.1. Introduction

Theoretically, drinking water for human consumption, has a small amount of pollutants and no pathogenic agents, as it goes through a purification process. Ozonation is one of the most frequent chemical disinfection processes. In the presence of bromide, this process leads to the formation of bromate (Farrel et al. 1995; von Gunten and Hoigné 1994). The concentration of bromate formed depends on the ozone dose and the level of bromide present in the water (Romele and Achilli 1998). Bromate is known to be a carcinogenic agent at very low concentrations (Crofton 2006; Kurokawa et al. 1990) causing different negative health effects such as hepatic toxicity, renal damage (Ohtomo et al. 2009) and damages in the nervous system and thyroid gland (Kurokawa et al. 1990). The World Health Organisation (WHO) has classified bromate into group 2B as possible human carcinogen (Kurokawa et al. 1990; Ohtomo et al. 2009) and established the health based limit value for drinking water as 2 μg L^{-1}. However, based on the instrumental difficulties to reach the desired detection limits and on the technological difficulties in removing bromate from drinking water, the European Union Directive (CEU 1998) and the WHO set the limit of 10 μg L^{-1} for drinking waters. Moreover, the Environmental Protection Agency (EPA) set the maximum level of bromate in drinking waters at 0 μg L^{-1} as a future goal. Analytical methods applied to bromate determination should be capable of detecting at least 2.5 μg L^{-1} with good accuracy and precision (Koscielna 2004).

For all these reasons, the development of analytical methods able to reach these features are imperative. Within existing methods for bromate determination, ion chromatography (IC) is commonly used and is a reference method by US Environmental Protection Agency (EPA) (Achilli and Romele 1999; Almendral-Parra et

al. 2008; Ingrand et al. 2002). Within IC methods, there are different detection approaches such as conductivity detection (Borba et al. 2005; Schminke and Seubert 2000), spectrophotometric detection after post-column reaction (Achilli and Romele 1999; Valsecchi et al. 1999) and inductively coupled plasma-mass spectrometry (Divjak et al. 1999; Yamanaka et al. 1997). However, these approaches involve different disadvantages related to the detection method used, for instance the need for sample pretreatment to remove interferences or to pre-concentrate bromate (Butler et al. 2005; Koscielna 2004), long analysis times and complex/costly equipment (Alonso-Mateos et al. 2008; Mitrakas et al. 2010). These disadvantages limit their use for routine in-situ monitoring.

Flow analysis techniques are very suitable for routine analysis because, along with increased accuracy and precision, elevated sample throughput, good reproducibility, low equipment cost, reduction in the consumption of samples/reagents and production of effluents, allow in-situ determinations with simplified sample handing and high degree of automation (Cerdà et al. 2007; Segundo and Rangel 2002). Within flow analysis systems for bromate determination, different detection systems were used such as spectrophotometric (Alonso-Mateos et al. 2008; Gordon and Bubnis 1995; Gordon et al. 1994; Oliveira et al. 2011; Uraisin et al. 2006; van Staden et al. 2004), fluorescent (Almendral-Parra et al. 2008; Ohtomo et al. 2009), potentiometric (Ohura et al. 2004), chemiluminescent (Silva et al. 2001) and ICP-MS (Elwaer et al. 2000).

In this chapter, we propose a spectrophotometric procedure using a liquid waveguide capillary cell with 1.0 m of optical pathlength. The use of LWCC allows reaching low levels of bromate with an elevated determination rate without the use of

preconcentration step that would increase the complexity of the flow manifold. In the LWCC, the optical pathlength is increased without light attenuation (Fuwa et al. 1984).

To carry out the spectrophotometric reaction, two colour reagents were tested chlorpromazine and trifluoperazine. Both reagents are oxidised by bromate under acidic conditions and converted to coloured compounds (Butler et al. 2005; Farrel et al. 1995; Koscielna 2004). The two reagents were compared in the determination of bromate in waters at low levels.

We tested the use of a multi-pumping flow system to handle the reaction chemistries. For comparison purposes, we also used a flow injection system system for the same purpose.

7.2. Materials and methods

7.2.1. Reagents and solutions

Chlorpromazine colour reagent was daily prepared by dissolving 44 mg of $C_{17}H_{19}ClN_2S \cdot HCl$ (Sigma-Aldrich) in 25 mL of deionized water.

Trifluoperazine colour reagent was daily prepared by dissolving 60 mg of $C_{21}H_{24}F_3N_3S \cdot 2HCl$ (Sigma-Aldrich) in 25 mL of deionized water.

7.2.2. MPFS and FIA procedures and system configuration

The MPFS (Fig. 7.1) and FIA manifold (Fig. 7.2) were designed to allow the determination of bromate in waters at low levels.

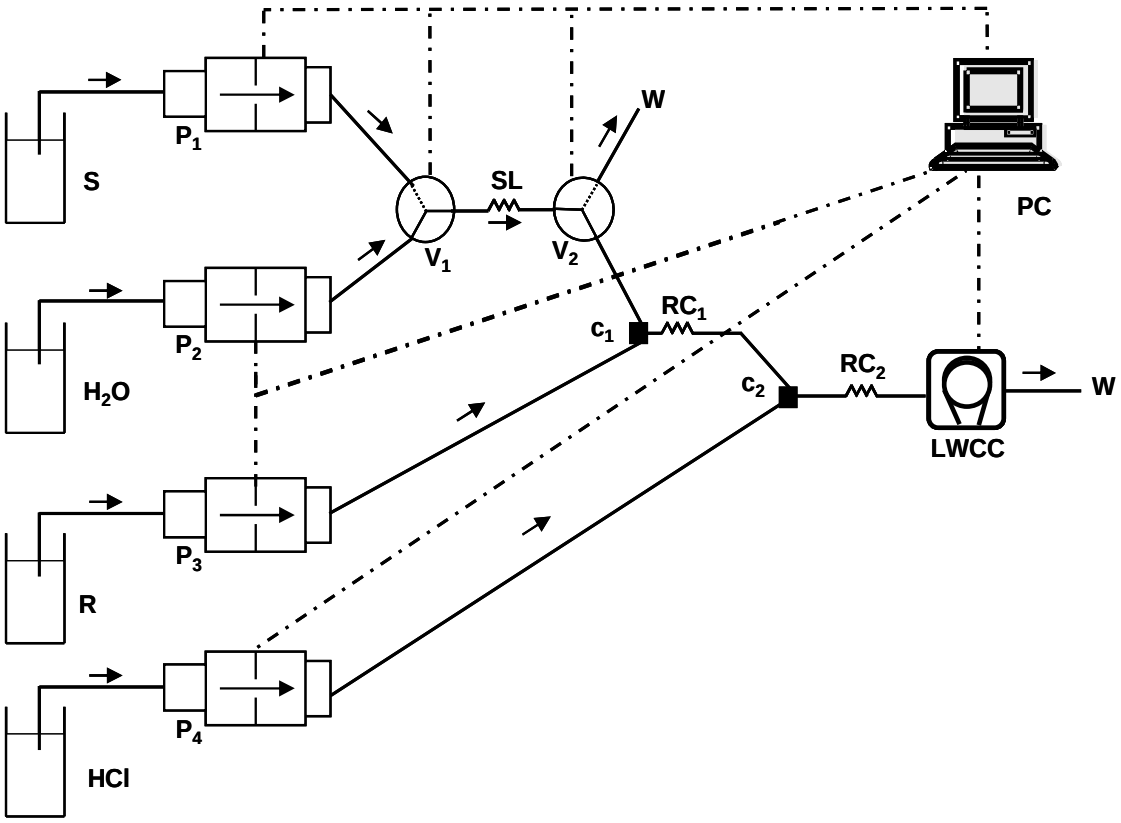

Figure 7.1. Multi-pumping flow system for the determination of bromate. P_i: pumps; V_i: solenoid valves; SL: sample loop (450 µL); RC_1: reaction coil (60 cm); RC_2: reaction coil (125 cm); c_i: confluences; LWCC: detector (100 cm optical path, 535 and 505 nm for chlorpromazine and trifluoperazine, respectively); PC: computer; W: waste; S: sample or standard; R: colour reagent (chlorpromazine or trifluoperazine); HCl: hydrochloric acid.

The set-up of MPFS (Fig. 7.1) included four solenoid pumps and two commutation valves. For the solenoid valves, the exchange options were classified in on/off lines. The “on” line was assigned to the flow manifold and the “off” line to the solutions represented with a solid line and dotted line, respectively, on Fig. 7.1.

The volume of the sample introduced into the MPFS can be set by controlling the volume dispensed in each stroke and/or the frequency of the strokes, however the analytical repeatability is affected by the variation of the dispensed volume. Therefore, an option for a volume-based sample injection was taken, instead of a time-based injection approach. To implement this strategy, two commutation valves were used and the sample volume was controlled by the length of the sample loop placed between them. In previous studies (Páscoa et al. 2011), an improvement in the repeatability was verified using this sampling mode.

The volumes dispensed by the micro-pumps in each stroke were verified and are shown in Table 7.1.

Table 7.1. Volumes propelled by the MPFS micro-pumps.

Micro-pump	Nominal volume per stroke (µL)	Actual volume propelled per stroke (µL)[a]
P_1	20	13.8 ± 0.5
P_2	20	17.3 ± 0.8
P_3	30	20.4 ± 0.5
P_4	30	25.5 ± 1.2

[a] Mean and standard deviation of 20 replicates.

The confluences (c_1 and c_2) were added downstream to promote mixing between sample/standard with colour reagent and hydrochloric acid. The reaction occurs in two steps. Firstly, sample/standard were mixed with the colour reagent at confluence c_1 and than this mixture joined the hydrochloric acid solution at confluence c_2 since the

reaction involving bromate and colour reagent only occurs under acidic conditions (Butler et al. 2005; Farrel et al. 1995; Koscielna 2004).

The operation sequence of the MPFS consists in four steps and is listed in Table 7.2.

Table 7.2. MPFS sequence of operations for the determination of bromate.

Step	Pump	Volume (mL)*	Flow rate (mL min^{-1})	Valve position V1	Valve position V2	Action
1	P_2	1	5	1	1	Sampling
2	P_1 and P_3	0.3 S 0.3 R	1 for each pump	0	0	Propel mixture of sample with colour reagent at the confluence (c_1)
3	P_1 and P_4	0.5 S + R 0.51 A	1 for each pump	0	0	Propel the final mixture of sample/standard with colour reagent and hydrochloric acid at the confluence (c_2)
4	P_1	3	2	0	0	Propell the final mixture to the detector and signal registration

S -sample/standard; R -colour reagent; A -hydrochloric acid; 0 -off; 1 -on;

* theoretical volume based on the nominal volume of each pump.

The first step consisted in the introduction of sample/standard (50 strokes) in the SL by pump 2. Afterwards, the sample/standard (15 strokes) and colour reagent (10 strokes) were propelled to the confluence (c_1) by pump 1 and 3, respectively.

Subsequently, the resulting mixture (25 strokes, pump 1) was propelled to the confluence (c2) where it was mixed with hydrochloric acid (17 strokes, pump 4). In the final step, the mixture was propelled to the detector and the analytical signal was registered.

The set-up of FIA manifold (Fig. 7.2) included one peristaltic pump and one four-way injection valve. The volume of sample introduced into this manifold was controlled by the length of the sample loop placed in the injection valve. The confluences were added with the objective to promote efficient mixing of the solutions. The flow rates were set as 1.9 mL min^{-1} for hydrochloric acid and 0.9 mL min^{-1} for carrier and colour reagent.

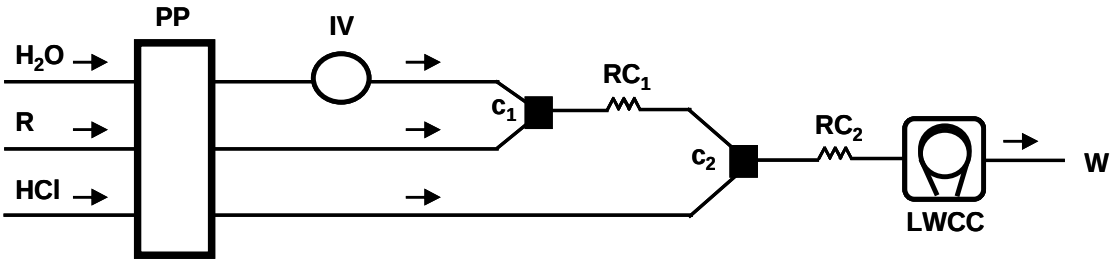

Figure 7.2. FIA manifold for the determination of bromate. PP: peristaltic pump; IV: injection valve; RC_1: reaction coil (60 cm); RC_2: reaction coil (125 cm); c_i: confluences; LWCC: detector (100 cm optical path, 535 and 505 nm for chlorpromazine and trifluoperazine, respectively); W: waste; R: colour reagent (chlorpromazine or trifluoperazine); HCl: hydrochloric acid.

The spectrophotometric measurements were carried out at 505 and 535 nm using trifluoperazine and chlorpromazine solutions, respectively.

At the end of a working day, the LWCC was sequentially washed in counter current with HCl (0.05 mol L^{-1}) and NaOH (0.05 mol L^{-1}) solutions, and finally with water.

7.3. Results and discussion

7.3.1. Study of physical and chemical parameters of the MPFS and FIA system

The initial study of physical and chemical parameters was performed in the MPFS with the chlorpromazine reagent.

Several physical parameters such as plug size, reaction coil length and sample/reagents volumes were varied.

The real volumes dispensed by the micro-pumps were estimated. Two different flow rates (1 and 5 mL min^{-1}) and two different volumes (1 and 5 mL) were used to evaluate each micro-pump; this experiment was performed with deionised water and with each micro-pump individually and not connected to the system. The results obtained represent the mean of twenty replicates (five replicates for each flow rate and volume) and are summarised in Table 7.1.

The sequence of reagent addition (Fig. 7.1 and Table 7.2) was established based on previous works (Farrell et al. 1995; Oliveira et al. 2011), resulting higher sensitivity with low absorbance blank values. To implement the reaction sequence in the flow set-up, two reaction coils (RC_1 and RC_2) were introduced. The influence of the length of both coils was evaluated one by one. The RC_1 and RC_2 were varied in the range of 50 to 85 cm and 50 to 150 cm and the higher sensitivity as well as lower relative standard deviations were obtained with 60 and 125 cm, respectively.

Following this study, the influence of the sample loop volume was studied between 200 and 1000 µL; the values of sensitivity and blank absorbance remained constant. Based on these results, a sampling loop of 450 µL was selected, being a

central value of this interval. The colour reagent and acid solution volume were varied within 200-500 and 300-700 µL. The best compromise between higher sensitivity and lower blank absorbance values were obtained with 300 and 500 µL of colour reagent and acid, respectively. It should be highlighted that the sample/standard, colour reagent and acid volumes values referred here are related to the nominal volume of the micro-pumps.

Afterwards, the influence of the chemical parameters was also evaluated.

The effect of chlorpromazine concentration was tested within 2.1-21.1 mmol L^{-1} and the selected value was 4.2 mmol L^{-1}. Similarly, the concentration of trifluoperazine was studied between 2 and 12 mmol L^{-1} and the concentration of 5 mmol L^{-1} was selected. These values were set to obtain a good sensitivity and low the blank absorbance values.

Finally, the acid concentration (HCl) was varied over the range of 1 to 4 mol L^{-1} with both reagents. The best sensitivities were attained with the concentration of 2 and 3 mol L^{-1} of HCl for trifluoperazine and chlorpromazine, respectively.

During the optimisation studies, some problems were identified in the MPFS set up. The major problem was the lack of day-to-day and even within day repeatability of the system, and this behaviour was noticed with both reagents. The sensitivity changed under the same experimental conditions. Therefore, to assess if this problem was due to some physical reason, additional experiments were planned.

This additional experiment consisted in using the bromothymol blue instead of sample (P_2) and reagents (P_3 and P_4) to test the repeatability of each micro-pump, separately. This experiment was performed in two consecutive days. The results obtained are summarised in Table 7.3.

Table 7.3. Repeatability study using a colour dye (BTB) in each micro-pump.

Micro-pump	Relative standard deviation[a]	
	Day 1	Day 2
P_2	2.8 %	0.8 %
P_3	1.2 %	1.3 %
P_4	2.1 %	3.2 %

[a] - relative standard deviation of ten replicates.

The results obtained (RSD < 5%) in this study demonstrate good repeatability for each micro-pump (Bio Chem Fluidics 2011) and it seems that the micro-pump performance do not justify the lack of repeatability attained with MPFS. However, the results in Table 7.3 were obtained by the introduction of diluted BTB solution into a carrier stream of borate buffer of 0.01 mol L^{-1} and not under the conditions of the bromate determination where up to 4 mol L^{-1} HCl is used.

During the optimisation of the MPFS, it was also observed that, when flow rates higher than 2 mL min^{-1} were tested, the volume delivered for each cycle at the end of the tubing did not increase proportionally. The volume was measured using a graduated cylinder at the end of the LWCC. This indicates that the micro-pumps were not able to deliver the solution due to the back pressure generated by the LWCC at flow rates superior than 2 mL min^{-1}. It should be emphasized that the internal diameter of the LWCC is 0.55 mm and the entire flow manifold was made with PTFE tubes with 0.8 mm id. This demonstrates an important drawback on the utilisation of micro-pumps.

In an attempt to explain these results, an option was made to set-up a FIA system (Fig. 7.2) for the same reaction chemistry and detector, and subsequently compare the features of both systems with the goal of understanding the lack of repeatability.

Several physical (reaction coil length, sample volume, flow rate) and chemical (reagents and acid concentration) parameters were also studied for this FIA manifold.

Firstly, the propelling Tygon tubes were selected with the objective of a mixture ratio approximately of 1:1 in both confluences (c_1 and c_2). For this purpose, the tubes selected allowed a flow rate of 1.9 mL min^{-1} for hydrochloric acid and 0.9 mL min^{-1} for carrier and colour reagent, resulting in a total flow rate of 3.7 mL min^{-1}.

Afterwards, the sample volume was studied within 50-350 μL, for both reagents, and 250 μL was chosen, since for higher volumes the sensitivity remained constant.

Following this study, both reaction coils length (RC_1 and RC_2) were evaluated. With chlorpromazine, the RC_1 and RC_2 length was varied over the range of 30 to 100 cm and 10 to 150 cm, respectively and the best results were obtained with 30 cm for RC_1 and 75 cm for RC_2. With trifluoperazine, the RC_1 and RC_2 length was varied in the range of 30 to 100 cm and 75 to 150 cm, respectively. The results obtained showed no significant difference. The 30 and 75 cm for RC_1 and RC_2, respectively was adopted for further experiments with both reagents.

Finally, the influence of the reagent and acid concentration were also evaluated.

The chlorpromazine and trifluoperazine concentration were tested between 2 and 10 mmol L^{-1}. Surprisingly, there were no variations of neither sensitivity nor absorbance values in all the concentrations tested. A concentration of 5 mmol L^{-1} was selected for both reagents.

Finally, the acid concentration (HCl) was varied within 1 - 4 mol L^{-1} and 3 mol L^{-1} was selected since for higher concentrations the sensitivity remained constant.

7.3.2. Figures of merit of the MPFS and FIA system

The analytical features achieved for the MPFS and the FIA system are summarised in Table 7.4 and 7.5, respectively. The limit of detection (LOD) and limit of quantification (LOQ) were calculated as the concentration corresponding to the intercept value plus three and ten times the statistic $s_{y/x}$ (Miller and Miller 2005).

Table 7.4. Figures of merit of the MPFS with both reagents.

Parameters	Values	
	Trifluoperazine	Chlorpromazine
Limit of detection (µg L^{-1})	1.5	1.2
Limit of quantification (µg L^{-1})	5.1	4.0
Analytical working range (µg L^{-1})	Up to 100	Up to 100
Analytical throughput(h^{-1})	23	23
Reagent consumption per assay (µmol)		
Colour reagent	1.5[a]	1.3[a]
Hydrochloric acid	1000[a]	1500[a]
Waste produced per assay (mL)	2.5[a]	2.5[a]

[a] -values estimated based on the nominal volumes of the micro-pumps.

In the MPFS, chlorpromazine reagent presents lower detection limit, quantification limit and colour reagent consumption with higher consumption of hydrochloric acid.

As referred above, the major problem in this MPFS was the lack of repeatability.

Table 7.5. Figures of merit of the FIA system with both reagents.

Parameters	Values	
	Trifluoperazine	Chlorpromazine
Limit of detection ($\mu g\ L^{-1}$)	1.0	0.7
Limit of quantification ($\mu g\ L^{-1}$)	3.4	2.4
Analytical working range ($\mu g\ L^{-1}$)	Up to 100	Up to 100
Analytical throughput(h^{-1})	60	60
Reagent consumption per assay (μmol)		
Colour reagent	4.65	4.65
Hydrochloric acid	5550	5550
Waste produced per assay (mL)	3.9	3.9

By using the FIA system, the only difference noticed with the use of the two reagents was the lower detection and quantification limit observed with chlorpromazine.

Using the FIA system, no stability problems were detected along all experiment, presenting a good repeatability with both reagents. This might lead to the conclusion that the problems encountered while using the MPFS set up were related to solenoid pump functioning.

The solenoid pumps deliver a pulsed flow which is characterized by a short go period corresponding to the sudden delivery of the solution stroke, followed by a

relatively long stop period. During the go period, turbulent mixing is noted, and the feature leads a fast homogenization of the involved solutions (Bio Chem Fluidics 2011). The manufacturer (Bio Chem Fluidics) refers that one micro-pump can generate around 5 PSI pressure. The applied pressure and fluid flow rate through the LWCC obeys the Hagen-Poiseuille relationship described:

$$\left(\Delta P = \frac{8\mu L Q}{\pi r^4} \right)$$

where (ΔP) is the difference of pressure, (L) is the length of the tube, (Q) is the flow rate, (μ) is the dynamic viscosity and (r) is the internal tube radius.

Therefore, the flow rate is proportional to pressure and to the fourth power of the diameter of the fluid capillary, as well as reciprocal to the length of the capillary and fluid dynamic viscosity. Based on the above equation and according to the LWCC manufacturer (World Precision Instruments 2011) description a one-meter length of 550 μm ID waveguide requires approximately 1.5 PSI for water flow of 1 mL min^{-1}. Therefore, the difference in the fluid dynamic viscosity can explain the difficulties encountered in MPFS where up to 3 mol L^{-1} HCl solution can be necessary.

7.4. Conclusion

The developed work presents interesting findings although it is not concluded. The LWCC offers a reliable alternative for achieving low levels of bromate in both systems without any complex sample pretreatment. The FIA system attained lower detection and quantification limits for both reagents when compared with MPFS without the lack of repeatability registered in the MPFS. Moreover, the FIA system

presents a higher sampling rate although has some important disadvantages such as high reagent consumption and manual operation.

It is also important to point out that there is a difficulty of the micro-pumps to overcome the back pressure generated by the LWCC.

An additional conclusion is that chlorpromazine reagent presents a lower detection limit for bromate determination in both systems than trifluoperazine.

The developed work reveals some important features namely low detection limit and high determination rate when compared with other flow methods described at Table 7.6.

This work is not finished since no interference studies were carried out and no application to water samples for both systems was carried out.

Table 7.6. Comparison of analytical figures of different flow methods for bromate determination (continuining).

System	Detection mode	Range ($\mu g\ L^{-1}$)	Detection limit ($\mu g\ L^{-1}$)	Determination rate (h^{-1})	Reagent consumption ($\mu mol\ assay^{-1}$)	Matrix	Preconcentra-tion	Reference
FIA	Spectrophoto-metry	Up to 160	10	8	2 ODA	Mineral water	n.a.	Alonso-Mateos et al. 2008
FIA	Spectrophoto-metry	Up to 40	0.8	24	0.056 CPZ	Aqueous standards	Solid phase extraction before injection in the system	Gordon and Bubnis 1995
MSFIA	Spectrophoto-metry	Up to 750	6	35	7 CPZ	Tap water	n.a.	Oliveira et al. 2011
FIA	Spectrophoto-metry	Up to 130	2.3	44	1.5 PCP	Tap and drinking water	n.a.	Uraisin et al. 2006
SIA	Spectrophoto-metry	Up to 3000	150	45	1.6 SCN^- and 1.8 PADAP	Water samples	n.a.	van Staden et al. 2004
FIA	Fluorescence	Up to 200	0.9	10	0.2 CARB	Mineral and well water	n.a.	Almendral-Parra et al. 2008
FIA	Fluorescence	15	1.5	18	0.2 TPZ	Mineral and tap water	n.a.	Ohtomo et al. 2009

Table 7.6. Comparison of analytical figures of different flow methods for bromate determination (continuinuation)

System	Detection mode	Range ($\mu g\ L^{-1}$)	Detection limit ($\mu g\ L^{-1}$)	Determination rate (h^{-1})	Reagent consumption ($\mu mol\ assay^{-1}$)	Matrix	Preconcentra-tion	Reference
FIA	Potentiometric	Up to 125	2.5	30	n.a.	Aqueous standards	n.a.	Ohura et al. 2004
FIA	Chemilumines-cence	Up to 62000	10	60	2.5 sulphite	Aqueous standards	n.a.	Silva et al. 2001
FIA	ICP-MS	Up to 50	0.13	6	n.a.	Tap, mineral, ozonated and hypochlorite trated waters	n.a.	Elwaer et al. 2000
MPFS	Spectrophoto-metry	Up to 100	1.2	23	1.3 CPZ	Aqueous standards	n.a.	Developed method
FIA	Spectrophoto-metry	Up to 100	0.7	60	4.65 CPZ	Aqueous standards	n.a.	Developed method

n.a. –not applied; ODA – 3-3'-dimethoxybenzidine; CPZ – chlorpromazine; PCP – prochlorperazine; PADAP – 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol; CARB – carbostyril; TPZ- trifluoperazine; ICP-MS – inductively coupled plasma mass spectrometry;

Chapter 8

General conclusions and suggestions for future work

In this chapter, an overview of the developed methods presented in this thesis is given. This overview includes the analytical features as well as the main contributions of each developed work. At last, some future possibilities and perspectives using the LWCCs are presented.

8.1. General conclusions and suggestions for future work

In this thesis, the LWCC was applied to the spectrophotometric determination of different analytes in several types of water samples. This type of detection cell proved to be very effective when determinations of trace levels are in focus, this way avoiding complex and time-consuming sample pre-treatment and analyte preconcentration steps. This cell provided good signal stability and repeatability, robustness as well as simple working mode under flow analysis conditions. Coupling of the long path-length cell to a flow-based method can be a relatively straightforward process and often does not require modifications in the chemical or hydrodynamic parameters of an existing method. The work presented in this thesis was focused on further improvement of the spectrophotometric analytical methods and reached better analytical features beyond the simple physical replacing of the detection cell. By using the LWCC instead of the U type flow cells, lower detection limits and reagents consumption ("Green Chemistry") were obtained.

The different flow approaches applied throughout this thesis contributed to a better understanding of the real differences between each approach. It can be stated that within all the flow approaches described in the Introduction Chapter, there is no single perfect and universal flow approach; the choice of the flow system should depend on the main objective to be achieved in the respective methodology.

All the developed methodologies presented a reduction in the reagents consumption without compromising other analytical features and also presented a high degree of automation (exception should be made to the FIA system in Chapter 7).

In Chapter 3, a comparison between two colorimetric reagents for the spectrophotometric determination of low levels of iron in natural waters was performed.

For this reason, a SIA system coupled with a LWCC was used. The developed system allowed the reduction in the reagents consumption with a high sampling determination. Another very important feature was the low detection limits achieved for both reagents in different types of water without the need of any complex preconcentration step. Although with neither of the reagents was possible to reach the necessary detection limits for the determination of iron in ocean waters, the objective of the determination of iron in estuarine waters (where the expected levels are higher) was satisfied. The comparison within reagents revealed that ferrozine reagent presents a higher sensitivity for iron and that it is also more prone to interference from other species when compared to 1,10-phenathroline reagent.

In Chapter 4, the main objective of the developed work was the spectrophotometric determination of iron in ocean waters. With this in mind, a preconcentration unit was added to a MSFIA system coupled with a LWCC. The use of the MSFIA approach allowed the application of preconcentration units since MSFIA is able to work under moderate back pressure. In the developed system, two colour reagents and two preconcentration resins were used. The low detection limit and reagent consumptions achieved with a considerable analytical throughput when using a preconcentration unit are the main features of this method. A lower detection limit could be reached by increasing the sampling load but with a lower analytical throughput. The comparison of the performance between the resins revealed that the NTA Superflow had better operational stability without the need for an additional conditioning solution. The ferrozine method presented a lower sensitivity than the ammonium thiocyanate method. It should also be emphasized that this developed method was the first to combine a LWCC with a preconcentration resin in a MSFIA system. The major drawback of this method was the high absorbance blank values obtained. These high absorbance blank

values were related to high reagent concentrations, drowning the attention to the importance of using high purity reagents as one way to diminish the high blank absorbance value.

In Chapter 5, a multi-parametric spectrophotometric determination of zinc and copper at low levels in natural waters was accomplished. Both analytes were determined using the same colour reagent. This approach, although not new in flow analysis applications, allowed to exploit the versatility of the MSFIA set up in liquid handling. The low detection limits and reagent consumptions achieved with a high sampling rate are the principal features. The copper determination presented a lower detection limit and a lower RSDs when compared to the zinc determination. The higher RSDs in the zinc determination were expected since these deviations are affected by the deviations obtained in copper determination. This method, when compared to other previous flow methods displaying similar working ranges, is simpler since it was not necessary to use any preconcentration step. It should also be mentioned that this method was the first one that involves the use of LWCC in a multi-parametric determination.

In Chapter 6, a MPFS was coupled with a LWCC with the goal of determining titanium at trace levels. The determination was based on a colorimetric reaction, known to have low sensitivity in batch analytical procedures. This methodology was successfully applied to different types of samples, including waters of different origin, digested commercial sunscreen formulations and a lake sediment. This analytical procedure presented as principal features, a low detection limit, low reagent consumption and a high analytical throughput. An additional important feature of this procedure is the low-power supply voltage required of the micro-pumps (important aspect for portable units) and the application to a wide variety of samples.

The work developed in Chapter 7 was the spectrophotometric determination of bromate in natural waters at low levels using a MPFS. The same spectrophotometric procedure was later applied to a FIA system. In order to attain low levels of bromate, a LWCC was coupled to both flow systems and two possible colorimetric reagents were tested. The FIA system provided lower detection limits, a higher sampling rate with a very good repeatability for both reagents when compared with the developed MPFS. Moreover, the micro-pumps in the MPFS revealed an extreme difficulty to overcome the back pressure generated by the LWCC. However, the FIA system presents some important disadvantages such as higher reagent consumption and manual operation. The chlorpromazine reagent presented lower detection limit than trifluoperazine reagent in both flow systems. The FIA procedure is not fully optimized at this stage of the work since the interference studies were not carried out and no application to water samples was done. Nevertheless, it reveals some important and promising features when compared to other alternative flow methods described in literature.

The major drawbacks detected for this cell were the high blank absorbance values obtained with some reagents and also the limitation of using very concentrated acids/bases (at concentrations superior than 4 M) with the purpose of protecting the fused silica layer.

The flow systems used in this thesis are attractive tools for several analytical procedures. The remarkable degree of automation (exception should be made to the FIA system in Chapter 7) and large versatility presented combined with the simple working mode and the low cost of the equipment are the main interesting features. The in-situ

application of these systems is an interesting option and the future works should exploit this field.

The application of the LWCC in fluorescence determinations represents an attractive possibility since this equipment is more versatile and has a low cost when compared to other commercial fluorimeters.

The possibility to use the LWCC coupled with preconcentration or separation units is another interesting option in the future to be exploited to reach even more attractive analytical features and with this, the multi-parametric determinations at trace levels become easy and simple.

The determination of other analytes and the application to other matrices are other possible alternatives.

References

Achilli M. and L. Romele. 1999. Ion chromatographic determination of bromate in drinking water by post-column reaction with fuchsin. Journal of Chromatography A 847:271.

Achterberg, E.P., T.W. Holland, A.R. Bowie, R.F.C. Mantoura and P.J. Worsfold. 2001. Review: Determination of iron in seawater. Analytica Chimica Acta 442:1.

Adornato, L.R., E.A. Kaltenbacher, T.A. Villareal and R.H. Byrne. 2005. Continuous in situ determinations of nitrite at nanomolar concentrations. Deep-Sea Research Part I 52:543.

Aggarwal S.G. and K.S. Patel. 1998. Flow injection analysis of Zn and Co in beverages, biological, environmental and pharmaceutical samples. Journal of Analytical Chemistry 362:571.

Alegret, S., J. Alonso, J. Bartroli, A.A.S.C. Machado, J.L.F.C. Lima and J.M. Paulis. 1987. Construction of equipment for potentiometric determinations in flow injection analysis. Química Analítica 6:749.

Almendral-Parra, M.J., A. Alonso-Mateos and M. S. Fuentes-Prieto. 2008. Online monitoring of bromate in ozonized water without a previous separation process. Journal of Fluorescence 18:1169.

Alonso-Mateos, A., M.J. Almendral-Parra and M.S. Fuentes-Prieto. 2008. Sequential and simultaneous determination of bromate and chlorite (DPBs) by flow techniques-kinetic differentiation. Talanta 76:892.

Alwarthan, A.A. and A. Townshend. 1987. Chemiluminescence determination of iron(II) and titanium(III) by flow-injection analysis based on reactions with and without luminol. Analytica Chimica Acta 196:135.

Amornthammarong, N. and J.-Z. Zhang. 2009. Liquid-waveguide spectrophotometric measurement of low silicate in natural waters. Talanta 79:621.

Anastas, P.T.. 1999. Green chemistry and the role of analytical methodology development. Critical Reviews in Analytical Chemistry 29:167.

Anastasio, C. and T. Robles. 2007. Light absorption by soluble chemical species in Arctic and Antarctic snow. Journal of Geophysical Research 112(D24304):1.

Anthemidis, A.N. and K.I.G. Ioannou. 2009. On-line sequential injection dispersive liquid-liquid microextraction system for flame atomic absorption spectrometric determination of copper and lead in water samples. Talanta 79:86.

Avivar, J., L. Ferrer, M. Casas and V. Cerdà. 2010. Automated determination of uranium(VI) at ultra trace levels exploiting flow techniques and spectrophotometric detection using a liquid waveguide capillary cell. Analytical and Bioanalytical Chemistry 397:871.

Avivar, J., L. Ferrer, M. Casas and V. Cerdà. Lab on valve-multisyringe flow injection system (LOV-MSFIA) for fully automated uranium determination in environmental samples. Talanta article in press

Ayora-Cañada, M.J., M.I. Pascual-Reguera and A. Molina-Díaz. 1998. Continuous flow-through solid phase spectrophotometric determination of trace amounts of zinc. Analytica Chimica Acta 375:71.

Belz, M., P. Dress, A. Sukhitskiy and S. Liu. 1999. Linearity and effective optical pathlength of liquid waveguide capillary cells. In: Internal Standardization and Calibration Architectures for chemical sensor, from Proceedings of the society of photo-optical instrumentation engineers.Vol: 3856; p. 271-281.

Belz, M., P. Dress, K.-F. Klein, W.J.O. Boyle, H. Franke and K.T.V. Grattan. 1998. Liquid core waveguide with fiber optic coupling for remote pollution monitoring in the deep ultraviolet. Water Science and Technology 37:279.

Bio Chem Fluidics site. 2011. (https://www.biochemfluidics.com/pdf/micro-pump_brochure_IBPMP-01_r3.pdf) (consulted in 5.04.2011).

Björklund, E., F. Maya, S.A. Bak, M. Hansen, J.M. Estela and V. Cerdá. 2011. Possibilities and limitations of the sequential injection chromatography technique for the determination of anticoccidial agents in water, pharmaceutical formulations and feed. Microchemical Journal article in press.

Blain, S. and P. Treguer. 1995. Iron(II) and iron(III) determination in seawater at the nanomolar level with selective online preconcentration and spectrophotometric determination. Analytica Chimica Acta 308:425.

Bonifácio, V.G., L.C. Figueiredo, L.H. Marcolino. and O. Fatibello. 2007. An improved flow system for chloride determination in natural waters exploiting solid-phase reactor and long pathlength spectrophotometry. Talanta 72:663.

Borba, B.M.D., J.S. Rohrer, C.A. Pohl and C. Saini. 2005. Determination of trace concentrations of bromate in municipal and bottled drinking waters using a hydroxide-selective column with ion chromatography. Journal of Chromatography A 1085:23.

Burguera-Pascu, M., A. Rodríguez-Archilla, J.L. Burguera, M. Burguera, C. Rondón and P. Carrero. 2007. Flow injection on-line dilution for zinc determination in human saliva with electrothermal atomic absorption spectrometry detection. Analytica Chimica Acta 600:214.

Butler, R., L. Lytton, A,R. Godley, I.E. Tothill and E. Cartmell. 2005. Bromate analysis in groundwater and wastewater samples. Journal of Environmental Monitoring 7:999.

Byrne, R.H. and E. Kaltenbacher. 2001. Use of liquid core waveguides for long pathlength absorbance spectroscopy: principles and practice. Limnology and Oceanography 46:740.

Byrne, R.H., W. Yao, E. Kaltenbacher and R. D. Waterbury. 2000. Construction of a compact spectrofluorometer:spectrophotometer system using a flexible liquid core waveguide. Talanta 50:1307.

Byrne, R.H., X. Liu, E.A. Kaltenbacher and K. Sell. 2002. Spectrophotometric measurement of total inorganic carbon in aqueous solutions using a liquid core waveguide. Analytica Chimica Acta 451:221.

Cai, R., Y. Kubota, T. Shuin, H. Sakai, K. Hashimoto and A. Fujishima. 1992. Introduction of cytotoxicity by photoexcited TiO2 particles. Cancer Research 52:2346.

Callahan, M.R., E. Kaltenbacher and R. Byrne. 2004. In-situ measurements of Cu in an estuarine environment using a portable spectrophotometric analysis system. Environmental Science and Technology 38:587.

Callahan, M.R., J. B. Rose and R. H. Byrne. 2002. Long pathlength absorbance spectroscopy: trace copper analysis using a 4.4 m liquid core waveguide. Talanta 58:891.

Caruso, R.A., M. Antonietti, M. Giersig, H.P. Hentze and J. Jia. 2001. Modification of TiO2 network structures using a polymer gel coating technique. Chemistry of Materials 13:1114.

Cerdà, V.. 2003. Multisyringe flow injection analysis, a young and promising technique. Journal of Flow Injection Analysis 20:203.

Cerdà, V., J.M. Estela, R. Forteza, A. Cladera, E. Becerra, P. Altimira and P. Sitjar. 1999. Flow techniques in water analysis. Talanta 50:695.

Cerdà, V., R. Forteza and J.M. Estela. 2007. Potential of multisyringe flow-based multicommutated systems. Analytica Chimica Acta 600:35.

Chan, M.S. and S.H. Huang. 2000. Direct determination of cadmium and copper in seawater using a transversely heated graphite furnace atomic absorption spectrometer with Zeeman-effect background corrector. Talanta 51:373.

Chen, J.L. and Z. Chang-Qing. 2007. Fluorescence enhancement assay for trace iron(II) using Pyr-tempo as a spin label fluorescence probe. Microchimica Acta 156:307.

Cheng, X., Z. Zhang and S. Tian. 2007. A novel long path length absorbance spectroscopy for the determination of ultra trace organophosphorus pesticides in vegetables and fruits. Spectrochimica Acta Part A 67:1270.

Cho, H.-R., E.C. Jung, K.K. Park, K. Song and J.-I. Yun. 2010. Effect of reduction on the stability of Pu(VI) hydrolysis species. Radiochimica Acta 98:555.

Christiansen, M.B., N. Gopalakrishnan, K.S. Sagar, S. Ndoni and A. Kristensen. 2010. Di-block co-polymer derived nanoporous polymer liquid core waveguides. In: Nanoengineering: Fabrication, properties, optics and devices VII, from Proceedings of SPIE-The International Society for Optical Engineering. Vol: 7764; p. 77640J.

Clesceri, L.S., A.E. Greenberg and A.A. Eaton. 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, American Water Works Association and Water Environment Federation. 3100 B Sampling and Sample preparation; p. 3-2.

Clesceri, L.S., A.E. Greenberg and A.A. Eaton. 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, American Water Works Association and Water Environment Federation. 3500 Fe Iron; p. 3-76.

Coale, K.H., K.S. Johnson, S.E. Fitzwater, R.M. Gordon, S. Tanner, F.P. Chavez, L. Ferioli, C.M. Sakamoto, P. Rogers, F. Millero, P. Steinberg, P. Nightingale, D. Cooper, W.P. Cochlan, M.R. Landry, J. Constantinou, G. Rollwagen, A. Trasvina and R. Kudela. 1996. A massive phytoplankton bloom induced by an ecossystemscale iron fertilization experiments in the equatorial Pacific Ocean. Nature 383:495.

Coelho, L.H.G., W.R. Melchert, F.R. Rocha, F.R.P. Rocha and I.G.R. Gutz. 2010. Versatile microanalytical system with porous polypropylene capillary membrane for calibration gas generation and trace gaseous pollutants sampling applied to the analysis of formaldehyde, formic acid, acetic acid and ammonia in outdoor air. Talanta 83:84.

Collado-Sánchez, C., J. Pérez-Peña, M.D. Gelado-Caballero, J.A. Herrera-Melian and J.J. Hernández-Brito. 1996. Rapid determination of copper,lead and cadmium in unpurged seawater by adsorptive stripping voltammetry. Analytica Chimica Acta 320:19.

Costa, R.C.C. and A.N. Araújo. 2001. Determination of Fe(III) and total Fe in wines by sequential injection analysis and flame atomic absorption spectrometry. Analytica Chimica Acta 438:227.

Crofton, K.M. 2006. Bromate: Concern for developmental neurotoxicity? Toxicology 221:212.

Croot, P.L., A.R. Bowie, R.D. Frew, M.T. Maldonado, J.A. Hall, K.A. Safi, J. LaRoche, P.W. Boyd and C.S. Law. 2001. Retention of dissolved iron and FeII in an iron induced Southern Ocean phytoplankton bloom. Geophysical Research Letters 28: 3425.

Croot, P.L., J.W. Moffett and L.E. Brand. 2000. Production of extracellular Cu complexing ligands by eukaryotic phytoplankton in response to Cu stress. Limnology and Oceanography 45:619.

[CEU] Council of the European Union. 1998 Dec 12. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. Official Journal of the European Communities L 330/30-L 330/52. (http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1998:330:0032:0054:EN:PDF) (consulted in 3.11.09).

D'Sa, E.J., R.G. Steward, A. Vodacek, N.V. Blough and D. Phinney.1999. Determining optical absorption of colored dissolved organic matter in seawater with a liquid capillary waveguide. Limnology and Oceanography 44:1142.

Dallas, T. and P.K. Dasgupta. 2004. Light at the end of the tunnel: recent analytical applications of liquid-core waveguides. Trac-Trends in Analytical Chemistry 23:385.

Dasgupta, P.K., I.Y. Eom, K.J. Morris and J. Li. 2003. Light emitting diode-based detectors. Absorbance, fluorescence and spectroelectrochemical measurements in a planar flow-thorough cell. Analytica Chimica Acta 500:337.

Dasgupta, P.K., G. Zhang, J. Li, C.B. Boring, S. Jambunathan and R. Al-Horr. 1999. Luminescence detection with a liquid core waveguide. Analytical Chemistry 71:1400.

Dasgupta, P.K., Z. Genfa, S.K. Poruthoor, S. Caldwell and S. Dong. 1998. High-sensitivity gas sensors based on gas-permeable liquid core waveguides and long-path absorbance detection. Analytical Chemistry 70:4661.

Datta, A., I.Y. Eom, A. Dhar, P. Kuban, R. Manor, I. Ahmad, S. Gangopadhyay, T. Dallas, M. Holtz, F. Temkin and P.K. Dasgupta. 2003. Microfabrication and characterization of teflon AF-coated liquid core waveguide channels in silicon. IEEE Sensors Journal 3:788.

de Jong, J., V. Schoemann, D. Lannuzel, J.L. Tison and N. Matielli. 2008. High-accuracy determination of iron in seawater by isotope dilution multiple collector inductively coupled plasma mass spectrometry (ID-MC-ICP-MS) using nitrilotriacetic acid chelating resin for pre-concentration and matrix separation. Analytica Chimica Acta 623:126.

Deng, G., L. Wei and G.E. Collins. 2003. Sensitive detection of beryllium using a fiber optic liquid waveguide cell. Talanta 60:9.

Dias, A.C.B., E.P. Borges, E.A.G. Zagatto and P.J. Worsfold. 2006. A critical examination of the components of the Schlieren effect in flow analysis. Talanta 68:1076.

Divjak, B., M. Novic and W. Goessler. 1999. Determination of bromide, bromate and other anions with ion chromatography and an inductively coupled plasma mass spectrometer as element-specific detector. Journal of Chromatography A 862:39.

Dress, P. and H. Franke. 1996. A cylindrical liquid-core waveguide. Applied Physics B 63:12.

Dress, P. and H. Franke. 1997. Increasing the accuracy of liquid analysis and pH-value control using a liquid-core waveguide. Review of Scientific Instruments 68:2167.

Dress, P., M. Belz, K.-F. Klein, K.T.V. Grattan and H. Franke. 1998. Physical analysis of teflon coated capillary waveguides. Sensors and Actuators B 51:278.

Dutra, R.L., H.F. Maltez and E. Carasek. 2006. Development of an on-line preconcentration system for zinc determination in biological samples. Talanta 69:488.

EC (European Commission) Technical Guidance Document in support of commission directive 93/67/EEC on risk assessment for new notified substances. Part II, Environmental Risk Assessment. (2003) Office for official publications of the European Communities, Luxembourg.

Ellis, P.S., A.J. Lyddy-Meaney, P.J. Worsfold and I.D. McKelvie. 2003. Multi-reflection photometric flow cell for use in flow injection analysis of estuarine waters. Analytica Chimica Acta 499:81.

Ellis, P.S., B.S. Gentle, M.R. Grace and I.D. McKelvie. 2009. A versatile total internal reflection photometric detection cell for flow analysis. Talanta 79:830.

Elrod, V.A., K.S. Johnson and K.H. Coale. 1991. Determination of subnanomolar levels of iron(II) and total dissolved iron in seawater by flow injection analysis with chemiluminescence detection. Analytical Chemistry 63:893.

Elwaer, A.R., C.W. McLeod and K.C. Thompson. 2000. On-line separation and determination of bromate in drinking waters using flow injection ICP mass spectrometry. Analytical Chemistry 72:5725.

Farrell, S., J.F. Joa and G.E. Pacey. 1995. Spectrophotometric determination of bromate ions using phenothiazines. Analytica Chimica Acta 313:121.

Federici, G., B.J. Shaw and R.D. Handy. 2007. Toxicity of titanium dioxide nanoparticles to rainbow trout, (Oncorhynchus mykiss): gill injury, oxidative stress, and other physiological effects. Aquatic Toxicology 84:415.

Feres, M.A. and B.F. Reis. 2005. A downsized flow set up based on multicommutation for the sequential photometric determination of iron(II)/iron(III) and nitrite/nitrate in surface water. Talanta 68:422.

Fernandes, S.M.V., A.O.S.S. Rangel and J.L.F.C. Lima. 1995. Colorimetric determination of iron in beer by flow injection analysis using the merging zones technique. Journal of the Institute of Brewing 101:281.

Floge, S.A., K.R. Hardy, E. Boss and M.L. Wells. 2009. Analytical intercomparison between type I and type II long-pathlength liquid core waveguides for the measurement of chromophoric dissolved organic matter. Limnology and Oceanography: Methods 7:260.

Fuwa, K., W. Lei and K. Fujiwara. 1984. Colorimetry with a total reflection long capillary cell. Analytical Chemistry 56:1640.

Galbám, J., S. Marcos, I Sanz, C. Ubide and J. Zuriarrain. 2010. CCD detectors for molecular absorption spectrophotometry. A theoretical and experimental study on characteristics and performance. Analyst 135:564.

Ganranoo, L., S.K. Mishra, A.K. Azad, A. Shigihara, P.K. Dasgupta, Z.S. Breitbach, D.W. Armstrong, K. Grudpan and B. Rappenglueck. 2010. Measurement of nitrophenols in rain and air by two-dimensional liquid chromatography - chemically active liquid core waveguide spectrometry. Analytical Chemistry 82:5838.

Gimbert, L.J. and P.J. Worsfold. 2007. Environmental applications of liquid-waveguide-capillary cells coupled with spectroscopic detection. Trac-Trends in Analytical Chemistry 26:914.

Gimbert, L.J., P. M. Haygarth and P. J. Worsfold. 2007. Determination of nanomolar concentrations of phosphate in natural waters using flow injection with a long path length liquid waveguide capillary cell and solid-state spectrophotometric detection. Talanta 71:1624.

Gledhill, M. and C.M.G. van den Berg. 1994. Determination of complexation of iron(III) with natural organic complexing ligands in seawater using cathodic stripping voltammetry. Marine Chemistry 47:41.

Gomes, D.M.C., M.A. Segundo, J.L.F.C. Lima and A.O.S.S. Rangel. 2005. Spectrophotometric determination of iron and boron in soil extracts using a multi-syringe flow injection system. Talanta 66:703.

González-San Miguel, H.M., M. Fernández, J.M. Estela and V. Cerdà. 2009. Contribution of multi-commuted flow analysis combined with monolithic columns to low-pressure, high-performance chromatography. Trac-Trends in Analytical Chemistry (article in press).

Gooijer, C., G.P. Hoornweg, T. de Beer, A. Bader, D.J. van Iperen and U.A.T. Brinkman. 1998. Detector cell based on plastic liquid-core waveguides suitable for aqueous solutions: one-to-two decades improved detection limits in conventional-size column liquid chromatography with absorption detection. Journal of Chromatography A 824:1.

Gordon, G. and B. Bubnis. 1995. The measurement of very low level bromate ion. Ozone Science and Engineering 17:551.

Gordon, G., B. Bubnis, D. Sweetin and C. Kuo. 1994. A flow injection, non-ion chromatographic method for measuring low level bromate ion in ozone treated waters. Ozone Science and Engineering 16:79.

Granger, J. and N.M. Price. 1999. The importance of siderophores in iron nutrition of heterotrophic marine bacteria. Limnology and Oceanography 44:541.

Greenwood, R., G.A. Mills and B. Roig. 2007. Introduction to emerging tools and their use in water monitoring. Trac-Trends in Analytical Chemistry 26:263.

Hanrahan, G., S. Ussher, M. Gledhill, E.P. Achterberg and P.J. Worsfold. 2002. High temporal and spatial resolution environmental monitoring using flow injection with spectroscopic detection. Trac-Trends in Analytical Chemistry 21:233.

Hashihama, F. and J. Kanda. 2010. Automated colorimetric determination of trace silicic acid in seawater by gas-segmented continuous flow analysis with a liquid waveguide capillary cell. La mer 47:119.

Hirata, S., H. Yoshihara and M. Aihara. 1999. Determination of iron(II) and total iron in environmental water samples by flow injection analysis with column preconcentration of chelating resin functionalized with N-hydroxyethylethylenediamine ligands and chemiluminescence detection. Talanta 49:1059.

Hirata, S., Y. Umezaki and M. Ikeda. 1986. Determination of chromium(III), titanium, vanandium, iron(III), and aluminium by inductively coupled plasma atomic emission-spectrophotometry with an online preconcentration ion-exchange column. Analytical Chemistry 58:2602.

Hong, H. and D.R. Kester. 1986. Redox state of iron in the offshore waters of Peru. Limnology and Oceanography 31:512.

Huang, Y., D. Yuan, J. Ma, M. Zhang and G. Chen. 2009. Rapid speciation of trace iron in rainwater by reverse flow injection analysis coupled to a long path length liquid waveguide capillary cell and spectrophotometric detection. Microchimica Acta 166:221.

Huang, Y.-Z., W.-B. Du, J.-Z. Pan and Q. Fang. 2008. Microfluidic chip-based valveless flow injection analysis system with gravity-driven flows. Analyst 133:1237.

Hund-Rinke, K. and M. Simon. 2006. Ecotoxic effect of photocatalytic active nanoparticles (TiO2) on Algae and Daphnids. Environmental Science and Pollution Research 13:225.

Hutchins, D.A., A.E. Witter, A. Butler and G.W. Luther III. 1999. Competition among marine phytoplankton for different chelated iron species. Nature 400:858.

Infante, C.M.C. and F.R.P. Rocha. 2008. A critical evaluation of a long pathlength cell for flow-based spectrophotometric measurements. Microchemical Journal 90:19.

Ingrand, V., J.L. Guinamant, A. Bruchet, C. Brosse, T.H.M. Noij, A. Brandt, F. Sacher, C. McLeod, A.R. Elwaer, J.P. Croué and P. Quevauviller. 2002. Determination of bromate in drinking water: developments of laboratory and field methos. Trac-Trends in Analytical Chemistry 21:1.

[IUPAC] International Union of Pure and Applied Chemistry, Analytical Chemistry Division, Commission on General Aspects of Analytical Chemistry. 2002. Use of the terms “recovery” and “apparent recovery” in analytical procedures. Pure Appl. Chem. 74:2201.

[IUPAC] International Union of Pure and Applied Chemistry, Analytical Chemistry Division, Commission on Spectrochemical and Other Optical Procedures for Analysis. 1976. Nomenclature, symbols, units and their usage in spectrochemical analysis-II. data interpretation: (Rules approved 1975). Pure Appl. Chem. 45:99.

Jambunathan, S., P.K. Dasgupta, D.K. Wolcott, G.D. Marshall and D.C. Olson. 1999. Optical fiber coupled light emitting diode based absorbance detector with a reflective flow cell. Talanta 50:481.

Kahru, A. and H-C Dubourguier. 2010. From ecotoxicology to nanoecotoxicology. Toxicology 269:105.

Keller, B.K., M.D. DeGrandpre and C.P. Palmer. 2007. Waveguiding properties of fiber-optic capillaries for chemical sensing applications. Sensors and Actuators B 125:360.

Kika, F.S. and D.G. Themelis. 2007. Selective stopped-flow sequential injection method for the spectrophotometric determination of titanium in dental implant and natural Moroccan phosphate rock. Talanta 71:1405.

King, D.W., R.A. Aldrich and S.E. Charnecki. 1993. Photochemical redox cycling of iron in NaCl solutions. Marine Chemistry 44:105.

Kirkpatrick, G.J., D.F. Millie, M.A. Moline and O. Schofield. 2000. Optical discrimination of a phytoplankton species in natural mixed populations. Limnology and Oceanography 45:467.

Korampally, V., S. Mukherjee, M. Hossain, R. Manor, M. Yun, K. Gangopadhyay, L. Polo-Parada and S. Gangopadhyay. 2009. Development of a miniaturized liquid core waveguide system with nanoporous dielectric cladding-a potential biosensing platform. IEEE Sensors Journal 9:1711.

Koscielna, H. 2004. Determination of Bromate(V) in drinking water- A review. Chemia Analityczna 49:445.

Kostal, V., M. Zeisbergerova, K. Slais and V. Kahle. 2005. Fluorescence detection system for capillary separations utilizing a liquid core waveguide with an optical fibre-coupled compact spectrometer. Journal of Chromatography A 1081:36.

Kostal, V., M. Zeisbergerova, Z. Hrotekova, K. Slais and V. Kahle. 2006. Miniaturized liquid core waveguide-based fluorimetric detection cell for capillary separation methods: Application in CE of amino acids. Electrophoresis 27:4658.

Koupparis, M.A. and P.I. Anagnostopoulou. 1986. Automated flow injection spectrophotometric determination of zinc using zincon: Applications to analysis of waters, alloys and insulin formulations. Analyst 111:1311.

Kozuka, S., K. Saito, K. Oguma and R. Kuroda. 1990. Simultaneous determination of trace amounts of iron(III) and titanium(IV) by flow injection with spectrophotometric detection. Analyst 115:431.

Kurokawa, Y., A. Maekawa, M. Takahashi and Y. Hayashi. 1990. Toxicity and carcinogenicity of potassium bromate- A new renal carcinogen. Environmental Health Perspectives 87:309.

Lapa, R.A.S., J.L.F.C. Lima, B.F. Reis, J.L.M. Santos and E.A.G. Zagatto. 2002. Multi-pumping in flow analysis concepts, instrumentation, potentialities. Analytica Chimica Acta 466:125.

Larsson, H. and P.K. Dasgupta. 2003. Liquid core waveguide-based optical spectrometry for field estimation of dissolved BTEX compounds in groundwater: a feasibility study. Analytica Chimica Acta 485:155.

Lee, S-W., S-M. Kim and S-M. J. Choi. 2009. Genotoxicity and ecotoxicity assays using the freshwater crustacean Daphnia magna and the larva of the aquatic midge Chironomus riparius to screen the ecological risks of nanoparticle exposure. Environmental Toxicology and Pharmacology 28:86.

Leelasattarathkul, T., S. Liawruangrath, M. Rayanakorn, B. Liawruangrath, W. Oungpipat and N. Youngvises. 2007. Greener analytical method for the determination of copper (II) in wastewater by micro flow system with optical sensor. Talanta 72:126.

Leelasattarathkul, T., S. Liawruangrath, M. Rayanakorn, W. Oungpipat and B. Liawruangrath. 2006. The development of sequential injection analysis coupled with lab-on-valve for copper determination. Talanta 70:656.

Lei, W., K. Fujiwara and K. Fuwa. 1983. Determination of phosphorus in natural waters by long-capillary-cell absorption spectrometry. Analytical Chemistry 55:951.

Li, J. and P.K. Dasgupta. 1999. Chemiluminescence detection with a liquid core waveguide Determination of ammonium with electrogenerated hypochlorite based on the luminol-hypochlorite reaction. Analytica Chimica Acta 398:33(b).

Li, J. and P. K. Dasgupta. 2000. Measurement of atmospheric hydrogen peroxide and hydroxymethyl hydroperoxide with a diffusion scrubber and light emitting diode-liquid core waveguide-based fluorometry. Analytical Chemistry 72:5338-5347.

Li, J. and P.K. Dasgupta. 2001. Measurement of gaseous hydrogen peroxide with a liquid core waveguide chemiluminescence detector. Analytica Chimica Acta 442:63.

Li, J. and P.K. Dasgupta. 2003. Selective measurement of gaseous hydrogen peroxide with light emitting diode-based liquid-core waveguide absorbance detector. Analytical Sciences 19:517.

Li, J., P. K. Dasgupta and G. Zhang. 1999. Transversely illuminated liquid core waveguide based fluorescence detection. Fluorometric flow injection determination of aqueous ammonium/ammonia. Talanta 50:617.

Li, J., P.K. Dasgupta, G. Zhang and M.A. Hutterli. 2000. Measurement of atmospheric formaldehyde with a diffusion scrubber and light-emitting diode-liquid-core waveguide based fluorometry. Field Analytical Chemistry and Technology 5:2.

Li, Q., K.J. Morris, P.K. Dasgupta, I.M. Raimundo and H. Temkin. 2003. Portable flow injection analyzer with liquid-core waveguide based fluorescence, luminescence and long path length absorbance detector. Analytica Chimica Acta 479:151.

Li, Q.P. and D.A. Hansell. 2008. Intercomparison and coupling of magnesium-induced co-precipitation and long-path liquid-waveguide capillary cell techniques for trace analysis of phosphate in seawater. Analytica Chimica Acta 611:68.

Li, Q.P., D.A. Hansell and J.-Z. Zhang. 2008. Underway monitoring of nanomolar nitrate plus nitrite and phosphate in oligotrophic seawater. Limnology and Oceanography: Methods 6:319.

Li, Q.P, J.-Z. Zhang, F.J. Millero and D.A. Hansell. 2005. Continuous colorimetric determination of trace ammonium in seawater with a long-path liquid waveguide capillary cell. Marine Chemistry 96:73.

Lide, D. R. [ed]. 1993-1994. Handbook of chemistry and physics, 74th ed. CRC Press. Section 14: Geophysics, astronomy and acoustics, p. 14-19.

Liu, R., D. Liu, A. Sun and G. Liu. 1995. Simultaneous determination of copper and zinc in the hair of children by pH gradient construction in a flow-injection system. Analyst 120:569.

Liu, X., Z.A. Wang, R.H. Byrne, E.A. Kaltenbacher and R.E. Bernstein. 2006. Spectrophotometric measurements of pH in-situ: laboratory and field evaluations of instrumental performance. Environmental Science and Technology 40:5036.

Lohan, M.C., A.M. Aguilar-Islas and R.P. Franks. 2005. Determination of iron and copper in seawater at pH 1.7 with a new commercially avaible chelating resin, NTA Superflow. Analytica Chimica Acta 530:121.

Lovern, S.B. and R. Klaper. 2006. Daphnia magna mortality when exposed to titanium dioxide and fullerene (C60) nanoparticles. Environmental Toxicology and Chemistry 25:1132.

Lovern, S.B., J.R. Stricker and R. Klaper. 2007. Daphnia magna when exposed to nanoparticle suspensions (Titanium dioxide, Nano-C60, and C60HxC70Hx). Environmental Science and Technology 41:4465.

Lu, Z., M. Dai, K. Xu, J. Chen and Y. Liao. 2008. A high precision, fast response, and low power consumption in situ optical fiber chemical pCO 2 sensor. Talanta 76:353.

Lupetti, K.R., F.R.P. Rocha and O. Fatibello. 2004. An improved flow system for phenols determination exploiting multicommutation and long pathlength spectrophotometry. Talanta 62:463.

Lunvongsa, S., M. Oshima and S. Motomizu. 2006. Determination of total dissolved amount of iron in water samples using catalytic spectrophotometric flow injection analysis. Talanta 68:969.

Lunvongsa, S., T. Takayanagi, M. Oshima and S. Motomizu. 2006. Novel catalytic oxidative coupling reaction of N,N-dimethyl-p-phenylenediamine with 1,3-

phenylenediamine and its applications to the determination of copper and iron at trace levels by flow injection technique. Anal. Chim. Acta 576:261.

Luo, Y., Y. Li, B. Lv, Z. Zhou, D. Xiao and M.M.F. Choi. 2009. A new luminol derivate as a fluorescent probe for trace analysis of copper (II). Microchimica Acta 164:411.

Ma, J., D. Yuan, M. Zhang and Y. Liang. 2009. Reverse flow injection analysis of nanomolar soluble reactive phosphorus in seawater with a long path length liquid waveguide capillary cell and spectrophotometric detection. Talanta 78:315.

Ma J., P.K. Dasgupta, W. Blackledge and G.R. Boss. 2010. Cobinamide-based ayanide analysis by multiwavelength spectrometry in a liquid core waveguide. Analytical Chemistry 82:6244.

Madrid, Y. and Z.P. Zayas. 2007. Water sampling: traditional methods and new approaches in water sampling strategy. Trac-Trends in Analytical Chemistry 26:293.

Malcolme-Lawes, D.J., G.A. Milligan and R. Newton. 1987. A novel-approach to non-segemented flow analysis. 1. An experimental system. Journal of Automatic Chemistry 9:179.

Masciongioli, T. and W-X. Zhang. 2003. Environmental technologies at nanoscale. Environmental Science and Technology 37:102A.

Matsumiya, H., N. Iki and S. Miyano. 2004. Sulfonylcalix[4]arenetetrasulfonate as pre-column chelating reagent for selective determination of aluminium(III), iron(II), and titanium(IV) by ion-pair reversed-phase high-performance liquid chromatography with spectrophotometric detection. Talanta 62:337.

Maya, F., J.M. Estela and V. Cerdà. 2009. Multisyringe flow injection analysis hyphenated with liquid core waveguides for the development of cleaner spectroscopic analytical methods: improved determination of chloride in waters. Analytical and Bioanalytical Chemistry 394:1577.

McKelvie, I.D., D.M.W. Peat, G.P. Matthews and P.J. Worsfold. 1997. Elimination of the Schlieren effect in the determination of reactive phosphorus in estuarine waters by flow-injection analysis. Analytica Chimica Acta 351:265.

Measures, C.I., J. Yuan and J.A. Resing. 1995. Determination of iron in seawater by flow injection analysis using in-line preconcentration and spectrophotometric detection. Marine Chemistry 50:3.

Melchert, W.R. and F. R.P. Rocha. 2008. An improved procedure for flow-based turbidimetric sulphate determination based on a liquid core waveguide and pulsed flows. Analytica Chimica Acta 616:56.

Melchert W.R., F.R.P. Rocha, I.M. Raimundo and B.F. Reis. 2008. Construction of a long-pathlength flow cell for spectrophotometric measurements. Química Nova 31:427.

Melchert, W.R. and F.R.P. Rocha. 2010. A greener and highly sensitive flow-based procedure for carbaryl determination exploiting long pathlength spectrophotometry and photochemical waste degradation. Talanta 81:327.

Melchert, W.R., D.R. Oliveira and F.R.P. Rocha. 2010. An environmentally friendly flow system for high-sensitivity spectrophotometric determination of free chlorine in natural waters. Microchemical Journal 96:77.

Milani, M.R. and P.K. Dasgupta. 2001. Measurement of nitrogen dioxide and nitrous acid using gas-permeable liquid core waveguides. Analytica Chimica Acta 431:169.

Miller J.C. and J.N. Miller. 1993. Statistics for analytical chemistry, 3rd ed. Ellis Horwood. Errors in instrumental analysis, p. 120-124.

Miller, J.N. and J.C. Miller. 2005. Statistics and chemometrics for analytical chemistry. Harlow: Pearson Education Ltd.

Miller, R.L., M. Belz, C. Del Castillo and R. Trzaska. 2002. Determining CDOM absorption spectra in diverse coastal environments using a multiple pathlength, liquid core waveguide system. Continental Shelf Research 22:1301.

Miller, W.L., D.W. King, J. Lin and D.R. Kester. 1995. Photochemical redox cycling of iron in coastal seawater. Marine Chemistry 50:63.

Millero, F.J. 1989. Effect of ionic interactions on the oxidation Fe(II)) and Cu(I) in natural waters. Marine Chemistry 28:1.

Millero, F.J. and S. Sotolongo. 1989. The oxidation of Fe(II) with H2O2 in seawater. Geochimica et Cosmochimica Acta 53:1867.

Mincher, B.J., L.R. Martin and N.C. Schmitt. 2008. Tributylphosphate extraction behavior of bismuthate-oxidized americium. Inorganic Chemistry 47:6984.

Miró M., V. Cerdà and J.M. Estela. 2002. Multisyringe flow injection analysis; characterization and applications. Trac-Trends in Analytical Chemistry 21:199.

Mitrakas, M., R. Tzimou-Tsitouridou and V. Keramidas. 2010. Interference of humic substances in the spectrophotometric determination of bromate by phenothiazines in natural waters. International Journal of Environmental Analytical Chemistry 78:343.

Moffett, J.W., L.E. Brand, P.L. Croot and K.A. Barbeau. 1997. Cu speciation and cyanobacterial distribution in harbors subject to anthropogenic Cu inputs. Limnology and Oceanography 42:789.

Morel, F.M.M., R.J.M. Hudson and N.M. Price. 1991. Limitation of productivity by trace-metals in the sea. Limnology and Oceanography 36:1742.

Morais, I.P.A., M.R.S. Souto and A.O.S.S. Rangel. 2005. A double-line sequential injection system for the spectrophotometric determination of copper, iron, manganese and zinc in waters. Journal of AOAC International 88:639.

Munoz, M., J. Alonso, J. Bartrolí and M. Valiente. 1990. Automated spectrophotometric determination of Titanium(IV) in water and brines by flow injection analysis based on its reaction with hydrogen peroxide. Analyst 115:315.

Neves, M.S.A.C., M.R.S. Souto, I.V. Toth, S.M.A. Victal, M.C. Drumond and A.O.S.S. Rangel. 2008. Spectrophotometric flow system using vanadomolybdophosphate detection chemistry and a liquid waveguide capillary cell for the determination of phosphate with improved sensitivity in surface and ground water samples. Talanta 77:527.

Nowicki, J.L., K.S. Johnson, K.H. Coale, V.A. Elrod and S.H. Lieberman. 1994. Determination of zinc in seawater using flow injection analysis with fluorometric detection. Analytical Chemistry 66:2732.

Oakes, M., N. Rastogi, B.J. Majestic, M. Shafer, J.J. Schauer, E.S. Edgerton and R.J. Weber. 2010. Characterization of soluble iron in urban aerosols using near-real time data. Journal of Geophysical Research 115(D15302):1.

Obata, H., H. Karatani and E. Nakayama. 1993. Automated determination of iron in seawater by chelating resin concentration and chemiluminescence detection. Analytical Chemistry 65:1524.

Oberdörster, G., E. Oberdörster and J. Oberdörster. 2005. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environmental Health Perspectives 113:823.

Ohtomo, T., R. Yatabe, Y. Tanaka, J. Kato and S. Igarashi. 2009. Fluorescence detection- FIA for ppb levels of bromate with trifluoperazine. Journal of Flow Injection Analysis 26:127.

Ohura M., T. Imato, K. Kameda and S. Yamasaki. 2004. Potentiometric determination of bromate using an Fe(III)-Fe(II) potential buffer by circulatory flow-injection analysis. Analytical Sciences 20:513.

Okada, T. 2007. Liquid-core waveguide in CE. Electrophoresis 28:3414.

Olivares, J.A., P.C. Stark and P. Jackson. 2002. Liquid core waveguide for full imaging of electrophoretic separations. Analytical Chemistry 74:2008.

Oliveira, C.C., R.P. Sartini, B.F. Reis and E.A.G. Zagatto. 1996. Multicommutation in flow analysis. Part 4. Computer-assisted splitting for spectrophotometric determination of copper and zinc in plants. Analytica Chimica Acta 332:173.

Oliveira, S.M., M.A. Segundo, A.O.S.S. Rangel, J.L.F.C. Lima and V. Cerdà. 2011. Spectrophotometric determination of bromate in water using multisyringe flow injection analysis. Analytical Letters 44:284.

Oliveira, S.M., T.I.M.S. Lopes, I.V. Toth, and A.O.S.S. Rangel. 2007. A multicommuted flow injection system with a multi-channel propulsion unit placed

before detection: Spectrophotometric determination of ammonium. Analytica Chimica Acta 600:29.

Otero-Romaní, J., A. Moreda-Piñeiro, P. Bermejo-Barrera and A. Martin-Esteban. 2009. Inductively coupled plasma-optical emission spectrometry/mass spectrometry for the determination of Cu, Ni, Pb and Zn in seawater after ionic imprinted polymer based solid phase extraction. Talanta 79:723.

Pan, J.-Z., B. Yao and Q. Fang. 2010. Hand-held photometer based on liquid-core waveguide absorption detection for nanoliter-scale samples. Analytical Chemistry 82:3394.

Pappas, A.C., C.D. Stalikas, Y.C. Fiamegos and M.I. Karayannis. 2002. Determination of hydrogen peroxide by using a flow injection system with immobilized peroxidase and long pathlength capillary spectrophotometry. Analytica Chimica Acta 455:305.

Paprocki, E.D., B.K. Keller, C.P. Palmer, W.R. Laws and M.D. DeGrandpre. 2008. Characterization of long pathlength capillary waveguides for evanescent fluorescence sensing applications. Sensors and Actuators B 135:145.

Páscoa, R.N.M.J., I.V. Tóth, A.A. Almeida and A.O.S.S. Rangel. 2011. Spectrophotometric sensor system based on a liquid waveguide capillary cell for the determination of titanium: Application to natural waters, sunscreens and a lake sediment. Sensors and Actuators B (article in press).

Páscoa, R.N.M.J., I.V. Tóth and A.O.S.S. Rangel. 2009. Sequential injection trace determination of iron in natural waters using a long-pathlength liquid core waveguide and different spectrophotometric chemistries. Limnology and Oceanography: Methods 7:795.

Pascual-Reguera, M.I., I. Ortega-Carmona and A. Molina-Díaz. 1997. Spectrophotometric determination of iron with ferrozine by flow-injection analysis. Talanta 44:1793-.

Patey, M.D., M.J.A. Rijkenberg, P.J. Statham, M.C. Stinchcombe, E.P. Achterberg and M. Mowlem. 2008. Determination of nitrate and phosphate in seawater at nanomolar concentrations. Trac-Trends in Analytical Chemistry 27:169.

Pinto, J.J., C. Moreno and M. García-Vargas. 2004. A very sensitive flow system for the direct determination of copper in natural waters based on spectrophotometric detection. Talanta 64:562.

Pons, C., R. Forteza and V. Cerdà. 2005. Multi-pumping flow system for the determination, solid-phase extraction and speciation analysis of iron. Analytica Chimica Acta 550:33.

Pons, C., R. Forteza, A.O.S.S. Rangel and V. Cerdà. 2006. The application of multicommutated flow techniques to the determination of iron. Trac-Trends in Analytical Chemistry 25:583.

Pressman, M.A.S. and J.H. Aldstadt. 2005. A remote in situ monitor based on continuous flow analysis for the quantitation of sub-micromoal levels of hexavalent chromium in natural waters. Journal of Environmental Monitoring 7:809.

Ramsden, C.S., T.J. Smith, B.J. Shaw and R.D. Handy. 2009. Dietary exposure to titanium dioxide nanoparticles in rainbow trout, (Oncorhynchus mykiss): no effect on growth, but subtle biochemical disturbances in the brain. Ecotoxicology 18:939.

Rastogi, N., M.M. Oakes, J.J. Schauer, M.M. Shafer, B.J. Majestic and R.J. Weber. 2009. New technique for online measurement of water-soluble Fe(II) in atmospheric aerosols. Environmental Science and Technology 43:2425.

Reis B.F., M.F. Giné, E.A.G. Zagatto, J.L.F.C. Lima and R.A. Lapa. 1994. Multicommutation in flow-analysis. 1. Binary sampling-concepts, instrumentation and spectrophotometric determination of irons in plant digests. Analytica Chimica Acta 293:129.

Resnick, P.R. and W.H. Buck. 1993. Teflon AF- a family of amorphous fluoropolymers with extraordinary properties. In: Antec 93: Be in that number, from Society of plastics engineers technical papers. Vol: 1-3; p. 1904-1907.

Richter, P., M.I. Toral, A.E. Tapia and E. Fuenzalida. 1997. Flow Injection Photometric Determination of zinc and copper with zincon based on the variation of the stability of the complexes with pH. Analyst 122:1045-1048.

Robles, T., Paige, D. and C. Anastasio. 2007. Lens-coupled liquid core waveguide for ultraviolet-visible absorption spectroscopy. Reviews of Scientific Instruments 77(073103):1.

Rocha, F.R.P. and L.S.G. Teixeira. 2004. Strategies to increase sensitivity in UV-Vis spectrophotometry. Quimica Nova 27:807.

Rocha, F.R.P., L.S.G. Teixeira and J.A.N. Nobrega. 2009. Green strategies in trace analysis: a glimpse of simple alternatives for sample pretreatment and analyte determination. Spectroscopy Letters 42:418.

Roerdink, A.R. and J.H. Aldstadt. 2005. Sequential injection absorption spectrophotometry using a liquid-core waveguide: Determination of p-arsanilic acid in natural waters. Analytica Chimica Acta 539:181.

Romele, L. and M. Achilli. 1998. Spectrophotometric determination of low levels of bromate in drinking water after reaction with fuchsin. Analyst 123:291.

Rubí, E., M.S. Jiménez, F. Bauzá de Mirabó, R. Forteza and V. Cerdà. 1997. Preconcentration and atomic absorption determination of iron by sequential injection analysis. Talanta 44:553.

Rue, E.L. and K.W. Bruland. 1997. The role of organic complexation on ambient iron chemistry in the equatorial Pacific Ocean and the response of a mesoscale iron addition experiment. Limnology and Oceanography 42:901.

Ruedas-Rama, M.J., A. Ruiz-Medina and A. Molina-Díaz. 2005. Resolution of biparamentric mixtures using bead injection spectroscopic flow-through renewable surface sensors. Analytical Sciences 21:1079.

Rumori, P. and V. Cerdà. 2003. Reversed flow injection and sandwich sequential injection methods for the spectrophotometric determination of copper(II) with cuprizone. Analytica Chimica Acta 486:227.

Ruzicka J. 1992. The second coming of flow-injection analysis. Analytica Chimica Acta 261:3.

Ruzicka J. 2000. Lab-on-valve: universal microflow analyzer based on sequential and bead injection. Analyst 125:1053.

Ruzicka J. and E.H. Hansen. 1975. Flow injection analysis. Part I. A new concept of fast continuous flow analysis. Analytica Chimica Acta 78:145.

Ruzicka J. and G.D. Marshall. 1990. Sequential injection: a new concept for chemical sensors, process analysis and laboratory assays. Analytica Chimica Acta 237:329.

Ruzicka, J. and L. Scampavia. 1999. From flow injection to bead injection. Analytical Chemistry 4:257A.

Salami, F.H., V.G. Bonifacio, O. Fatibello and L.H. Marcolino. 2009. Spectrophotometric flow determination of chlorine in water using a long pathlength optic cell and multicommutation. Quimica Nova 31:112.

Sanchez, M.A. and F.R.P. Rocha. 2008. A flow-based analytical procedure for salbutamol determination exploiting chemiluminescence in a liquid-core waveguide. Analytical Letters 41:1579(a).

Sanchez, M.A. and F.R.P. Rocha. 2008. A critical evaluation of a flow-cell based on a liquid core waveguide for chemiluminescence measurements. Luminescence 23:410(b).

Santana-Casiano, J.M., M. Gonzalez-Davila and F.J. Millero. 2005. Oxidation of nanomolar levels of Fe(II) with oxygen in natural waters. Environmental Science and Technology 39:2073.

Santelli, R.E. and R.C.S. Araujo. 1992. Spectrophotometric flow-injection determination of titanium in rocks. Analyst 117:1519.

Schminke G. and A. Seubert. 2000. Simultaneous determination of inorganic disinfection by-products and the seven standard anions by ion chromatography. Journal of Chromatography A 890:295.

Segundo, M.A. and A.O.S.S. Rangel. 2002. Flow analysis: a critical view of its evolution and perspectives. Journal of Flow Injection Analysis 19:3.

Segura, R., M.I. Toral and V. Arancibia. 2008. Determination of iron in water samples by adsorptive stripping voltammetry with a bismuth film electrode in the presence of 1-(2-piridylazo)-2-naphthol. Talanta 75:973.

Shams, E., A. Babaei and M. Soltaninezhad. 2004. Simultaneous determination of copper, zinc and lead by adsorptive stripping voltammetry in the presence of Morin. Analytica Chimica Acta 501:119.

Shipway, A.N., E. Katz and I. Willner. 2000. Nanoparticle arrays on surfaces for electronic, optical and sensor applications. Chemphyschem 1:18.

Shpigun, L.K., Y.V. Shushenachev and P.M. Kamilova. 2006. Kinetic separation in flow injection spectrophotometry: Simultaneous determination of copper and zinc in a single run. Analytica Chimica Acta 573-574:360.

Shpigun, L.K., Y. V. Shushenachev and P. M. Kamilova. 2007. Simultaneous Spectrophotometric Determination of Copper(II) and Zinc(II) Based on Their Kinetic Separation in Flow-Injection Systems. Journal of Analytical Chemistry 62:623.

Silva, J.C.G.E.D., J.R.M. Dias and J.M.C.S. Magalhaes. 2001. Factorial analysis of a chemiluminescence system for bromate detection in water. Analytica Chimica Acta 450:175.

Silva, M.L.S., M.B.Q. Garcia, J.L.F.C. Lima and E. Barrado. 2007. Voltammetric determination of food colorants using a polyallylamine modified tubular lelctrode in a multicommuted flow system. Talanta 72:282.

Silva, S.G., J.A. Nóbrega and F.R.P. Rocha. 2011. Exploiting Mn(III)/EDTA complex in a flow system with solenoid micro-pumps coupled to long pathlength spectrophotometry for fast manganese determination. Microchemical Journal 98:109.

Skeggs L.T. 1957. An automatic method for colorimetric analysis. American Journal of Clinical Pathology 28:311.

Skrabal, S.A. and C.M. Terry. 2002. Distributions of dissolved titanium in porewaters of estuarine and coastal marine sediments. Marine Chemistry 77:109.

Skrabal, S.A. 1995. Distributions of dissolved titanium in Chesapeake Bay and the Amazon River Estuary. Geochimica et Cosmochimica Acta 59:2449.

Song, G., I. Villanueva-Fierro, S.-I. Ohira, S. Mishra, H. Bailiff, C.R. Savage and P.K. Dasgupta. 2008. Capillary scale liquid core waveguide based fluorescence detectors for liquid chromatography and flow analysis. Talanta 77:901.

Steimle, E.T., E.A. Kaltenbacher and R.H. Byrne. 2002. In situ nitrite measurements using a compact spectrophotometric analysis system. Marine Chemistry 77:255.

Stookey, L.C. 1970. Ferrozine – A new spectrophotometric reagent for iron. Analytical Chemistry 42:779.

Sugiya, K., M. Harada and T. Okada. 2009. Water-ice chip with liquid-core waveguide functionality: toward lab on ice. Lab on a Chip 9:1037.

Sun, M., W.-B. Du and Q. Fang. 2006. Microfluidic liquid-liquid extraction system based on stopped-flow technique and liquid core waveguide capillary. Talanta 70 392.

Sun, Z.H., W.-X. Cao, J. Zhao, C. Li and W. Zhou. 2008. Construction and test of long pathlength automated analyzer of trace nutrients in seawater. Spectroscopy and Spectral Analysis 28:3000.

Sunda, W.G. and S.A. Huntsman. 1995. Iron uptake and growth limitation in oceanic and coastal phytoplankton. Marine Chemistry 50:189.

Suteerapataranon, S., J. Jakmunee, Y. Vaneesorn and K. Grudpan. 2002. Exploiting flow injection and sequential injection anodic stripping voltammetric systems for simultaneous determination of some metals. Talanta 58:1235.

Szpunar, J., J. Bettmer, M. Robert, H. Chassaigne, K. Cammann, R. Lobinski and O.F.X. Donard. 1997. Validation of the determination of copper and zinc in blood plasma and urine by ICP MS with cross-flow and direct injection nebulisation. Talanta 44:1389.

Takiguchi, H., A. Tsubata, M. Miyata, T. Odake, H. Hotta, T. Umemura and K.-I. Tsunoda. 2006. Liquid core waveguide spectrophotometry for the sensitive determination nitrite in river water samples. Analytical Sciences 22:1017.

Tao, S., S. Gong, L. Xu and J.C. Fanguy. 2004. Mercury atomic absorption by mercury atoms in water observed with a liquid core waveguide as a long path absorption cell. Analyst 129:342.

Teshima, N., J. Li, K. Toda and P.K. Dasgupta. 2005. Determination of acetone in breath. Analytica Chimica Acta 535:189.

Teshima, N., S. Gotoh, K. Ida and T. Sakai. 2006. One-shot flow injection spectrophotometric simultaneous determination of copper, iron and zinc in patients' sera with newly developed multi-compartment flow cell. Analytica Chimica Acta 557:387.

Tsunoda, K.-I., T. Umemura, T. Watanebe, H. Takiguchi, H. Asano, H. Itabashi, Y. Ishibashi and S. Sato. 2003. Characteristics of liquid core waveguide cell made of low-refractive-index polymer and its application to the spectrophotometric: determination of sulfur in a steel sample. Tetsu to Hagane- Journal of Iron and Steel Institute of Japan 89:978.

Uraisin K, T. Takayanagi, D. Nacapricha and S. Motomizu. 2006. Novel oxidation reaction of prochlorperazine with bromate in the presence of synergistic activators and its application to trace determination by flow injection/spectrophotometric method. Analytica Chimica Acta 580:68.

Valsecchi, S., A. Isernia, S. Polesello and S. Cavalli. 1999. Ion chromatography determination of trace level bromate by large volume injection with conductivity and

spectrophotometric detection after post column derivatisation Journal of Chromatography A 864:263.

van Staden J.F. and S.I. Tlowana. 2002. On-line separation, simultaneous dilution and spectrophotometric determination of zinc in fertilisers with a sequential injection system and xylenol orange as complexing agent. Talanta 58:1115.

van Staden, J.F., L.V. Mulaudzi and R.I. Stefan. 2004. Spectrophotometric determination of bromate by sequential injection analysis. Talanta 64:1196.

Vanloot, P., B. Coulomb, C. Brach-Papa, M. Sergent and J.-L. Boudenne. 2007. Multivariate optimization of solid-phase extraction applied to iron determination in finished waters. Chemosphere 69:1351.

Vendramini, D., V. Grassi and E.A.G. Zagatto. 2006. Spectrophotometric flow-injection determination of copper and nickel in plant digests exploiting differential kinetic analysis and multi-site detection. Analytica Chimica Acta 570:124.

von Gunten, U. and J. Hoigné. 1994. Bromate formation during ozonation of bromide containing water: Interaction of ozone and hydrozyl radical reactions. Environmental Science and Technology 28:1234.

Wada, S., T. Kawatsu, Y. Hashizume, T. Yabutani and J. Motonaka. 2006. A long pathlength spectrophotometric tegafur determination using a liquid-core waveguide. International Journal of Modern Physics B 25:4046.

Wada, A., M. Harada and T. Okada. 2008. Kinetic monitoring of electrophoretically induced solute reaction by axial absorption detection with liquid-core waveguide. Analytical Chemistry 78:4709.

Wang, S.-L., X.-J. Huang and Z.-L. Fang. 2001. A miniaturized liquid core waveguide-capillary electrophoresis system with flow injection sample introduction and fluorometric detection using light-emitting diodes. Analytical Chemistry 73:4545.

Wang, S.-L., X.-F. Fan, Z.-R. Xu and Z.-L. Fang. 2005. A simple microfluidic system for efficient capillary electrophoretic separation and sensitive fluorimetric detection of DNA fragments using light-emitting diode and liquid-core waveguide techniques. Electrophoresis 26:3602.

Wang, Z.A., X. Liu, R.H. Byrne, R. Wanninkhof, R.E. Bernstein, E.A. Kaltenbacher and J. Patten. 2007. Simultaneous spectrophotometric flow-through measurements of pH, carbon dioxide fugacity and total inorganic carbon in seawater. Analytica Chimica Acta 596:23.

Wang, Z.A., Y. Wang, W.-J. Cai and S.-Y. Liu. 2002. A long pathlength spectrophotometric pCO 2 sensor using a gas-permeable liquid-core waveguide. Talanta 57:69.

Wang, Z.A., W.-J. Cai, Y. Wang and B. L. Upchurch. 2003. A long pathlength liquid-core waveguide sensor for real-time pCO 2 measurements at sea. Marine Chemistry 84:73.

Ward, N.I. in: F.W. Fifiel and P.J. Haines, (Eds). Environmental Analytical Chemistry, 2nd ed., Oxford, 2000, Chapter 15, p. 363.

Watanabe, K., H. Miyamoto and M. Itagaki. 1999. Determination of zinc(II) using micellar enhanced chemiluminescence of 1,10-phenanthroline. Bunseki Kagaku 48:705-710.

Waterbury, R.D., W. Yao and R.H. Byrne. 1997. Long pathlength absorbance spectroscopy: trace analysis of Fe(II) using a 4.5 m liquid core waveguide. Analytica Chimica Acta 357:99.

Watson, A.J., D.C.E. Bakker, A.J. Ridgwell, P.W. Boyd and C.S. Law. 2000. Effect of iron supply on Southern Ocena CO2 uptake and implications fro glacial atmospheric CO2. Nature 407:730.

Wilson, R.E., Y.-J. Hu and H. Nitsche. 2005. Detection and quantification of Pu(III, IV, V, and VI) using a 1.0 meter liquid core waveguide. Radiochimica Acta 93:203.

World Precision Instruments site (http://www.wpiinc.com/pdf/lwcc-im-040208.pdf) and (http://www.wpiinc.com/index.php/General/General/Liquid-Waveguide-Capillary-Cell.html) (both consulted in 05.04.2011).

Wu, J. and E.A. Boyle. 1997.Low blank preconcentration technique for the determination of lead, copper and cadmium in small-volume seawater samples by isotope dilution ICPMS. Analytical Chemsitry 69:2464.

Xin, J., Y. He, J. Hu, Y. Duan and X. Hou. 2009. A compact spectrophotometer using liquid core waveguide and handheld charge coupled device: for green method and ultrasensitive speciation snalysis of Cr(III) and Cr(VI). Spectroscopy Letters 42:351.

Xu, Z.-R., Q. Li, X.F. Fan, H.-D. Zhang and J. Fang. 2010. A miniaturized spatial temperature gradient capillary electrophoresis system with radiative heating and automated sample introduction for DNA mutation detection. Electrophoresis 31:3137.

Yabutani, T., H. Kurokawa, Y. Hayashi, H. Higuchi, Y. Iwata, Y. Hashizume, S. Wada, Y. Hayashi and J. Motonaka. 2010. Development of a compact system for the determination of lead using a liquid core waveguide and a blue diode as a light source. Analytical Sciences 26:407.

Yamanaka, M., T. Sakai, H.Kumagai and Y. Inoue. 1997. Specific determination of bromate and iodate in ozonized water by ion chromatography with postcolumn derivatization and inductively coupled plasma mass spectrometry. Journal of Chromatography A 789:259.

Yamane, T. 1995. The use of large sample volume injection in flow injection analysis and its application. Journal of Flow Injection Analysis 12:176.

Yan, X.P., M.J. Hendry and R. Herrich. 2000. Speciation of dissolved iron(III) and iron(II) in water by on-line coupling of flow injection separation and preconcentration with inductively coupled plasma mass spectrometry. Analytical Chemistry 72:1879.

Yang, K.L., T.J. Jiang and J. Hwang. 1996. Determination of titanium and vanadium in water samples by inductively coupled plasma mass spectrometry with on-line preconcentration. Journal of Analytical Atomic Spectrometry 11:139.

Yang, T., W. Qin and W. Liu. 2004. Study on the new fluorescence enhancement system of Zn–bis-(trimethylolaminomethane)-4-tert-butyl-disalicylicimine in the presence of cyclodextrin and its analytical application. Talanta 62:451.

Yao, W., R.H. Byrne and R.D. Waterbury. 1998. Determination of nanomolar concentrations of nitrite and nitrate in natural waters using long path length absorbance spectroscopy. Environmental Science and Technology 32:2646.

Yao, W. and R.H. Byrne. 1999. Determination of trace chromium(VI) and molybdenum(VI) in natural and bottled mineral waters using long pathlength absorbance spectroscopy (LPAS). Talanta 48:277.

Zagatto, E.A.G., M.A.Z. Arruda, A.O. Jacintho and I.L. Mattos. 1990. Compensation of the Schlieren effect in flow injection analysus by using dual wavelength spectrophotometry. Analytica Chimica Acta 234:153.

Zamzow, H., K.H. Coale, K.S. Johnson and C.M. Sakamoto. 1998. Determination of copper complexation in seawater using flow injection analysis with chemiluminescence detection. Analytica Chimica Acta 377:133.

Zhang, J.-Z. 2000. Shipboard automated determination of trace concentrations of nitrite and nitrate in oligotrophic water by gas-segmented continuous flow analysis with a liquid waveguide capillary flow cell. Deep-Sea Research Part I 47:1157.

Zhang, J.-Z. 2006. Enhanced sensitivity in flow injection analysis using a long pathlength liquid waveguide capillary flow cell for spectrophotometric detection. Analytical Sciences 22:57.

Zhang, J.-Z. and J. Chi. 2002. Automated analysis of nanomolar concentrations of phosphate in natural waters with liquid waveguide. Environmental Science and Technology 36:1048.

Zhang, J.-Z., C. Kelble and F.J. Millero. 2001. Gas-segmented continuous flow analysis of iron in water with a long liquid waveguide capillary flow cell. Analytica Chimica Acta 438:49.

Zhang, M., D. Yuan, G. Chen, Q. Li, Z. Zhang and Y. Liang. 2009. Simultaneous determination of nitrite and nitrate at nanomolar level in seawater using on-line solid phase extraction hyphenated with liquid waveguide capillary cell for spectrophotometric detection. Microchimica Acta 165:427.

Zhang, T., Q. Fang, S.-L. Wang, L.-F. Qin, P. Wang, Z.-Y. Wu and Z.-L. Fang. 2005. Enhancement of signal-to-noise level by synchronized dual wavelength modulation for light emitting diode fluorimetry in a liquid-core-waveguide microfluidic capillary electrophoresis system. Talanta 68:19.

Zhang, X., S. Hongwen, Z. Zhang, Q. Niu, Y. Chen and J.C. Crittenden. 2007. Enhanced bioaccumulation of cadmium in carp in the presence of titanium dioxide nanoparticles. Chemosphere 67:160.

Zhuang, G.S., Z. Yi, R.A. Duce and P.R. Brown. 1992. Link between iron and sulphur cycles suggested by detection of Fe(II) in remote marine aerosols. Nature 355:537.