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The 2nd Latin American Analitica Congress will make interaction between the academic and industrial sectors its main objective. As well as speeches, symposiums and round tables, the congress will also have a Panel Exposition Area, where university, research center and industry studies will be discussed in order to share information and increase academic-industrial interaction.

The congress will be held simultaneously with the 11th edition of Analitica Latin America – International Exhibition of Laboratory Technology, Analyses, Biotechnology and Quality Control. In its last edition, the event hosted around 10,000 visitors and 500 brands on exhibition.

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MÍDIA OFICIAL / *Official Media:* BrJAC Brazilian Journal of Analytical Chemistry

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Editorial

Starting to set roots

The issue number 2 of BrJAC arrives after an excellent reception of the earlier ones. Some comments received by the Editorial Board are summarized below: *"I would like to congratulate the BrJAC that highlights as an important space to publish Analytical Chemistry and related researches. It is especially interesting to see in BrJAC a new media to promote Forensic Sciences and its interfaces"* as reported by Rodrigo G. Garrido, Forensic Scientist – 1ST Class of IPPGF/DGPTC/PCERJ.

We also received other letters highlighting the importance of the publication for the Brazilian Analytical Chemistry and the quality of its articles, as in the letter by Dr. Maria Rosecler M. Rosseto and in the letter by FAPEMIG, represented by its Scientific Director, Dr. José Policarpo G. de Abreu.

However, we believe that the seed of integration between academia, research centers and industry must be fostered by dreams and concrete actions, and the launching of BrJAC represents a good example of engagement and the implementation of a mechanism to disseminate the scientific knowledge.

In the face of the great challenges of the chemical industries today – being sustainable, considering the raw materials, the industrial processes, the energy consumption, the economic competitiveness, the need for innovation and the preservation of life quality in the planet – only a real academic-industrial integration would be able to provide the conditions for sustainable growth.

From the viewpoint of industries, Analytical Chemistry is spread throughout organizations; from the development laboratories to industrial control equipment, from the research analytical departments to process and quality control laboratories, from the environmental monitoring laboratories to specific process analyzers.

Thus, the wide application of the intrinsic know-how of Analytical Chemistry inside organizations can be improved by the scientific knowledge shared in the articles and sessions of BrJAC. Moreover, the network formed by the readers of BrJAC can produce a different perception of the routine activities in the labs, stimulate discussions of known practices and complete and feed the knowledge cycle, breaking some paradigms.

That is the reason we invite you to join us in consolidating BrJAC as an innovative tool to achieve development and economic growth, based on the academic-industrial integration in the knowledge era.

Cristina M. Schuch
Editor

O Encontro da Tecnologia Analítica na América Latina

11ª EDIÇÃO

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O evento é o local de encontro com os principais fornecedores e compradores dos setores de tecnologia laboratorial, biotecnologia e controle de qualidade.Na última edição o evento contou com **500 marcas** em exposição e **10 mil visitantes** de mais de **20 países**.

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2º CONGRESSO ANALITICA LATIN AMERICA

Revista Oficial:

BrJAC
Brazilian Journal of Analytical Chemistry

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SINDUSFARMA
SINDICATO DA INDÚSTRIA DE PRODUTOS FARMACÊUTICOS NO ESTADO DE SÃO PAULO

2011 AIQ ANO INTERNACIONAL DA QUÍMICA

Sociedade Brasileira de Química

International Year of CHEMISTRY 2011

Organização:

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Editorial Board

Summary

Expedient

BrJAC
Brazilian Journal of Analytical Chemistry

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Letter

This section is reserved for you to send comments, suggestions or reviews about the articles or published reports by BrJAC. You may also submit comments on issues related to the Analytical Chemistry in Brazil and abroad. Join us in this project! Be part of that!

Analytical Chemistry: A multidisciplinary Science

It is an honor to have the opportunity to write some words about Analytical Chemistry in this Journal, which has arisen to provide us not only with a scientific forum of innovation in techniques, analysis, methods, devices, and materials, among others, but also with a mechanism for achieving an important relationship among Academy, Research Centers and Industry.

Since Analytical Chemistry is an interdisciplinary area, it has played a significant role in the understanding of basic science for a range of useful applications, such as quality of pharmaceuticals, medical care, food, water, drink, and the environment. Nowadays, the importance of analytical chemistry can be noticed in the fields of health care, diagnosis, and even sports. Analytical chemistry has also aided the construction of devices and the design of methodologies and studies that will affect our society and will have an impact on forensic, police force and regulatory activities, agriculture, and quality control.

As professionals working in both academic institutions and industry, we feel obliged to work together in order to provide students with the necessary instruction regarding the area of chemical analysis, so that the future professionals can successfully compete in the modern industrial environment as well as in academia. Graduates need a solid background in basic chemistry, experience with modern analytical equipment, and critical analytical thinking skills. Industrial analytical chemists must be involved in developing solutions to this problem. A partnership with the industry can provide the university with additional resources and the student with analytical perspectives.

Considering all that was exposed above, I believe that the Brazilian Journal of Analytical Chemistry has been created at a consolidated moment of maturity of Analytical Chemistry in Brazil. It will surely meet the requirements of innovation and entrepreneurship and will be an important instrument for implementing the university-industry partnership.

Considering the statistic in terms of scientific publications and graduate programs in Analytical Chemistry in Brazil, I am sure that contributions to the BrJAC will put this journal in a position of highly outstanding merit.

Congratulations to BrJAC and to everyone that is involved in this important mission.

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AN OPTIMISTIC VIEW UPON SCIENTIFIC RESEARCH IN BRAZIL

Glaucius Oliva, President of the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq)

When it comes to scientific research, Brazil is still considered a "young country, with many challenges ahead". Despite this, the outlook is positive in the opinion of the Professor Glaucius Oliva, President of the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq), the most the most important federal Brazilian agency for research support.

Professor Oliva thinks that the country has progressed significantly, promoting a continuous growth in the generation of new knowledge and training qualified personnel for R, D & I. According to the President of CNPq, Brazil currently produces 2.7% of all world science and this number tends to grow.

In an exclusive interview to BrJAC, Professor Glaucius also spoke about the key challenges faced by the Brazilian Science, the underserved areas of science and what is needed to meet them, the criteria adopted by the CNPq for the evaluation of researchers, the contributions provided by the Laboratory of Protein Crystallography and Structural Biology, Institute of Physics of São Carlos University of São Paulo, among other issues.

Brazilian Journal of Analytical Chemistry was launched in May 2010, during the 33rd Annual Meeting of the Brazilian Chemical Society, with the aim of increasing the frequency, the follow up and expand the dissemination of scientific results to a larger number of science and technology professionals in activity in universities, industries and research centers, fostering greater integration between the academy and business companies. What is your opinion about the importance of this type of initiative for the scientific and technological development in Brazil?

I consider it very important to have, in Brazil, journals of international quality in all areas of knowledge, allowing the dissemination of the research that is conducted here and in also other countries. To be successful and to attract our best scientists, the journal should have a peer review policy, which should be selective, a well-defined focus, technical excellence and a clear open access policy. Also, if the journal publishes innovative research with potential application in business, the circulation of the journal inside the industries will be timely, thus fostering closer relation between universities and companies.

As the new President of CNPq (the main federal agency for research funding in the country), what is your view on the science policies of the country?

The scenario is highly positive, since the Brazilian science has advanced

expressively. Even though Brazil is still a young country when it comes to scientific research, and with many challenges ahead, we have managed to promote a continuous growth in the generation of new knowledge and training qualified personnel for R, D & I. An important component of this process has been the definition of science and technology policies clearly expressed in the Plan of Action for Science, Technology and Innovation. Currently, we produce 2.7% of all the world science and this number tends to grow. We are recognized as leaders in several areas of knowledge such as tropical agriculture, geophysics and engineering in the field of exploration of oil and gas in deep waters. Our scientific community is recognized and respected throughout the world. Since 2001, the number of masters (MsC) and doctors (PhD) in Brazil has doubled. In 2010 only, 12,000 received a PhD degree and 41,000 obtained their master degrees. In the CNPq Lattes Platform (a curriculum and institutions database of science and technology areas in Brazil) more than 1.7 million resumes are now registered, including 135,000 PhDs and 237,000 MScs, distributed in more than 27,000 research groups registered in the Directory of Research Groups 2010 Census.

What are the main challenges to be faced by Brazilian science?

I think the two main challenges are to conduct research with greater impact and quality and to foster innovation. Despite the fact that we are advancing far in the area of S & T in Brazil, a huge barrier between science and the market still exists, i.e. there is a failure to transform the knowledge into wealth and useful services to society. In this sense, we need to make the universities be closer to Brazilian companies so

A brilliant career

At 51 years of age, besides commanding the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), Professor Glaucius Oliva works in important positions in education and research institutions and agencies. Prof. Oliva himself is a research fellow of CNPq, receiving grants in the Research Productivity category 1A.

At the Institute of Physics of São Carlos, University of São Paulo (IFSC/USP), Oliva is a Full Professor, where he teaches since 1982. In the IFSC/USP, he lead the creation of the area of Biomolecular Physics in the Graduate Program in Physics. Oliva founded and now he coordinates the National Institute of S & T of Structural Biotechnology in Medicinal Chemistry and Infectious Diseases and also the Laboratory of Protein Crystallography and Structural Biology. He heads the Center for Structural Molecular Biotechnology, one of the centers for Research, Innovation and Diffusion of FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) too.

Oliva's main research interests are focused on the structure and function of biological macromolecules and their application to planning and development of new drugs, with particular emphasis on tropical and infectious diseases. He actively fights for the consolidation of Crystallography of Proteins research line in Brazil and its integration into the national research on drugs and medicines. As part of this project, he coordinated several protein crystallization experiments in microgravity aboard NASA space shuttle, in the years 1997-98, a pioneer experience in Brazil.

Oliva got his Ph.D in Protein Crystallography at the University of London in 1988, where he worked with the renowned British scientist Sir Tom L. Blundell. He now leads a team of about 80 researchers, including physicists, biologists and chemists. He has supervised around 30 Ph.D. theses and dissertations.

that they become more competitive in foreign markets. It is necessary to disseminate the culture of innovation in supply chains, and also to reduce the bureaucracy and costs for patent registration, so that we can stimulate further more the technological entrepreneurs.

In your opinion, what are the scientific areas that are in most need of investment?

The areas of engineering and natural sciences must be reinforced and encouraged as soon as possible, because the economic and industrial development of the country depends heavily on these professionals. In recent years this area has advanced a bit but still falls short of our needs. Of the total number of students taking undergraduate courses, only 5% are in the area of engineering, while in China, this percentage reaches 30%. And, worsening the situation, many engineers in the area continue to quit their careers due to lack of stimulus. This needs to be quickly modified. Now we must join efforts and plans to encourage more young people into this area and to recognize the efforts of those already involved, providing them a broad, solid and continuous education. Only then we will be able to meet the challenges of Brazilian development.

"It is necessary to disseminate the culture of innovation in supply chains, and also to reduce the bureaucracy and costs for patent registration"

Many critics point out that large amounts of resources are invested in research in Brazil, but unsatisfactory return is obtained. Do you agree?

Indeed, the process of generating knowledge and transforming that knowledge into wealth for the society, despite having advanced greatly in recent years, is still incipient in Brazil. The problem is that the science and the market still do not communicate effectively here. In addition to increasing funding for S & T, we need primarily encourage development of leading edge technology in Brazilian companies, which can enhance our competitiveness internationally. The CNPq has developed various initiatives that encourage innovation, as the RHAE Program, aimed at training human resources in strategic areas, which contains a set of different grant types for technology development support, specially designed to add highly qualified personnel in R & D in the companies.

The criteria currently adopted by the CNPq for the evaluation of researchers have often been criticized for being quantitative rather than qualitative. In your opinion, are the mechanisms and criteria appropriate? What points could be improved?

We really need to rethink how to evaluate the projects supported from now on, because this process needs to respond to major challenges of Brazilian science. That is, an assessment is needed that can focus on relevance, impact, proximity to the frontier of knowledge, innovation, and insertion in multidisciplinary research. For that to happen, the Program for Strategic Reconfiguration of CNPq, conducted in partnership with the Center for the Management of Strategic Studies - CGEE, has been working with the aim of finding ways to optimize more and more the evaluation procedures, including the contact with similar agencies elsewhere in the world to get to know how they are addressing this problem.

In Brazil, few companies invest in research compared with developed countries. What mechanisms could be made to change this scenario?

The Brazilian science can not fail to further deepen its commitment to economic and social development of the country. For this, we need to implement new and more efficient instruments to stimulate innovation in the academic environment, but especially in business. Strategic areas which hold the future should be prioritized, such as green industrial chemistry, alternative sources of energy and bioenergy in particular, food production, nanotechnology, information technology and communication, the Amazon and the Sea, and innovative support to processing industry.

" The areas of engineering and natural sciences must be reinforced and encouraged as soon as possible, because the economic and industrial development of the country depends heavily on these professionals. "

You have established the Laboratory of Protein Crystallography and Structural Biology at the Institute of Physics of São Carlos. What are the contributions provided for scientific development in this area?

Since my graduation in engineering and physics at University of São Paulo, in São Carlos, I had an interest in studying the structure of the molecules that make up living things. I thought the structure would be the key to understanding its operation. However, in the 70 and 80s in Brazil had not yet research groups working in this area. I did my master's degree in crystallography of small molecules, studying the structure of organic and inorganic compounds. Then I decided to go for a PhD abroad, to work on Protein Crystallography, conducted at the University of London, under the supervision of Professor Sir Tom Blundell. Returning in the late 80's, we created, together with Prof. Richard Garratt, the first group of Protein Crystallography in Brazil, in the Institute of Physics of São Carlos, in which, over the past 20 years, trained over 60 researchers in this area, which spread across the country. We now have at least two dozen protein crystallography labs in the country. It is very pleasant to verify the proper development of this area in Brazil.

Currently, you are leading a research team with about 80 participants, including physicists, biologists and chemists. Is it possible to reveal some details of this research?

Our group joined other researchers from different institutions, first as a Research, Innovation and Diffusion Center (CEPID) from FAPESP, and more recently as a National Institute of Science and Technology, supported by CNPq, FAPESP and the Ministry of Health, focusing Structural Biotechnology in Medicinal Chemistry and Infectious Diseases. We studied the structure of target proteins of parasites that are causes of neglected tropical diseases such as Chagas disease, leishmaniasis, schistosomiasis and malaria, and subsequently we started to use the techniques of medicinal chemistry for the development of inhibitory compounds, drug candidates to treat these diseases. For this, we maintain close collaboration with researchers from the fields of organic synthesis and natural products. We have advanced our

research up to promising compounds in cases of Chagas disease and leishmaniasis.

You have more than 130 papers published in international journals, which have been cited nearly 2000 times. Does Brazil publish too little in your opinion? What are the challenges to increasing the scientific production of Brazilian researchers?

The growth of Brazilian scientific production in recent years is undeniable. In 1999 the country accounted for 1.29% of world production, and in 2009 (the most recent data available) the percentage jumped to 2.7%. Brazil has 54.42% of Latin America papers. There is no doubt that valuing people who do science plays a central role in the production of Brazilian scientists. Thinking about it, the CNPq has introduced the Research Productivity Grant and, more recently, the Productivity Scholarship in Innovative Technology Development and Extension, which today are essential to recognize and encourage our researchers. The CNPq has always been the main driver of scientific and technological development of the country, naturally added to the efforts of other agencies and government bodies, as the Ministry of Science and Technology, Capes (Coordination for the Improvement of Higher Education), the FINEP (Financier of Studies and Projects) and state agencies. In addition to providing grants for training and resources to promote the research, the CNPq maintains a careful system of peer review, through its committees, which motivates the scientific community and enhances the exposure of research results in national and international journals. However, the assessment outlines the research result. As our challenges now are to improve the impact and relevance of research, stimulating innovation and multidisciplinary, we have to improve the evaluation systems so as to favor this direction.

" It is definitely necessary to expand the frontiers of knowledge to ensure that all regions have technological platforms with skilled research centers, each with specific features and characteristics of their region "

The discussion on the exploitation of the Amazon for scientific research purposes has been common. What is your opinion on this?

The Amazon is now a wealth of opportunities for research. We are living in a new global context in which biodiversity, rainforests, water and mineral resources are valued. In this sense, it is necessary to produce more information about the Amazon for reducing deforestation, promoting social inclusion and encourage mainly of S, T & I in the region. Great news is that among the 122 INCTs, 8 are in the Amazon region, the result of a successful partnership with CNPq states of Amazonas and Pará. Still there is plenty of room to expand the training of qualified human resources in the region and to establish actions to attract technology-based businesses focused on biodiversity as well as stimulating programs that seek to reduce or even eradicate social deprivation. It is definitely necessary to expand the frontiers of knowledge to ensure that all regions have technological platforms with skilled research centers, each with specific features and characteristics of their region.

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Determination of aglucone isoflavones in non-transgenic and transgenic soybeans (*Glycine max* L.) by HPLC-DAD

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Abstract
Soybeans, an important global crop, is an excellent source of oil and proteins and also contain many other compounds, including isoflavones, which have been the subject of several studies demonstrating their potential in the treatment of menopause and osteoporosis. This paper describes a method for quantification of the aglucone isoflavones found in soybeans using high performance liquid chromatography with diode array detection after extraction of the total isoflavones followed by acid hydrolysis. The quantities of the total aglucone isoflavones (daidzein, glycitein and genistein) in non-transgenic (BRS133) and transgenic (BRS245RR) soybeans, planted in the same region (north of Parana State, Brazil) and in the same period of the year, in 100 g of soybeans, were 284 ±10 mg and 228±13 mg for the non-transgenic and transgenic soybean varieties, respectively.

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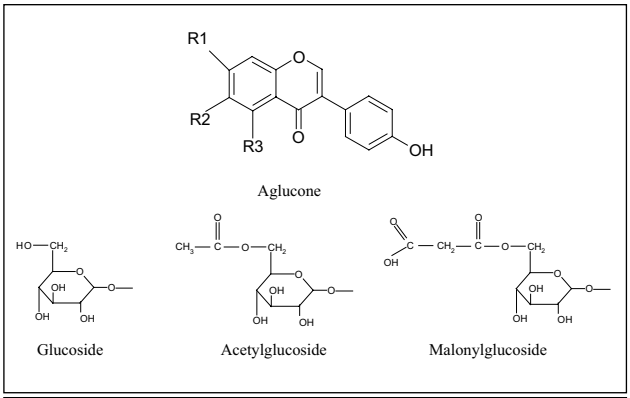
Keywords: soybeans, *Glycine max* L., aglucone isoflavones, transgenic, HPLC-DAD.

Introduction

Among worldwide agricultural products, soybeans have presented enormous expansion and now occupy a prominent position, because this leguminosae is a food that presents high nutritional value, is a source of antioxidants such as isoflavones, has a rich chemical composition including oils, vitamins and some minerals, such as calcium and iron, and contains more than 34% protein.[1,2] Soybeans stand out as a crop with high productivity that present easy adaptation for cultivation in almost all regions of the world. Brazil is the one of the largest producers of soybeans. For the 2007/08 harvest, soybeans occupied an area of more than 21 million hectares, with a total production of 60 million tons, being the second largest producer worldwide, behind the United States.

Isoflavones are naturally occurring phenolic compounds found in a number of plants. Also known as phytoestrogens, isoflavones are found in plant-based foods and appear to have estrogen-like activity.[3] Isoflavones are believed to have a potential preventive action against some prevalent chronic diseases, including hormone-related cancers[4] and osteoporosis[5], and have been indicated for alleviation of menopausal symptoms.[6]

The soybean contains three isoflavone nuclei that are normally present in several different forms (Figure 1), i.e., glucosidic (daidzin, glycitin, genistin); acetylglucosidic (acetyldaidzin, acetylglycitin and acetylgenistin); malonylglucosidic (malonyldaidzin, malonylglycitin, malonylgenistin) and the unconjugated structural forms, the aglucones (daidzein, glycitein, genistein). Soybean and defatted soybean meal contain mainly malonyl glucoside isoflavones, with smaller quantities of the glucoside form and even smaller amounts of acetyl glucoside conjugates.[7] Of these, the glucoside isoflavones genistin and daidzin are the main forms found in soybean, constituting 50 to 90% of flavonoids in soybean meal [8,9] although they show lower biological activity than their aglycone forms, genistein and daidzein.



Isoflavone	R1	R2	R3
Genistein	H	H	OH
Daidzein	H	H	H
Glycitein	H	OCH3	H
Genistin	Glucoside	H	OH
Daidzin	Glucoside	H	H
Glycitin	Glucoside	OCH3	H
Acetyl-genistein	Acetylglucoside	H	OH
Acetyl-daidzin	Acetylglucoside	H	H
Acetyl-glycitin	Acetylglucoside	OCH3	H
Malonyl-genistein	Malonylglucoside	H	OH
Malonyl-daidzin	Malonylglucoside	H	H
Malonyl-glycitin	Malonylglucoside	OCH3	H

Figure 1: Chemical structures of the isoflavones found in soybeans.

The concentration of isoflavones in soybeans is highly variable, [7,10-12] between 0.1 and 0.4% (w/w), and the amounts and compositions of isoflavones differ according to the year, genotype (transgenic or non-transgenic), location and climate [13], there are also differences between transgenic and non-transgenic soybeans, in their formation and in their metabolism, which can generate differences in the amount of isoflavones and other constituents in the soybeans. Most isoflavones are products of the shikimate pathway, the target pathway of the herbicide glyphosate.[14] Therefore, it is possible that glyphosate affects the levels of isoflavones in soybeans produced from transgenic seeds. Concern about phytoestrogen levels in herbicide-tolerant varieties that are typically oversprayed with chemicals was precipitated by preliminary data obtained for Phaseolus species: it was postulated that herbicide treatment may generate increased levels of phytoestrogens.[15] Preliminary phytoestrogen data on single soybean samples showed a wide range of values without overt evidence of differences in isoflavone levels between conventional and genetically modified, unsprayed Roundup Ready soybeans.[16] However, no data have been published on the composition of genetically modified soybeans sprayed with glyphosate in the normal course of production, compared with conventional soybeans.

Alezandro *et al.*[17] have used HPLC-DAD to evaluate the macronutrients and the isoflavones daidzein and genistein in the flour obtained from transgenic BRS243RR soybeans, while García-Villalba *et al.*[18] applied capillary electrophoresis-mass spectrometry to identify the principal components found in both transgenic and non-transgenic soybeans. In general, the same compounds were found in both varieties. However, no quantification was carried out by either of these groups and they did not hydrolyze to convert the isoflavone derivatives into the aglucone forms. Since transgenic crops were introduced in 1995, questions have been proposed regarding potential effects of the transgenes on food quality.[19] One study has indicated that transgenic soybeans contain lower levels of estrogenic isoflavones than non transgenic soybeans.[20] However in this work the solvent for extraction was methanol:water (80:20 v/v), which is not a good extractant, so that only the glycosidic forms, genistin and daidzin, were quantified.

Many procedures have been described for extraction of isoflavones from soybeans. Many are based on solvent extraction, but supercritical fluid extraction,[21] ultra sound-assisted extraction,[22] pressurized liquid extraction,[23] microwave-assisted extraction[24,25] and solid phase extraction [26] are also reported, as recently reviewed by Rostagno *et al.*[27]

For determination of isoflavones in soybeans, after appropriate extractions, the most common techniques for separation and quantification is high performance liquid chromatography with UV-Vis detection [10,28-31] while capillary electrophoresis,[18,32] and liquid chromatography–mass spectrometry has also been carried out. [10,26,33-37]

The present study describes the development and validation of a method for extraction, hydrolysis and quantification of total isoflavones in soybeans, using high performance liquid chromatography. The total quantity of the isoflavones was compared between two different genotypes of soybeans, non-transgenic and transgenic, that were planted in the same cultivation area and during the same period of the year.

Experimental

Chemicals and samples

HPLC-grade acetonitrila, methanol, DMSO, hydrochloric acid (37%), ammonium hydroxide (28-30%) and glacial acetic acid, all ACS grade, were purchased from Merck (Rio de Janeiro, Brazil). The aglucone isoflavone reference standards (genistein, daidzein and glycitein) were purchased from ChromaDex (Santa Ana, CA, USA). The soybean samples (non-transgenic, BRS133 and transgenic, BRS245RR) were donated by CLASPAR – Empresa Paranaense de Classificação de Produtos (Paraná, PR, Brazil). These samples were grown on adjacent plots

in central Parana State, Brazil, (24°45'S and 49°58'W), during the spring of 2005.

Stock solutions of isoflavone standards

Approximately 10 mg of each isoflavone standard (genistein, daidzein and glycitein) were accurately weighed in 100 mL volumetric flasks. Methanol:water (80:20 v/v) was added and the resulting solution was sonicated for 10 min before completing the volume. These stock solutions were stored at 4 °C.

Chromatographic equipment

The HPLC analyses were carried out on a Merck Hitachi LaChrom system from Merck, Darmstadt, Germany, consisting of a L-7100 quaternary pump, a L-7250 programmable autosampler, a L-7455 diode array detector and a L-7300 column oven. Merck HSM software version 4.1 was used for data treatment. The column (150 x 3.9 mm ID) contained Nova-Pak endcapped C-18 (4 µm particle size) from Waters. The mobile phases were: water containing 0.1% acetic acid (A) and acetonitrile, also with 0.1% acetic acid (B). The gradient program with a flow rate of 1.0 mL min^{-1} was: 14% of B for 8 min; 14% to 21% B in 4 min; 21% of B for 3 min; 21% to 29% B in 5 min; 29% to 40% B in 3 min; 40% to 50% B in 2 min; 50% of B for 5 min; 50% to 14% B in 5 min; 14% B for 9 min. The injection volume was 10 µL and detection was at 254 nm. The separations were carried out at 35 °C.

Extraction procedure

The extraction and hydrolysis procedure was based on the method of Griffith and Collison.[10] At the first step, crushed portions of 1.0 g were accurately weighed into 50 mL glass-stoppered flasks. After adding 10 mL of acetonitrile, the samples were swirled and 6 mL of deionized water were added. The samples were then sonicated for 2 h in an Ultrasonic Cleaner (Odontobras, Ribeirão Preto, Brazil). During sonication, the samples were heated to about 50–55 °C. After the samples had cooled to room temperature, 4 mL of deionized water was added in order to obtain a final liquid volume of 20 mL of a 50:50 water:acetonitrile (v/v) solution. The liquid phases were transferred to screw-capped tubes and centrifuged for 10 min at 7000 rpm. The clear solutions were stored at 4 °C until the hydrolysis studies.

Acid hydrolysis of the isoflavones

Four mL of extract were placed in screw-capped test tubes with 1 mL of 37% HCl. The tubes were heated for 2 h at 80 °C in a water bath and then were cooled to room temperature. Two mL of a 50:5:45 (v/v/v) solution of NH_4OH (28-30% in water)/glacial acetic acid/DMSO were added. The pH of the final solution was adjusted with 3 mol L^{-1} NH_4OH to obtain a neutral pH (tested with pH paper). The final solution was completed with H_2O to 10 mL and filtered thorough a 0.45 µm PTFE membrane before HPLC analysis.[30,32]

Method validation

Purity checks of the chromatographic peaks obtained for daidzein, genistein and glycitein were evaluated using the DAD detector, by recording the corresponding UV spectra at different points in the peak obtained after injection of the hydrolyzed samples of soybeans, to compare to the spectra of standards. Addition of isoflavones to the extracts provided additional confirmation of peak superposition.

The linearity for each compound was verified through external-standard calibration, obtaining the linear correlation coefficient (r) resulting from the regression line of five different concentrations of standards spiked into soybean extract.

The LOD and LOQ were calculated using the parameters from the analytical curve,[38-40] according to equations 1 and 2:

$LOD = 3.3 \times s/S$ **(equation 1)**

$LOQ = 10 \times s/S$ **(equation 2)**

where *s* is the estimated standard deviation of the linear coefficient error and S is the slope of the analytical curve.

The precision of the method, in terms of repeatability for determination of isoflavones, was verified by analyzing of six different samples of soybeans on the same day. Three injections for each of the extracted samples were made and the dispersion of results was verified for each isoflavone, according to ISO 5725.[41]

For the calculation of the repeatability limit (r'), equation 3 was used: [41]

$r' = 2.8 \times s_r$ **(equation 3)**

where s_r is the estimated standard deviation of each set of six samples.

To verify the accuracy of the method, 1 mL of reference standard containing 18.0 µg mL^{-1} for glycitein, 54.9 µg mL^{-1} for daidzein and 51.9 µg mL^{-1} for genistein were spiked into a sample before the extraction and hydrolysis and the accuracy was calculated from the recovery results. The results were expressed in percent recovery and the *Student t* test at the 95% confidence limits was applied,[42] comparing the experimental mean ($\overline{x}$), with the expected value (µ).

Analysis of soybean samples

Samples of BRS133 (non-transgenic soybeans) and samples of BRS245RR (transgenic soybeans), cultivated in the same region of Paraná state (Brazil) during the same period of the year (spring) were extracted, hydrolyzed and analyzed using the validated method. Statistic analyses were performed on six samples of each kind of soybean, using the *t* (significance) test for samples at the

95% confidence limit, for comparison between the two means of six samples each soybeans.[42]

Results and Discussion

Several methods of extraction were evaluated in this work, but the best extraction results were obtained with the method of Griffith and Collison [10] followed by acid hydrolysis according to Delmonte *et al.*[43] These procedures to recover the aglucone isoflavones are simple and require only small volumes of solvents. The aglucones were then separated using an optimized HPLC-DAD method. The DAD detector was preferred in this work because of its greater applicability to routine analysis. Identification of isoflavones was achieved by comparison of retention times and UV spectra of separated compounds with authentic standards. Figure 2 shows the separation of aglucone isoflavones in a soybean sample using the gradient elution program developed for this purpose, along with their respective spectra. Several chromatographic conditions were evaluated in both the isocratic and gradient modes. The best condition for the separation of aglucone isoflavones, especially considering the total time of the run, was by gradient elution where the mobile phase was water and acetonitrile, both containing 0.1% acetic acid.

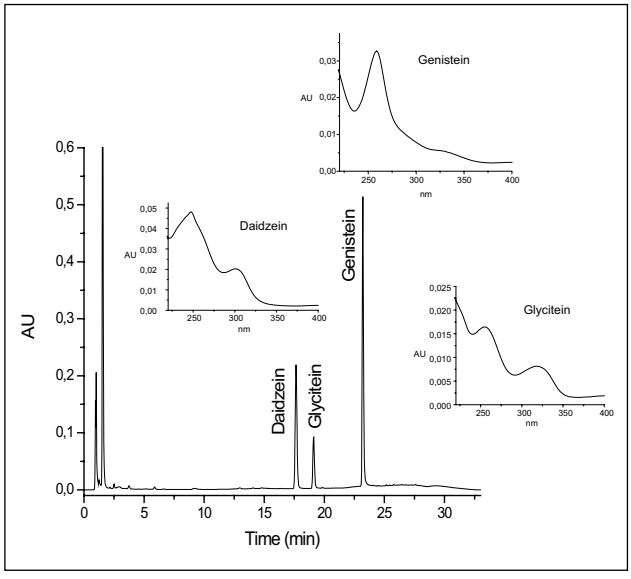


Figure 2: Chromatogram of isoflavone reference standards added to soybean samples. The inserts show the spectra from the 200 to 400 nm. Chromatographic conditions are described in the experimental section.

Samples of non transgenic soybeans after extraction but without hydrolysis were also injected into the chromatograph using the optimized conditions to show the presence of a number of isoflavone forms, with the aglucones forms in only small amounts. The malonylgenistein form produced a peak with the highest concentration and other glucosidic forms had peaks having smaller amounts. However, there was significant overlap of some peaks, that could complicate the determination of the isoflavones. The advantages of chromatographing after hydrolysis to reduce sample complexity are evident. Furthermore, malonyl and acetyl forms of the isoflavones are not available as reference standards for identification and quantification. In addition Rostagno *et al.*[27] indicate that chromatographic analysis time is considerably shorter and separation of target compounds is easier after hydrolysis since there are fewer compounds to be found.

Both acid hydrolysis and enzymatic attack break the bond between the isoflavone and the glucoside moieties, transforming all the isoflavone derivatives into their aglucone forms. On the other hand, basic hydrolysis breaks ester bonds, removing acid groups that are bonded to the sugar moiety of the isoflavone glucosides.[10] Thus, in this work, all the isoflavone forms were extracted from the soybeans and then transformed into the respective aglucone forms by acid hydrolysis.

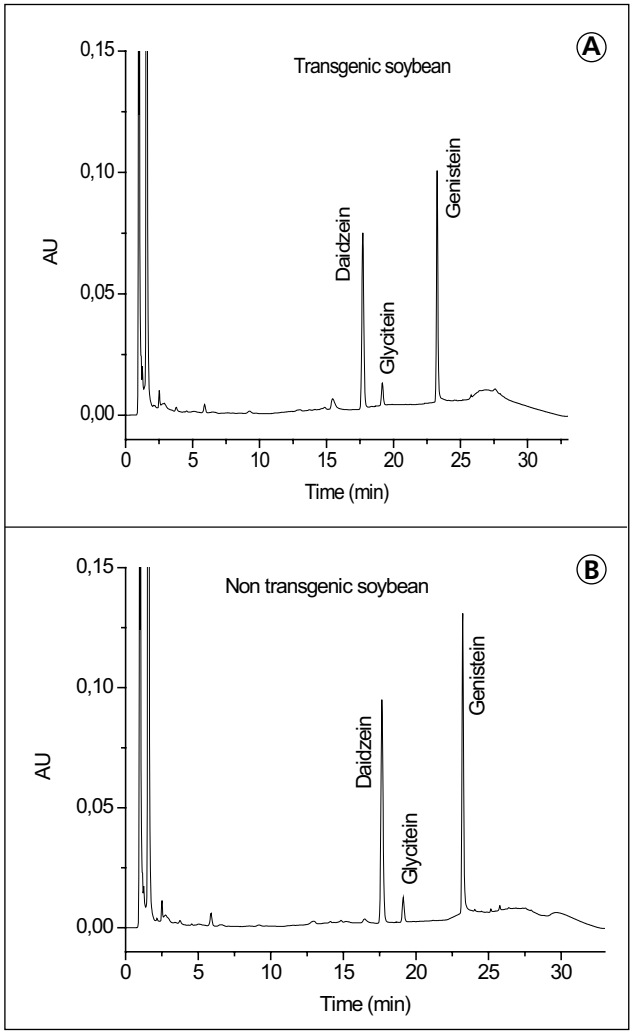


Figure 3: Chromatograms of samples of transgenic (a) and non-transgenic (b) soybean extracts after hydrolysis. Chromatographic conditions are described in the experimental section.

Method validation

Selectivity is the ability of a method to discriminate the analyte from all potential interfering substances. Thus the retention times of reference samples of daidzein, glycitein and genistein were compared with the retention times of samples in which potential interferences could be found. The selectivity was also verified by comparing the ultraviolet spectra of the extracted and hydrolyzed isoflavones with the spectra of standards where the spectra of each peak were completely superimposed on the spectra of the standards. To correct the quantitative errors that might arise from the influence of the matrix, an external-standard calibration with soybean extracts spiked with increasing amounts of the analytes was evaluated and compared with solutions of the analyte standards in mobile phase, showing that there is a slight influence of the soybean matrix on the analytical curves. Thus calibration with soybean extract as matrix was used to correct these small quantitative errors. The precision (% CV) showed values lower than 5% and the accuracy, evaluated by aglycone recovery, showed results higher than 97%. Table I presents the validation parameters of the method.

Table I: Analytical parameters, obtained using standard addition to the matrix for daidzein, glycitein and genistein.

Parameters	Daidzein	Glycitein	Genistein
Linearity range (μg mL^{-1})	28.5 – 110.9	2.9 – 29.9	11.7 – 89.7
Correlation coefficient r	0.9971	0.9974	0.9971
LOD (μg mL^{-1})	2.5	0.9	2.0
LOQ (μg mL^{-1})	7.4	2.9	6.1
Repeatability (% CV)	1.7	1.7	4.7
Recovery (%)	98.9	97.6	99.8

Analysis of soybean samples

Currently there is considerable controversy about the use of transgenic *vs* non transgenic soybeans as both human and animal food. Transgenic products are a relatively recent reality, with few studies relating the amounts the diverse components in the different varieties.

The results of determination of the three aglucones after acid hydrolysis of extracts of non-transgenic (BRS133) and transgenic (BRS245RR) soybeans are presented in Table 2, while Figure 3 show the relevant chromatograms. The three aglucones present significant individual differences. The total amounts of isoflavones in 100 g of soybeans were 284 ±10 mg and 228±13 mg for non-transgenic and transgenic soybean varieties, respectively, confirming that the transgenic form does, indeed present lower total amounts of these important constituents, as previously suggested by Setchell and collaborators.[20]

The results from the present study were submitted to a *Student t-test* that showed that a significant difference exists between the total aglucone isoflavone content of the conventional and transgenic soybeans. The quantities of total isoflavones for non-transgenic soybeans found in this work are within the range reported in the literature.[7,28] Cultivation of the same soybeans under different climatic and soil conditions has been reported to cause differences in isoflavone contents.[11-13] The content of isoflavones may change according to the cultivar, the location of planting, soil, climate and crop.[44,45] However, in the present study the soybeans were cultivated under similar conditions so that the significant differences in aglucone isoflavone content between these varieties of transgenic and non-transgenic soybeans do not arise from external conditions, but were inherent in the soybeans themselves.

Table II: Aglucone contents of transgenic and non-transgenic soybeans samples.

	Transgenic Soybeans (BRS245RR)			Non-transgenic Soybeans (BRS133)		
	$\overline{x}$ [a]	SD	CV (%)	$\overline{x}$ [a]	SD	CV (%)
Daidzein	146	8	5.7	181	7	3.9
Glycitein	15	1	6.0	16	1	4.7
Genistein	67	5	7.9	87	4	4.7
Total isoflavones	228	13	5.8	284	10	3.6

[a] in mg 100g^{-1}

The present study has described the development, validation and application of a method for extraction, hydrolysis to aglucones and quantification of the individual and total isoflavones in soybeans, using high performance liquid chromatography with diode array detection (HPLC-DAD). The quantities of the isoflavones found in two different genotypes of soybeans commercialized in Brazil, one a non-transgenic soybean (BRS133) and the other a glyphosate-tolerant transgenic (BRS245RR) soybean derived from BRS133, both cultivated in the same area and during the same time of the year, to reduce the influence of soil and climate on the results, were determined. Although several researchers have reported the isoflavone content of soy foods available in several countries,[11,44] none of these earlier studies evaluated transgenic and non-transgenic soybeans cultivated at the same location to eliminate the influence of external factors on the isoflavone content.

Conclusions

The sequence extraction-hydrolysis-HPLC-DAD presented statistically acceptable results for determining total isoflavones in soybeans. The validated method allowed verifying that the amounts of the individual isoflavones and the total amount of isoflavones in transgenic and non-transgenic soybean varieties cultivated

under very similar climatic and soil conditions were statistically different. However, other constituents in the soybeans need to be determined before reaching a definite conclusion about the overall differences between transgenic and non-transgenic soybeans. Moreover, considering the growing commercialization of genetically modified products, reliable and informative analytical methods are essential for the implementation of labeling requirements.

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METHOD DEVELOPMENT FOR THE DETERMINATION OF THE DISODIUM SALT OF BICYCLO[2.2.1]HEPTANE-2,3-DICARBOXYLIC ACID IN AN ISOTACTIC POLYPROPYLENE MATRIX USING LIQUID CHROMATOGRAPHY WITH EVAPORATIVE LIGHT SCATTERING DETECTION

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ABSTRACT
Sodium bicyclo [2.2.1] heptane-2,3-dicarboxylate, commercially known as ®Hyperform HPN-68L, represents a significant technological advance in conventionally used nucleation technology. This nucleating agent is added to polypropylene in order to improve the processes of molding and extrusion. According to the manufacturer the final concentration of the nucleating agent in the polymeric matrix should be in the range of 100 to 200 ppm. This control is very important because it can define the quality of the final product. The analytical procedure for HPN-68L in a polypropylene matrix needs an extraction step prior to instrumental analysis. In the present work, the extraction of the additive was done by refluxing with solvent, sonication in an ultrasound bath and using a microwave reactor, with three different solvent composition mixtures (methanol, acetonitrile, chloroform, decalin). The solvent mixture of acetonitrile, methanol and decalin show the best recovery (137%). High performance liquid chromatography with evaporative light scattering detection (HPLC-ELSD) was used to analyze the sample, as an alternative to gas chromatography (GC) and infra-red spectroscopy (FT-IR), which are the techniques commonly used for this system. HPLC-ELSD showed good selectivity and detectivity for the analysis of HPN-68L (LOD = 6 ppm and LOQ = 20 ppm).

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KEYWORDS: *HPN-68L, polypropylene, HPLC-ELSD, microwave reactor, ultrasound bath*

1. INTRODUCTION

In the last decades the use of polymers like isotactic polypropylene in the food industry or in the manufacture of surgical instruments has increased significantly and raised a series of analytical problems due to the necessity of detecting and quantifying low amounts of additives. These additives are important to improve the properties or to inhibit the degradation of the polymers, for example. In the case of isotactic polypropylene the use of nucleating agents improve both its mechanical and optical properties. In 2002 Miliken Chemical introduced the first hyper nucleating agent, bicyclo[2.2.1] heptane-2,3dicarboxylic acid, disodium salt, commercially known as *Hyperform®HPN-68L* (Figure 1). This nucleating agent represents a significant technological improvement over the conventional and advanced nucleation technologies used by the industry, accelerating molding and extrusion processes, improving isotropic shrinkage and reducing warpage. A main application of isotactic polypropylene nucleated with HPN-68L is as packaging material in the food industry. There are strict regulations for the amounts of additives that can be present in polymers if they are used in direct contact with food. According to the European Food Safety Authority (E.F.S.A.) the concentration of HPN-68L in polypropylene should not exceed 0.25%.

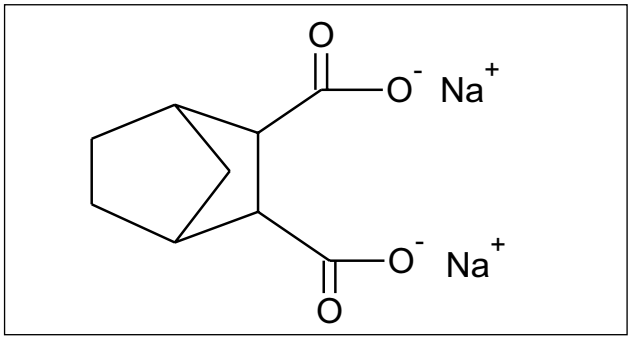

FIGURE 1. CHEMICAL STRUCTURE OF BICYCLO[2.2.1]HEPTANE-2,3DICARBOXYLIC ACID, DISODIUM SALT (HYPERFORM®HPN-68L).

Nowadays two methods are used to quantify *Hyperform®HPN-68L*. One of these methods uses gas chromatography with flame ionization detection (GC-FID). This method was developed by Milliken Chemical [1] and uses a very expensive and time consuming derivatization cocktail to analyze the HPN-68L. The other method uses Fourier transform infrared spectroscopy (FT-IR) and has some drawbacks as all other additives have to be in the same concentration in the sample and in the calibration curve, because their spectra superpose with that of HPN-68L. In addition this is a secondary method and needs the first one for confirmation [2]. Both methods are laborious or unsuitable for quality control. Industry needs fast and inexpensive methods. Therefore the development of an alternative methodology for the quantification of *Hyperform®HPN-68L* in isotactic polypropylene matrixes is very important in order to allow its use on a larger scale. The extraction of the nucleating agent from the matrix, and the required low detection limits for the analytical instruments to be used, are the main experimental difficulties in developing such a methodology.

The ELSD is based on the measurement of the amount of light scattered from solute particles or droplets passing through a light beam. The scattering intensity is proportional to the concentration of the solute in the light beam at any particular time. ELSD is a universal, non-specific detector that can provide a stable baseline even with gradient elution [3]. In recent years this detector has been increasingly used with analytes that have no chromospheres and therefore cannot be detected with UV-detectors. ELSD presents higher detectivity in comparison to refractive index detectors and can be used with gradient elution. On the other hand, obtaining reproducible and precise results with the ELSD is more troublesome in comparison to other detectors.

In recent years the use of the ELSD has increased significantly for the determination of analytes that do not have good responses or good detectivity with analytes conventional detectors used in liquid chromatography, such as UV, fluorescence and mass spectrometers. The combination of LC with ELSD has been used for many systems, such as the determination of four flavonoids and four cycloartanol glycosides from the aerial parts of *Sutherlandia frutescens* (L.) [4]; fatty acid methyl ester (FAME) in three different oils [3]; terpene marker compounds in commercially available Ginkgo biloba capsules [5]; aminoglycoside antibiotic amikacin and its precursor in pharmaceutical raw material [6]; triterpenic acids in jujube fruits [7]; saponines in Platycodo Radix [8]; sulforaphane in broccoli samples [9]; artemisinin from *Artemisia annua L*. [10]; lipid classes of buttermilk and milk from cows, goats and ewes [11]; phospholipids in infant formula and toddlers milk [12]; tocopherols and tocotrienols in Portuguese olive oils [13]; polyoxypregnane glycosides in Marsdenia tenacissima [14]; sugars from model enzyme-mediate pulp hydrolyzates [15]; acylglycerides in feces from soluble cellulose-fed hamsters [16]; terpene trilactones in Ginkgo biloba leaves [17]; triterpenolds in Radix Achyranthis Bidentatae [18]; fosfomycin trometamol and its related substances in the bulk drug [19]; isoflavones and saponines in Pueraria flowers [20]; asphaltene in crude oil and petroleum products [21]. The LC-ELSD has also been used for quality control in pharmaceutical products for the determination of impurities with better responses than those conventional systems [22-24].

The use of the LC-ELSD has been considered a potential technique for the determination of additives in polymer samples, due to the universality and detectivity of the detector [25]. For this reason this work presents a proposal to establish a protocol for the extraction of *Hyperform®HPN-68L* from an isotactic polypropylene matrix and to apply a method using high-performance liquid chromatography with evaporative light scattering detection (HPLC-ELSD) for the qualitative and quantitative evaluation of the additive.

2. METHODOLOGY

2.1. STANDARDS AND REAGENTS

The solvents: chloroform p.a., methanol p.a., isopropanol p.a., decalin p.a., dimethylformamide p.a., butanol p.a., acetonitrile HPLC grade, and methanol HPLC grade, were purchased from Merck KGA, Damstadt, Germany. The commercial polypropylene homopolymer was provided by Braskem S.A. The reagents pentaeritrol-tetra-cis[3-(3,5-di-tert-butyl-4-hydroxyphenyl)] propanate (Irganox 1010), Ciba; tri (2,4-di-tert-butylphenyl) phosphite (Irgafós-168), Ciba; glycerol monostearate (GMS), Danisco S.A., were also used.

2.2. SAMPLE PREPARATION

A typical formulation of isotactic polypropylene with *Hyperform® HPN-68L* also involves other additives that prevent degradation and improve the processability of the polymer. In order to simulate the normal extrusion conditions these additives were also added to the polymer prior to the extraction processes. Therefore standard solutions of the following additives in methanol were prepared: HPN-68L (1000 mg L^{-1}); Irganox 1010 (500 mg L^{-1}); Irgafós 168 (500 mg L^{-1}); Amide E (500 mg L^{-1}); glycerol monostearate (500 mg L^{-1}). The solutions were stored under controlled temperature in the dark. Polypropylene pellets without additives were produced specially for the experiments. Thereafter 1000 mg L^{-1} of HPN-68L and 500 mg L^{-1} of each other additive were

added to the polymer. The sample was extruded and pelletized. The pellets were ground in a knife mill using a 20 mesh sieve before being submitted to the different extraction processes.

2.3. Evaluation of HPN-68L extraction from the polypropylene matrix

Three methods were used in order to evaluate the extraction of the HPN-68L from the polypropylene matrix: solvent extraction, sonication in an ultrasound bath (Unique de 25 Hz) and the use of a microwave reactor (CEM, model Discover®, series DC-5051).

The extractions using the solvent were carried out with 5.0 g of the sample, the ones using the ultrasound bath with 1.5 g of the sample while 1.0 g of the sample was used with the microwave reactor.

The solvent mixtures used in the extraction processes were: **mixture A** (70% methanol and 30% acetonitrile); **mixture B** (70% methanol and 30% chloroform) e **mixture C** (90% (70% methanol and 30% acetonitrile) and 10% decalin). The solvents were chosen taking into account the solubility of the sample components.

2.4. Chromatographic Analysis

The optimization of chromatographic parameters took into account retention time, base peak width, peak area, and resolution. In the isocratic mode three different compositions of mobile phase were used: (A) 100% acetonitrile, (B) 100% methanol and (C) methanol:acetonitrile (70:30). Also a gradient mode using acetonitrile (eluent A) and methanol (eluent B) as mobile phase was used with the gradient: 0–5 min (30% B), 5–10 min (30–0% B), 10–20 min (0% B), 20-25 min (0-30% B) and then equilibrated with 30% B for 5 min at a flow of 1 mL min^{-1}.

HPLC of PP spiked standards was carried out on a Varian instrument ProStar model equipped with Evaporative Light Scattering Detector (ELSD) PL-ELS 2100 model, from Varian, U.S.A., with a manual injector. A Chromospher C18 column (250 mm × 4.6 mm, 5 μm particle size) from Varian, U.S.A., and a pre-column OminiSpher 5 C18 were used for the separations.

The response of the ELSD depends on several parameters, i.e., eluent composition, mobile phase flow-rate, gas flow-rate and nebulizer and evaporation (drift tube) temperature. These parameters were adjusted in order to obtain the best repeatability in peak area and retention time (n = 5). The experiments were carried out using a test solution of HPN-68L in the concentration of 1000 mg L^{-1}. In all experiments the injection volume was 20 μL.

2.5. Validation

Specificity and selectivity: a typical formulation provided by the manufacturer of the nucleating agent Hyperform ® HPN-68L for the production of polypropylene resins involves the use of other additives (Irganox 1010, glycerol monosterate (GMS), erucamida (Amide E) and Irgafos 168) in the stabilization and polymer processing. Therefore, in order to evaluate the specificity and selectivity of the proposed chromatography method with respect to these additives, individual solutions were prepared for each additive containing polypropylene homopolymer spheres free of additives. These solutions were: 500 mg L^{-1} of Irganox 1010, 500 mg L^{-1} of GMS, 500 mg L^{-1} of Amide E and 500 mg L^{-1} of Irgafos 168 in 70% methanol and 30% acetonitrile. They were analyzed using the same chromatographic conditions as for HPN-68L. Peaks were assigned by comparing the retention times of individual standard solutions prepared in the same solvent.

Linearity: the standard solutions in a solvent mixture, containing 70% of methanol and 30% of acetonitrile, containing 296, 611; 912, 1219 and 1496 mg L^{-1}, corresponding to concentration of the HPN-68L, were prepared in triplicate, 20 μL of each sample was injected into the HPLC column and the calibration curves were constructed. Calibration curves were plotted using peak area versus concentration of the HPN-68L. In the case of the ELSD, the response of the calibration curve can be non linear. In this case the curve is better represented by a log-log plot.

The nonlinear response for ELSD is because the relationship between the analyte mass and area response for ELSD can be described by:

$$A = aM^b \quad (1)$$

where A is the area response of the detector, M is the mass of the analyte injected and, **a** and **b** are numerical coefficients that depend on the analyte and chromatographic conditions [6]. However, the log–log plot of the peak area response versus analyte quantity is linear for the ELSD [24]. It can be described by the equation:

$$\log A = \log A + \log M \quad (2)$$

Precision: Considering the specific characteristics of the Evaporative Light Scattering Detector, determination of the precision of the retention time and area is important. The determination of the intra-day variability was determined, with a standard solution of HPN-68L in methanol in the concentration of 1000 mg L^{-1}. Variations were expressed by the relative standard deviation (RSD).

Limits of detection (LOD) and quantification (LOQ): in the present chromatographic conditions, LOD and LOQ were calculated according ANVISA Manual (resolution 899, 29/05/2003) [26]. The LOD estimation was done by the equation:

$$LOD = \frac{DP x\, 3}{IC}$$

where, DP is the standard deviation of the blank, and IC is the slope the regression equation from the calibration curve.

Similarly, the LOQ was determined by the equation:

$$LOQ = \frac{DP\,x\,10}{IC}$$

Recovery: the percent recoveries for the extraction procedures were determined by comparing the mass of HPN-68L added in the PP pellets and the mass of HPN-68L determined in the extracts after the extraction procedures described in 2.4.1. The recovery test was used to evaluate the accuracy of this method.

The ANVISA Resolution [26] was used for analyses of the data obtained in the validation.

3. Results and Discussions

3.1. Chromatographic conditions for the analysis of the *Hyperform*® HPN-68L

For LC analysis, the detector conditions were: nebulizer temperature: 70 °C, evaporator temperature: 70 °C, nebulizer gas flow: 0.9 L min^{-1}, LED intensity: 100% and *smoothing:* 50.

The selectivity of the LC method was evaluated by analyzing a standard solution containing the HPN-68L and the additives, in the isocratic mode with methanol and acetonitrile (70:30) as mobile phase. In this condition, the HPN-68L showed good resolution, but the additives did not elute adequately from the column, mainly Irgafós 168. Better separation was achieved using gradient conditions. In the gradient mode the HPLC conditions were as follows: eluent A, acetonitrile; eluent B, methanol; gradient, 0–5 min (30% B), 5–10 min (30–0% B), 10–20 min (0% B), 20-25 min (0-30% B) and then equilibrated with 30% B for 5 min at a flow of 1 mL min^{-1}. Figure 2 presents the chromatogram for the individual compounds and Table I presents the data obtained for the separation of these compounds using the HPLC-ELSD. In the established conditions it was possible analyze HPN-68L without interference of additives that can be present in the sample.

Table I. Chromatographic parameters for the compounds analyzed by HPLC-ELSD.

Additives	T_R (min)	Peak area (mV min^{-1})	Peak height (mV)
HPN-68L	2.18	28.70	154.4
I-1010	10.47	116.6	370.6
GMS 1	11.27	39.00	128.6
GMS 2	15.24	37.20	110.6
Amida E	18.73	18.60	55.40
I-168	31.43	103.1	86.00

T_R – retention time in minutes.

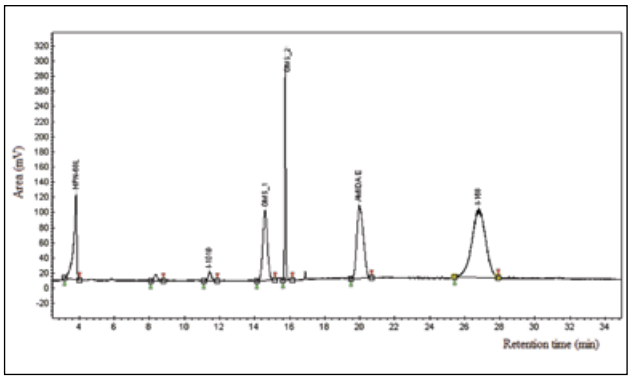

Figure 2. Chromatogram of HPN-68L and the additives present in a typical formulation.

The linearity of the method was evaluated with a standard solutions of HPN-68L analyzed using the established chromatographic conditions. Figure 3A presents the calibration curve obtained in linear coordinates.

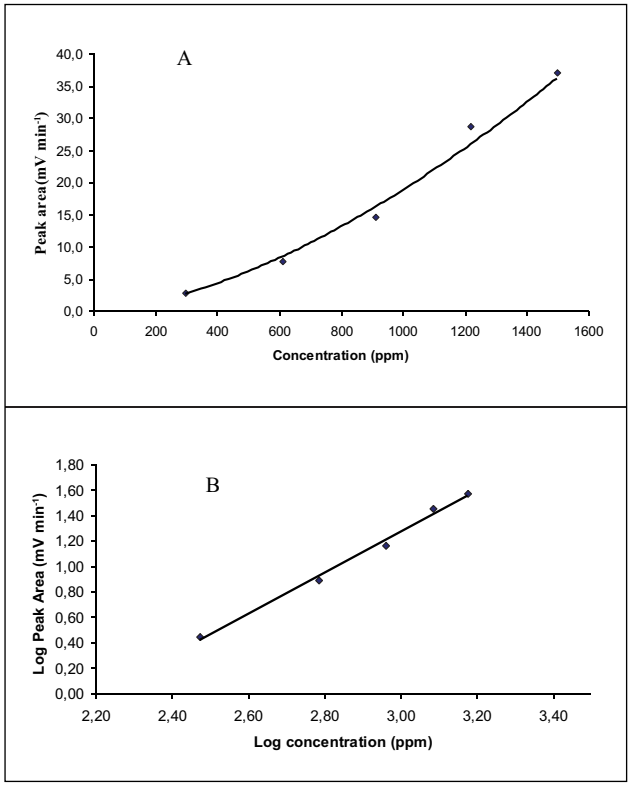

Figure 3. Calibration curve for HPN-68L in the concentration range of 611 to 1496 mg L^{-1}. (A) Linear coordinates; (B) logarithmic coordinates.

The curve was not linear, in agreement with many other papers using the same detector [3,5,27-30]. Better linearity was obtained when the curve was plotted in a logarithmic coordinate system (Figure 3B). The equation for the log plot was y = 1.6216x - 3.5909 with a correlation coefficient of 0.9919. This was a good result for the concentration range evaluated.

The linearity of the log plot determined by the ratio of the peak area and concentration ($(S/Q)_i$ %) demonstrate that the points are in the linear area of calibration. The points are consider inside the linear area if the ratio $(S/Q)_i$% is 5% above the angular coefficient of the curve [26] (Table II).

Table II. Evaluation of the linearity of the calibration curve for the HPN-68L in the concentration range of 269 to 1496 mg L^{-1}.

Log C (ppm)	Log A (mV min^{-1})	$(S/Q)_i$ %
2.4	0.4	0.057
2.8	0.9	0.053
3.0	1.0	0.052
3.1	1.4	0.051
3.1	1.57	0.050

A – Peak area

C – Concentration of the solutions

The precision was determined as the intra-day repeatability for the retention time and peak area with a confidence of 95%, using the solutions of the calibration curve. The values obtained as RSD% for retention time and area were: 0.4% and 11.4%, respectively. The ANVISA Resolution [26] establishes 5% as the maximum value acceptable for RSD%. The RSD% for retention time is lower and for the area is higher but can be accepted because the variation of the response for ELSD is higher compared to the other detector used in a liquid chromatography (Table III).

Table III. Statistical results of the intra-day repeatability for the retention time and peak area for a HPN-68L solution in methanol in the concentration of 1000 mg L^{-1}, analyzed by LC-ELSD.

Statistical Results	Retention Time HPN-68L (min)	Peak area HPN-68L (mV min^{-1})
Average	2.26	13.38
Standard deviation	0.009	1.532
Variance	0.000080	2.35
CV	0.40	11.45
RSD	0.40	11.45
T Student n^{-1}	2.776	2.776
Confidence (95%)	0.0079	1.34
Confidence interval	2.25 + 0.0048	20.34 + 7.02

The accuracy was determined as the recovery of the extraction procedure, and the data are reported in Table IV.

3.2. Extraction of HPN-68L from the polypropylene matrix

The polymer matrix containing HPN-68L was ground in a knife mill with 20 mesh screen. The solvent mixtures used in the extraction were: mixture A (70% methanol and 30% acetonitrile), mixture B (70% methanol and 30% chloroform) and mixture C (90% (70% methanol and 30% acetonitrile) and 10% decalin). The choice of the solvents for extraction took into account the solubility of the system, which in this case is a critical factor in the experimental conditions. In the procedures for the extraction using ultrasound and reflux with solvent, the amount of polypropylene sample, organic solvent volume and extraction time used were previously established in the quality control laboratory of Brasken S.A. for the determination of other additives (Irganox 1010 and Irganox 168) in the same sample. For the extraction with microwave the mass sample and organic solvent volume used were, respectively, 1.0 g and 5.0 mL, as indicated by the manufacturer. The extraction temperature was 130 °C. Table IV presents the recoveries obtained with the three extraction system procedures. Figure 4 shows the chromatogram of a sample of PP extracted with microwave and solvent mixture C, spiked with 1000 ppm of HPN-68L.

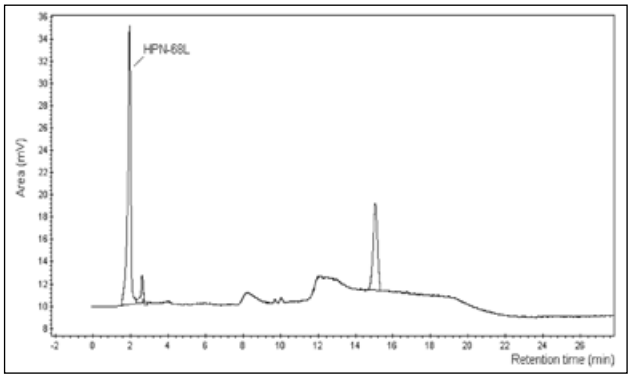

Figure 4. Chromatogram of a PP sample spiked with 1000 ppm of HPN-68L, after extraction in the microwave with solvent mixture C.

Table IV. Recovery for the extraction of HPN-68L from the PP matrix, using solvent extraction, ultrasonic bath and microwave and the three solvent mixtures. Extraction time for reflux and ultrasound was 30 min and for microwave was 5 min.

	Recovery (%)		
Solvent mixture	Reflux extraction	Ultrasound bath	Microwave reactor
A	54.7	33.8	40.9
B	33.8	33.8	43.2
C	70.8	38.5	137.0

The extraction with microwave and reflux showed the best recoveries. Moreover, the chromatogram of Figure 5 shows no interference from other peaks in the retention time of HPN-68L. With respect to mixtures of solvents used for extraction, the addition of decalin significantly improved the extraction of HPN-68L from the polypropylene matrix. This behavior can be attributed to the fact that decalin is able to swell the polymer, allowing better solvation and therefore freeing the HPN-68L more easily. The best extraction conditions were established using a microwave reactor with mixture C (90% (70% methanol and 30% acetonitrile) and 10% decalin) as solvent.

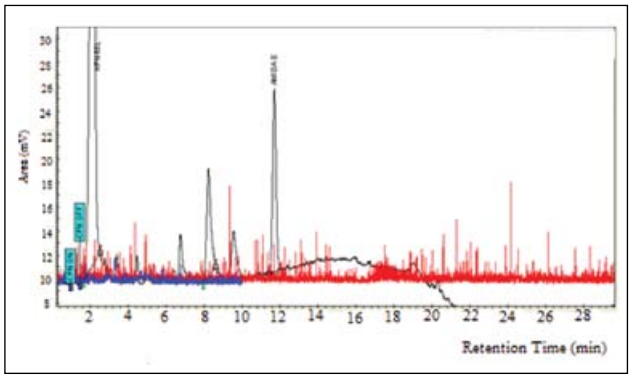

FIGURE 5. COMPARISON OF THE CHROMATOGRAM OF THE EXTRACTION SOLVENT (BLUE LINE), PP EXTRACTED WITH THE MIXTURE C (RED LINE) AND PP SPIKED WITH 1000 PPM OF HPN-68L (DARK LINE).

4. CONCLUSIONS

In our work the main goal was the comparison of the extraction methods in terms of which could provide the best extraction with the lowest interference as the matrix is very complicated to manipulate. The extraction with microwave demonstrates this is particularly suitable for the routine analysis of this product, mainly because it is fast with low solvent consumption, good recovery and uses a small quantity of sample. The solvent mixture consisting of 90% of methanol:acetonitrile (70:30) and 10% decalin, was shown to be the most adequate for the extraction step and the chromatographic analysis results obtained using HPLS-ELSD were satisfactory, showing selectivity and detectivity. The methodology proposed in this work, for extraction and analysis of HPN-68L in PP samples, was shown to be adequate for use in industrial quality control on a large scale.

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HUMIC ACID-METAL IONS AFFINITY THROUGH MODIFIED CARBON PASTE ELECTRODES. STUDY BY ANODIC STRIPPING VOLTAMMETRY

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ABSTRACT
Carbon paste electrodes (CPE) are built by a mixture of graphite powder with either mineral or vegetal oil. Its main characteristic is its easily renewable surface but it also has characteristics like large working windows in both cathodic and anodic potentials, low cost, and facility of manipulation. Humic substances (HS) are polyelectrolytic organic structures resulting from natural substance degradation. Due to their anionic functional groups, HS have affinity and make coordination complexes with metal ions. In this paper, a carbon paste electrode was modified with humic acids (CPE-HA) and used as a working electrode. The objective was to evaluate the complexation behavior of the HA/solution interface using an electrochemical tool. The anodic stripping voltammograms of CPE-HA, obtained at pH 4.5 and 5 mV s^{-1}, show a quasi-reversible process with a well defined oxidation step at about 0.01 V (SCE) and two reduction steps at -0.12 and -0.17 V. Cyclic voltammetry shows that the intensity of the second cathodic step increases compared to the first one, after each cycle. The first reduction step is more pronounced in concentrated solutions. All potentials are repetitive at pH values between 3.0 and 4.5, but from 5.0 up to 6.0, the second reduction step shifts in the cathodic direction. The two-step reduction behavior is not observed with a non-modified CPE. According to the results, the two reductions steps are representative, respectively, of linked and free (or weakly linked) Cu^{2+} with the HA present on the electrode surface. CPE-HA tool presents a good way to evaluate metal-HA interactions in heterogeneous systems.

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KEYWORDS: 1. Humic acids; 2. Cu^{2+}; 3.Stripping Voltammetry, 4.Carbon Paste Electrode.

1. INTRODUCTION

Carbon paste electrodes (CPE) are built by the mixture of graphite powder with either mineral or vegetal oil. Its main characteristic is the easily renewable surface but characteristics like large working windows both in cathodic and anodic potentials, low cost and facility of manipulation are also described [1,2]. Modifications in the paste are very simple and the modifying agent is chosen considering the sensitivity and/or selectivity of the analyte. The modifying agent changes the electrochemical behavior of the electroactive substance on the electrode surface as compared to the non-modified electrode. These changes occur by interactions between the analyte and the modifying agent.

Humic substances (HS) are polyelectrolytic organic macromolecules resulting from natural substance degradation. The main characteristics of HS are their amorphous and colloidal structures that are pH dependent, their large variety of molar masses and shapes, and their large quantity of oxygenated functional groups like quinones, carboxylic groups, and phenols. They are very important in an environmental setting due to their ubiquity. Practically all aspects of the presence of toxic metallic elements in soils are related to complex

formation with organic matter [3]. Humic acids (HA), the fraction soluble in basic solution, are, in general, the major component of natural humus. Their solubility varies for each substance and depends upon the molecular size and mass, and the distribution of functional groups [4].

The polyelectrolytic properties of HS have an important role in the environment due their influence in metallic ion speciation [5,6]. Either complex or chelate formation between HS and metallic ions can be observed through changes in chemical behavior, absorption spectrum, electrical conductance, pH, solubility, isolation of formed complexes [4-7] and changes of redox potentials [8]. In general, studies involving HS and metal ion interactions are observed in homogeneous solutions where both HS and metal are in soluble forms. This is not the case in other instances, mainly in soil conditions, where the HS are bonded to the mineral particles, and so the reactions occur in an interfacial region between particles and soil solution [3].

Voltammetric methods can improve the visualization of interfacial interactions by the change of redox potentials. In the literature, several papers discuss the utilization of CPE modified with HS in regard to the quantification of metallic ions [9-14] or organic substances [15,16]. Studies of electrochemical behavior of Cu^{2+} on CPE modified with HS are also described, but the electrochemical steps are not clearly discussed [10,17,18].

In a previous paper [8], we discussed the interactions between Cu^{2+} with HA in a CPE using cyclic voltammetry. The differences between the electrochemical behavior of copper on CPE as compared to CPE-HA show the interactions of the cation with the HA present in the paste.

This article reports HA-Cu^{2+} interaction studies by voltammetric stripping methods. The objective was to evaluate the complexation behavior of the HA/solution interface using an electrochemical tool. A carbon paste electrode was modified with HA (CPE-HA) and utilized as working electrode. The changes in the redox potential and current peaks are presented and compared to the non-modified CPE. Accumulation was done with and without potential application. The influence of pH, scan rate and Cu^{2+} concentration are discussed.

2. EXPERIMENTAL

2.1 CHEMICALS AND MATERIALS

The CPE was prepared with Carbone-Lorraine powder and castor oil (Fluka). The HA utilized to modify the electrode was extracted from a peat area from a border of the Mogi Guaçu River in São Paulo State - Brazil. The HA was extracted and purified according to the International Humic Substances Society method [19]. The main characteristics of this HA are shown in table I.

TABLE I: CHARACTERISTICS OF HA FROM PEAT UTILIZED IN THIS STUDY.

CHARACTERISTICS		VALUES
Acidity (mmol H^+ g^{-1})	Total	4.08 ± 0.23
	Carboxylic	2.29 ± 0.05
	Phenolic*	1.79 ± 0.28
Elemental composition (% w/w)	C	51.73
	H	5.15
	N	3.14
	O	39.98
Ash (% w/w)		2.2

*Obtained by difference

Copper (II) solution was prepared with $CuSO_4.5H_2O$ (P.A., Merck). A stock solution with a $1.57x10^{-2}$ mol L^{-1} of Cu^{2+} was prepared as an aqueous $5x10^{-3}$ mol L^{-1} H_2SO_4 solution. Dilutions were done when necessary.

The support electrolyte was 0.1 mol L^{-1} sodium acetate buffer prepared by mixing 0.1 mol L^{-1} H_3CCOOH (glacial - Panreac) with 0.1 mol L^{-1} $CH_3COONa.3H_2O$ (P.A. - Panreac) until the desired pH.

2.2 CPE PREPARATIONS

2.2.1 NON-MODIFIED CARBON PASTE ELECTRODE (CPE)

This electrode is prepared mixing carbon powder (76.4%) and castor oil (23.6%) in a mortar for 10 min. The paste obtained was packed in a polyethylene tube provided with a stainless steel contact. Before utilization, the electrode was stored in a refrigerator (4° C) for one night. The surface was renewed by discarding a tiny (~ 0.1 mm) part of the paste in the plastic tube followed by polishing it with a paper surface.

2.2.2 CARBON PASTE ELECTRODES MODIFIED WITH HUMIC ACID (CPE-HA)

The method applied for electrode modification can strongly determine its response and reproducibility, due to non-homogeneous distribution of the components in the paste. In a previous work [2], we developed the best method, which is described below.

Eight milliliters of $4x10^{-3}$ mol L^{-1} aqueous LiOH solution was utilized to solubilize 40 mg of HA. The solution so obtained was filtered to remove the non-soluble material. After, carbon powder (10 min under magnetic stirring) was added, followed by freeze-drying. The dry powder is sifted (80 µm thick) and the paste preparations do not change with regard to the CPE (23.6 % of castor oil and 76.4 % of modified powder).

2.3 ANALYSIS

Classical three electrode system was utilized. The CPE

is the working electrode, the reference is a saturated calomel electrode (SCE) and the counter electrode is a stainless steel wire. A fresh carbon paste surface was used for each measurement. Nitrogen is bubbled for 10 min before the measurements to minimize oxygen content in the solution.

A 552 model AMEL potentiostat with a 566 model AMEL function generator and a PL-3 LLOYD Instruments plotter were utilized. The solution was stirred by using a VARIOMAG stirrer with a numeric speed dial.

2.3.1 Anodic stripping voltammetry

Three steps composed the anodic stripping voltammetry:

a) The electrode is immersed in a 0.1 mol L^{-1} acetate buffer (pH 4.5) in the presence of Cu^{2+}. A potential of -0.5 V (SCE), under magnetic stirring, was imposed to reduce Cu^{2+} to Cu.
b) After a period of 5 min. the electrode was carefully rinsed with the acetate buffer solution.
c) The data were registered by linear sweep cyclic voltammetry from -0.5 V to +0.4 V (SCE) in acetate buffer solution.

2.3.2 Complexation

a) The working electrode was put in a cell with 0.1 mol L^{-1} acetate buffer (pH 4.5) and $1.6x10^{-4}$ mol L^{-1} Cu^{2+} solution under magnetic stirring for 20 min in an opened circuit.
b) The electrode was carefully rinsed with buffer solution
c) The electrode was put either in 0.1 mol L^{-1} acetate buffer solution or in acetate buffer with a low concentration ($3.2x10^{-6}$ mol L^{-1}) Cu^{2+} solution. Linear sweep voltammetry was done from +0.5 V to -0.5 V (SCE) at 5 mV s^{-1}.

3. Results and discussion

3.1 Complexation of Cu^{2+} on the CPE-HA surface

Cu^{2+} was first complexed on the electrode surface by immersing the modified electrode in $1.6x10^{-5}$ mol L^{-1} Cu^{2+} + buffer solution, in an opened circuit, during 10 min under magnetic stirring. The cyclic voltammetry was done from +0.5 V to -0.5 V (SCE) in the same solution at 5 mV s^{-1}. The voltammogram is shown in figure 1A.

In the voltammograms obtained with prior Cu^{2+} accumulation in an opened circuit (figure 1A) just one reduction step at -0.19 V (SCE) was observed in the first cycle. Without accumulation (figure 1B) the first cycle does not clearly present this step. The second reduction step, at about -0.23 V, is only visible from the second cycle. However, when the non-modified electrode (CPE) was used the adsorption of Cu^{2+} does not proceed and the cyclic voltammetry with prior "accumulation" is equal to the one without accumulation (not shown). The voltammogram presented in figure 1B is in accordance with the ones presented in previous articles [2,8,9,11].

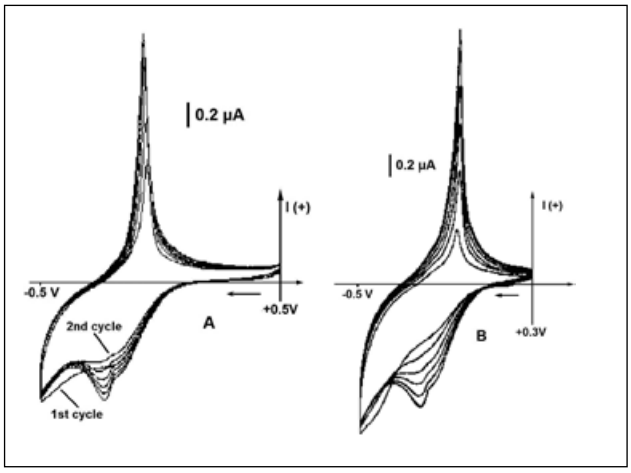

Figure 1: Cyclic voltammetry of Cu^{2+} (1.6x10-5 mol L^{-1}) obtained: (A) after 10 min. of accumulation (opened circuit) and (B) without accumulation. Eletrolyte: 0.1 mol L^{-1} acetate buffer solution (pH 4.5). Working electrode: CPE-HA. v = 5 mV s^{-1}.

3.2 Saturation of complexation sites of modified electrodes

In this experiment the modified electrode surface was saturated with Cu^{2+}. The electrode surface was immersed in $1.6x10^{-4}$ mol L^{-1} Cu^{2+} in 0.1 mol L^{-1} acetate buffer solution (pH 4.5), under magnetic stirring. The scanning potential was done in buffer solution without Cu^{2+} or with a small quantity of this cation ($3.2x10^{-6}$ mol L^{-1}). The voltammograms obtained by this procedure are shown in figure 2.

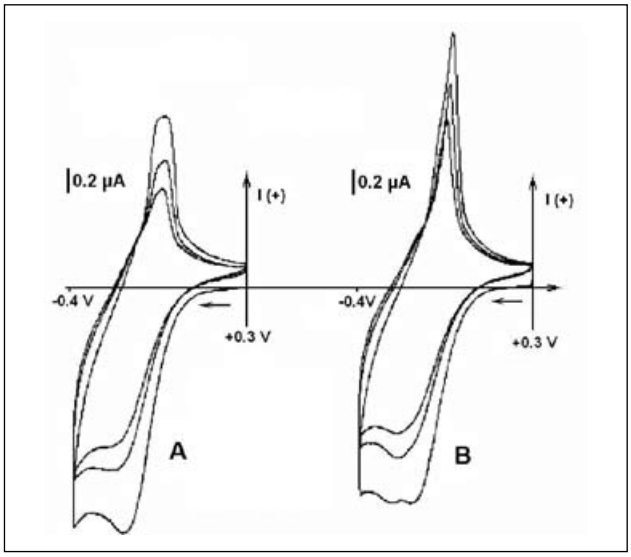

Figure 2: Cyclic voltammetry obtained after 20 min of accumulation with opened circuit in 0.1 mol L^{-1} acetate buffer solution (pH 4.5) with $1.6x10^{-4}$ mol L^{-1} Cu^{2+}. The scanning potential was done in acetate buffer solution: (A) without Cu^{2+} and (B) with $3.2x10^{-6}$ mol L^{-1} Cu^{2+}. Working electrode: CPE-HA. v = 5 mV s^{-1}.

The redox potentials obtained with different complexation times are shown in table II.

From table II, it is shown that the reduction steps are influenced by the pre-concentration time by the presence of low Cu^{2+} quantity in the "scanning" solution.

TABLE II: REDOX POTENTIALS OBTAINED BY "COMPLEXATION" PROCEDURE ON THE CPE-HA SURFACE. THE CONDITIONS ARE SHOWN IN FIGURE 2.

COMPLEXATION TIME (MIN)	"SCANNING POTENTIAL" SOLUTION	E_{red}. (V/SCE)	E_{ox}. (V/SCE)
15	buffer	-0.19	-0.01
15	buffer + $3.2x10^{-6}$ mol L^{-1} Cu^{2+}	-0.19	-0.02
20	buffer	-0.19	-0.02
20	buffer + $3.2x10^{-6}$ mol L^{-1} Cu^{2+}	-0.19/-0.27	-0.01
20 [(a)]	buffer	-	-

(a) non-modified carbon paste electrode.

After 15 min of pre-concentration, just one reduction step at -0.19 V was observed, either in buffer solution or in buffer plus Cu^{2+} solution (table II). This potential corresponds to the reduction of strongly adsorbed Cu^{2+} on the electrode surface. The sites present on the electrode surface are not still saturated with this accumulation time.

When 20 min of pre-concentration was used, probably all complexation sites on the electrode surface were saturated. One reduction step was observed when the potential scanning was done in buffer solution and when a pre-concentration time of 15 min was imposed (figure 2A). However, two reduction steps (-0.19 and -0.27V) were observed when the potential scanning was in buffer plus $3.2x10^{-6}$ mol L^{-1} Cu^{2+} solution (figure 2B). The cathodic step at -0.27 V corresponds to the reduction of Cu^{2+} that was in the diffusion layer.

These results show that the steps correspond to the reduction of strongly adsorbed Cu^{2+} (-0.19 V) and weakly adsorbed Cu^{2+} (-0.27 V) on the modified electrode surface.

3.3 COMPARISON BETWEEN CPE AND CPE-HA BY ANODIC STRIPPING VOLTAMMETRY AT PH 4.5

This methodology contains three steps. First, a proper electrode surface was immersed in buffered $1.6x10^{-5}$ mol L^{-1} Cu^{2+} solution. A potential of -0.5 V (SCE) was imposed for 5 min, under magnetic stirring (pre-concentration step). Second, the electrode was rinsed with buffer solution and finally, the cyclic voltammetry was done in buffer solution from -0.5 V to +0.5 V (SCE) at 10 mV s^{-1} to observe just the copper adsorbed on the electrode surface.

Figure 3A shows that copper presents one anodic peak (10.06 ± 1.01 µA; +0.07 V) at CPE and does not present a measurable cathodic current. The copper, after being re-oxidized on the CPE surface, goes to the solution bulk and, thus, its detection in the return scanning potential (+ 0.5 V to - 0.5 V) becomes difficult.

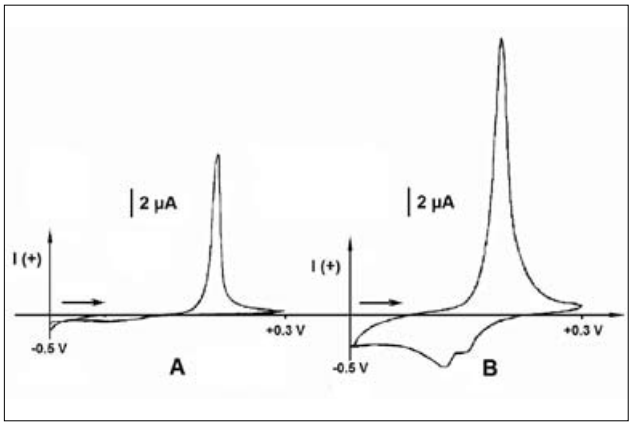

FIGURE 3: ANODIC STRIPPING VOLTAMMETRY OF Cu^{2+} ($1.6x10^{-5}$ MOL L^{-1}) ON: (A) CPE AND (B) CPE-HA. SUPPORT ELECTROLYTE: 0.1 MOL L^{-1} ACETATE BUFFER SOLUTION (PH 4.5). PRE-CONCENTRATION STEP: -0.5 V (SCE) FOR 5 MIN UNDER MAGNETIC STIRRING. V = 10 MV s^{-1} (POTENTIAL SCANNING WAS DONE IN BUFFER SOLUTION WITHOUT Cu^{2+}).

For the CPE-HA, the anodic current is larger (17.82 ± 0.22 µA, +0.05 V) than the one obtained by the non-modified electrode (figure 3). The reduction potential values are -0.12 and 0.17 V. This result shows that the HA present with the modified electrode, improves the accumulation phenomena of copper on the electrode surface when the potential was imposed. The HA present in the paste changes the reduction potential to positive values by ca. 50 mV and thus catalyses the reduction of Cu^{2+}. For CPE-HA in the return scanning the re-oxided copper was adsorbed by complexation on the electrode surface and so those two cathodic steps were observed.

These results show the complexation capability of the HA present in the paste. In a previous paper [8], we described that a surface modification by a simple adsorption of HA on a non-modified CPE can change the electrochemical behavior of Cu^{2+} in a very similar fashion.

3.4 SCAN RATE INFLUENCE ON ANODIC STRIPPING VOLTAMMETRY

By changes in the scan rate, it is possible to understand the kinetic parameters of the reaction. The scan rate influence from 5 to 200 mV s^{-1} was studied. In cation surface desorption process, the anodic peak currents are not proportional to the scan rate. At high scan rates, the peaks are not symmetric; they are broad and change their potential values to the positive regions (figure 4). This probably occurs since the oxidation process is slow so that the proportionality between scan rate and anodic peak current is hampered. However, the electrical charge (in Coulomb) will be constant and the anodic peak surfaces, S_{ap}, (µA V) will be proportional to the potential scan rate (figure 5). The cathodic poten-

tials are not markedly influenced by the scan rate.

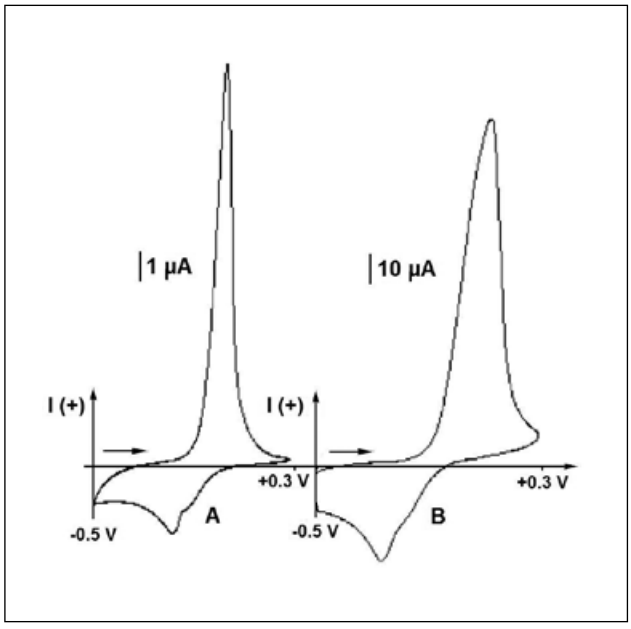

Figure 4: Anodic stripping voltammetry of the Cu adsorbed on CPE-HA surface. Scan rate: (A) 5 mV s^{-1} and (B) 100 mV s^{-1}.

Figure 5 indicates that the anodic peak surface is proportional to the potential scan rate. The cumulated charge at the electrode was about 203.3 µC and it corresponds to 6.34 x 10^{14} atoms Cu^{2+} reduced on the electrode surface.

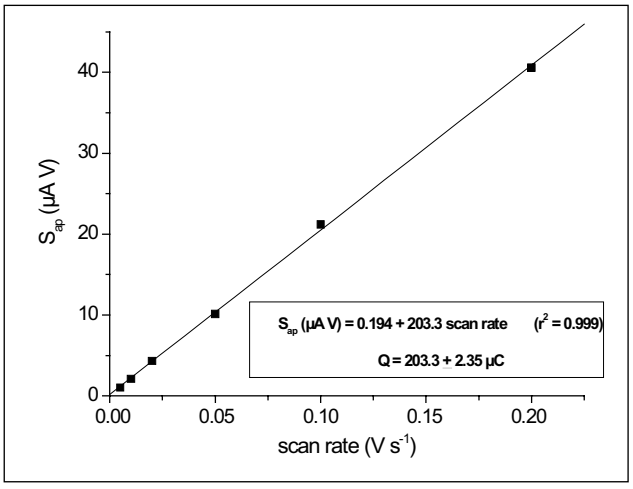

Figure 5: Influence of potential scan rate on anodic peak surfaces (S_{ap}). Results obtained by linear sweep anodic stripping voltammetry. WE: CPE-HA. Support electrolyte: 0.1 mol L^{-1} acetate buffer (pH 4.5). [Cu^{2+}] = 1.6x10^{-5} mol L^{-1}.

3.5 Influence of pH on the electrochemical behavior of Cu^{2+} on the modified electrode surface

The pH influences Cu^{2+} redox potentials on the modified electrode surface (table III). With a pH increase, the redox potentials (mainly anodic potentials) are displaced to regions of negative values. This occurs because at higher pH values the HA sites present on the electrode surface (mainly carboxylic groups) are ionized and thus the electrode surface largely changes. At pH values from 3.0 to 4.5 the potential differences between two reduction steps (ΔE_{red}) are constant (about 50 mV). Above pH 5.0 the ΔE_{red} value increases in opposition with the anodic peak current (I_{pa}) and the current signals are not repeatable (table III). Still above pH 5.0, the relative current of the second reduction step decreases in comparison to the first one (figure 6). When the non-modified electrode was used the electrochemical behavior of Cu^{2+} was similar to that obtained by CPE-HA, but only one cathodic step was observed and the repeatability was poorer. The results obtained by CPE-HA agree with those observed by the complexation experiment described previously. The first reduction step corresponds to complexed Cu^{2+} to Cu reduction on the modified electrode surface. The ionic hydrogen concentration influences the stabilization of Cu^{2+} in solution, besides influencing HA ionization sites. These results show that, as observed by other techniques in previous publications [3,5,6], pH represent a key point of the interaction strength between HS and Cu^{2+}, controlling the solubility of both metal ion and HA, and also the ionization of weak acid functions present in HS structure.

Table III: Redox potentials, obtained by anodic stripping voltammetry on CPE-HA, of Cu^{2+} (1.6x10^{-5} mol L^{-1}). Electrolyte: 0.1 mol L^{-1} acetate buffer solution. Accumulation conditions: -0.5 V (SCE) for 3 min. v= 10 mV s^{-1}.

pH	E red. (VxSCE)	ΔE red (V)	E ox. (VxSCE)	I_{PA} (µA)
3.0	-0.10/-0.15	0.05	+0.10	14.2 ± 0.5
3.5	-0.10/-0.15	0.05	+0.07	13.6 ± 0.8
4.0	-0.11/-0.16	0.05	+0.06	15.8 ± 0.9
4.5	-0.12/-0.17	0.05	+0.05	16.1 ± 0.6
5.0	-0.15/-0.22	0.07	+0.02	9.5 ± 1.3
5.5	-0.16/-0.24	0.08	+0.02	10.5 ± 1.0
6.0	-0.17/-0.33	0.25	+0.02	2.7 ± 0.9

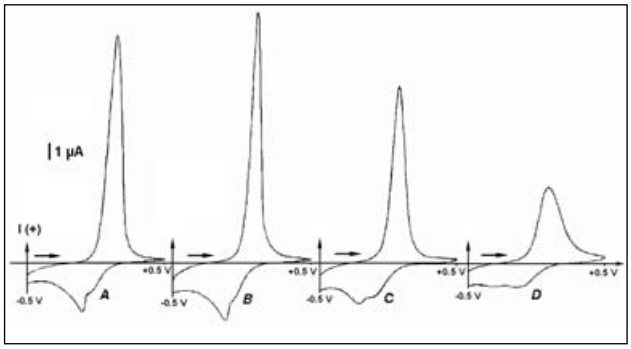

Figure 6: Anodic stripping voltammetry of Cu^{2+} (1.6x10^{-5} mol L^{-1}) obtained on CPE-HA at different pH values: (A) 3.0; (B) 4.5; (C) 5.5 and (D) 6.0. v = 10 mV s^{-1}. Accumulation conditions: -0.5 V (SCE) for 3 min.

4. Conclusion

The results presented in this article represent a good approach in terms of the complexation capability of the humic acids in a non-homogeneous systems. Different cathodic potentials observed for free or HA-linked Cu^{2+}, show how humic substances can act in the environment, controlling physical and chemical parameters of the metal, like solubility and redox potentials. As the electrochemical signal represents only the interface solution/electrode surface, the use of modified electrodes can promote a better understanding of the interfacial interactions in heterogeneous systems (very common in soil, waters and sediments).

Humic substances are not selective in terms of cation interactions and, thus, other metals in solution (mainly Fe^{3+} and Al^{3+}) compete for the same HA functional groups. In this case, other parameters, like formation kinetics and stability of the complexes must be evaluated before analyses.

5. Acknowledgments

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METAL TUBE ATOMIZER IN HYDRIDE GENERATION ATOMIC ABSORPTION SPECTROMETRY FOR ARSENIC DETERMINATION IN ANIMAL TISSUES AND SEDIMENT

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ABSTRACT

Arsenic determination was succesfully carried out using a metallic atomizer for hydride generation atomic absorption spectrometry. The optimized conditions were established as 1600 µL-injection loop, 30 cm-reaction coil, 40 mL min^{-1} of argon flow rate, 3:10 L min^{-1} for the acetylene:air ratio, 1.5 mm i.d. of the ceramic capillary, 43 mm^2 as total drilled hole area of the atomizer, and 1.4 mol L^{-1} and 0.7% (w/v) as HCl and $NaBH_4$ concentrations, respectively. With these conditions, the proposed method allows good figures of merit, with LOD and LOQ as 2.3 and 7.7 µg L^{-1}, respectively, a linear range from 7.7 to 80 µg L^{-1} and precision better than 5.8%. Certified reference materials were used for checking the accuracy of the method.

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INTRODUCTION

The first application of hydride generation atomic absorption spectrometry (HG-AAS) in the literature was reported in 1969 by Holak [1]. Since then, quartz tube atomizers (QTA) [2-5] are currently the devices employed.

Based on a similar atomizer, which is currently used in TS-FF-AAS applications [6-8], Figueiredo et al. [9] and Klassen et al. [10] recently reported an alternative to hydride atomizers based upon a flame-heated metal furnace. Besides its long life-time (> 2000 h) and long-term stability, as well as low cost (*ca.* US$ 7/tube), the authors highlighted good figures of merits regarding the proposed methods using a metallic atomizer for Sb [9] and Bi and Se [10] determinations in different samples such as medicines, urine and certified reference materials. Another important characteristic of these tubes is related to the new environments obtained, due to the constituents of each alloy used, to the flame characteristics (acetylene/air at 3:10 L min^{-1}), and to the possibility of promoting different reactions inside the system, which can be used for favoring hydride decomposition [9,10].

In this way, the present manuscript reports the optimization of the method for arsenic determination in animal and sediment samples by hydride generation atomic absorption spectrometry, using Inconel 600® as metallic atomizer.

EXPERIMENTAL

APPARATUS

A Perkin-Elmer Analyst 300 flame atomic absorption spectrometer with deuterium lamp background correction was used for As determination. All measurements were based on integrated absorbance. An electrodeless discharge lamp (EDL) was used as a primary radiation source (As = 193.7 nm) and the operating conditions were those recommended by manufacturer.

The flow system comprised an Ismatec peristaltic pump, (IPC - 12, Glattzbrugg, Switzerland) and a home-made three-piece injector-commutator device built-in polymethacrylate [11]. Polyethylene tubes (0.7 mm i.d.) were used as transmission lines, and Tygon® tubes for propelling the solutions towards the atomizer. The tube atomizer dimensions are similar to those ones already described for TS-FF-AAS applications [6-8]. It contains six holes facing the burner, allowing partial flame penetration inside the tube, as well as a lateral hole where a ceramic tube employed for sample introduction is partially inserted (*ca*. 2 mm). The Inconel 600® tube atomizer composition [> 72% (m/m) Ni, 14-17% (m/m) Cr and 6-10% Fe] was obtained from the manufacturer (Camacam, São Paulo, Brazil).

High purity deionized water was obtained from a Millipore model Milli-Q Plus water purification unit and a Provecto Analítica microwave oven, model DGT Plus (Jundiaí, Brazil), was used for sample decomposition.

REAGENTS, SOLUTIONS AND SAMPLES

All solutions were of analytical grade and deionized water (18.2 MΩ cm, 25 °C) was used. All glassware were washed with soap, and kept in a 10% (v/v) HCl for 24 h with posterior cleaning with ultra-pure water.

The reference solution (50 μg L^{-1}) was daily prepared in 1.44 mol L^{-1} HCl (Merck, Darmstadt, Germany) by serial dilution with deionized water of a standard stock solutions (1000 mg L^{-1} - Tec-Lab, Hexis Científica Ltda, Indaiatuba, SP, verified by SRM 3103a, NIST, USA). 0.7% (w/v) $NaBH_4$ (Aldrich, Steinheim, Germany) solutions were prepared daily in 0.8% (w/v) NaOH (Merck, Darmstadt, Germany).

Nitric and hydrofluoric acids (Merck, Darmstadt, Germany) were used for sample decomposition. For promoting the As(V) to As(III) reduction, KI and ascorbic acid were used at 2% (w/v) and 4% (w/v), respectively.

Three certified materials, SRM 1566a (Oyster Tissue) from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA), and CRM 422 (Cod Muscle) and CRM 320 (River Sediment) from BCR (Bureau Community of Reference, Brussels, Belgium), respectively, were analyzed for accuracy check.

SAMPLE PREPARATION

The proposed procedure for cod muscle and oyster tissue sample preparation was adapted from Ribeiro et al [12]. In this way, *ca.* 250 mg of the certified materials plus 1.5 g of magnesium nitrate were placed in a ceramic vessel and 10 mL of conc. HNO_3 were added under gentle heating (*ca*. 70 °C) until almost dryness. Then, the sample was transferred to a muffle, and the initial temperature adjusted to 200 °C, being increased by 50 °C each 30 min up to 450 °C. Finally, this temperature was held constant for 1 h. The white ash obtained after dry decomposition of both BCR 422 and SRM 1566a samples, was then dissolved into 4.2 mL HCl. The final solutions were filtered (qualitative filter, 14 μm-porosity) and transferred to a 50 mL and a 25 mL volumetric flask, respectively and treated with 2% (w/v) KI and 4% (w/v) ascorbic acid in 4 mol L^{-1} HCl for As(V) to As(III) reduction.

For river sediment, *ca.* 250 mg of BCR 320 sample were weighted, and a pre-digestion carried out using 5 mL of *aqua regia*. After 40 min, 2.5 mL conc. HF were added. A microwave-assisted digestion (MW) was then carried out, with the program time (min) *versus* power (W) as follow: 3@200, 5@400, 5@600 and 20@700. After the decomposition, *ca*. 200 mg of boric acid was added to the solution, allowing fluoride elimination. Then, the vessels were gentle heated (*ca.* 70 °C) almost to dryness, and the filtered samples were transferred to a 50 mL volumetric flask. From this solution, 2 mL were transferred to a 25 mL volumetric flask, containing 2% (w/v) KI, 4% (w/v) ascorbic acid and 4 mol L^{-1} HCl, for As(V) to As(III) reduction.

FLOW INJECTION SYSTEM

The arsine was obtained as the product of the chemical reaction between As(III) and $NaBH_4$ in an acid medium (1.44 mol L^{-1} HCl). The hydride generated was introduced into a gas-liquid separator (GLS) [13], being transported by a constant argon flow rate (*ca.* 40 mL min^{-1}) towards the atomizer. The atomizer was fixed on the burner of the flame atomic absorption spectrometer using a lab-made steel support with four ceramic pins, as previously reported [6-8].

Figure 1 shows the system used. In the sampling position (Figure 1A) the L_1 (samples) and L_2 ($NaBH_4$) loops (1600 μL each) are filled. When the central portion of the injector is switched to the alternative position (Figure 1B), the volumes contained in both L_1 and L_2 are transported by C_1 and C_2 carrier streams (deionized water) towards the analytical pathway. Then, the acidified sample and the $NaBH_4$ are mixed at the *x* confluence point, and the hydride is generated in the reactor through the reaction between BH_4^- and As(III) in an acidic medium. Then, the arsine generated is separated using the gas-liquid separator and transported to its upper part through a constant argon flow rate (40 mL min^{-1}). The remaining sample is aspirated through a lateral hole in the GLS and then discarded, the hydride being transported towards the ceramic capillary (1.5 mm i.d., 2.0 mm o.d. and 100 mm length), reaching the heated Inconel 600® tube atomizer, and allowing the determination of arsenic.

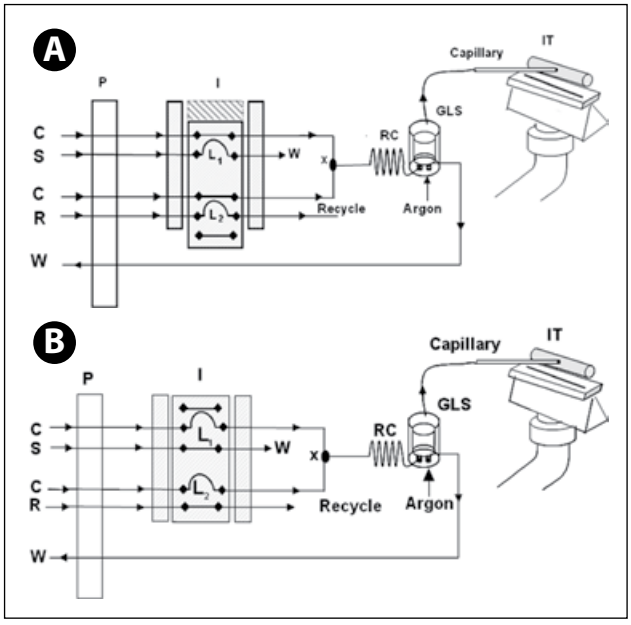

FIGURE 1. SCHEMATIC DIAGRAM OF THE PROPOSED FLOW INJECTION SYSTEM. [P]: PERISTALTIC PUMP; [I]: INJECTOR-COMMUTATOR; [*x*]: CONFLUENCE POINT; [RC]: REACTION COIL; [GLS]: GAS-LIQUID SEPARATOR; CERAMIC CAPILLARY AND INCONEL 600® TUBE (IT); [C] CARRIER SOLUTION (DEIONIZED WATER); [S] STANDARD/SAMPLE; [R] $NaBH_4$ IN NaOH; [Ar]: ARGON; [W] WASTE; [L_1] SAMPLE; [L_2] $NaBH_4$ IN NaOH. (A) SYSTEM IN THE SAMPLING POSITION; (B) SYSTEM IN INJECTION POSITION.

Results and discussion

Optimization of the variables

The physical and chemical variables were performed in a univariated way, and the maximum integrated absorbance obtained with a 50 μg L^{-1} standard solution, which was used for optimizing the method.

Pre-conditioning of the inconel 600® tube

A nickel tube is commonly used in the TS-FF-AAS technique [6-8], and it was also tested as atomizer for this work. However, it yielded imprecise results (*ca.* 35% RSD) and poor sensitivity (*ca*. 30% when compared to the Inconel 600® tube). These results are in agreement to those ones recently obtained [9], and they can be attributed to the reaction between the Ni present in the surface atomizer and As, which is constantly injected into the system. As a result, the formation of $Ni_3(AsO_4)_2$ is presumed, such species being thermodynamically favorable ($\Delta^{\circ}G_f$ = -1581,73 kJ mol^{-1}) [14]. The formation of $NiAs_2O_6$ is not probable, because the literature reports its synthesis occurs only after one week of reaction between NiO and As_2O_5 at 700 °C [15]. Since the decomposition of the arsine occurs at 700°C [16], it is more probable that the As reacts with the nickel oxide (present in the atomizer alloy), forming $Ni_3(AsO_4)_2$, as previously mentioned.

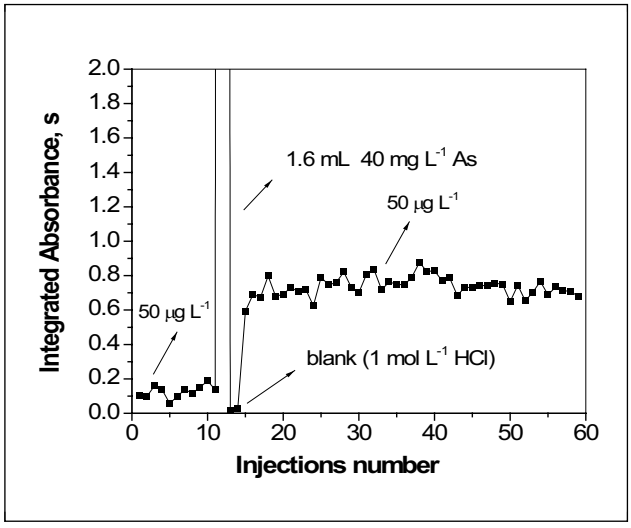

Figure 2. Evaluation of the As signal stability (50 μg L-1) after tube pretreatment by injecting a high concentration of As (40 mg L-1).

As similarly described for Sb [9], a concentrated As standard (40 mg L^{-1}) was injected into the Inconel 600® atomizer for increasing the sensitivity of the method. Subsequently, many blank solutions were also injected for checking any problem related to carryover effects. After these procedures, the analytical signal for As (50 μg L^{-1}) increased 65%, remaining constant after 60 consecutive injections. Then, 44 consecutive injections of 50 μg L^{-1} As solution were carried out, the SD and the RSD being 0.0577 and 7.8%, respectively, indicating the good efficiency of the treatment. The results obtained are shown in Figure 2. Apparently, a decrease of the nickel atomizer surface concentration is required for signal stabilization and is of utmost important for attaining good results. Then some changes in the inner atomizer environment were necessary for stabilizing the As signal. Similar behavior has also been noted in the literature [9].

Effects of the injection volume and reaction coil

The L_1 and L_2 loop volumes (300, 600, 1000, 1300, 1600 and 2000 μL) were simultaneously studied, the increase of the integrated absorbance signal proportional being to the sample volume. As a compromise between sensitivity and the analytical frequencies of the method, 1600 μL was then selected as injection volume for further measurements.

As better mixing conditions between the sample and $NaBH_4$ are promoted by the reaction coil, its length needs to be sufficient for hydride release. The reactor length was evaluated from 10 to 90 cm, and the best analytical signal was obtained with 30 cm, this length being selected for future experiments.

Effects of carrier gas flow rate and acetylene-air flow rates

As argon is frequently used for transporting the hydride to the atomizer, its flow rate was tested from 10 to 200 mL min^{-1}, the best conditions being achieved at 40 mL min^{-1}.

The acetylene-air flow rates in the flame were verified from 1 to 3 L min^{-1} and from 8 to 12 L min^{-1}, respectively. The results are shown in Figure 3, indicating that, when minimal acetylene and maximum air flow rates were used, the analytical signal was lower. In Figure 3B, a low background signal can be seen, which was obtained after the optimization of the total hole area. This result suggests that the atomization mechanisms of the arsine, when a metallic atomizer is employed, is similar to that already proposed and, considering the QTA atomizer [17], where the authors reported that for the atomization of hydrides the presence of hydrogen radical and a low oxygen flow rate is necessary. Thus, the flow rate of acetylene and air was 3 and 10 L min^{-1}, respectively.

Effect of the ceramic capillary inner diameter

The inner diameter of the ceramic capillary was also evaluated, using 0.5; 1.0 and 1.5 mm i.d. The best result was obtained with the largest i.d. With 0.5 mm i.d., the hydride was not efficiently transported, the arsine being hydrolyzed quickly, because it remains constantly in contact with the liquid phase.

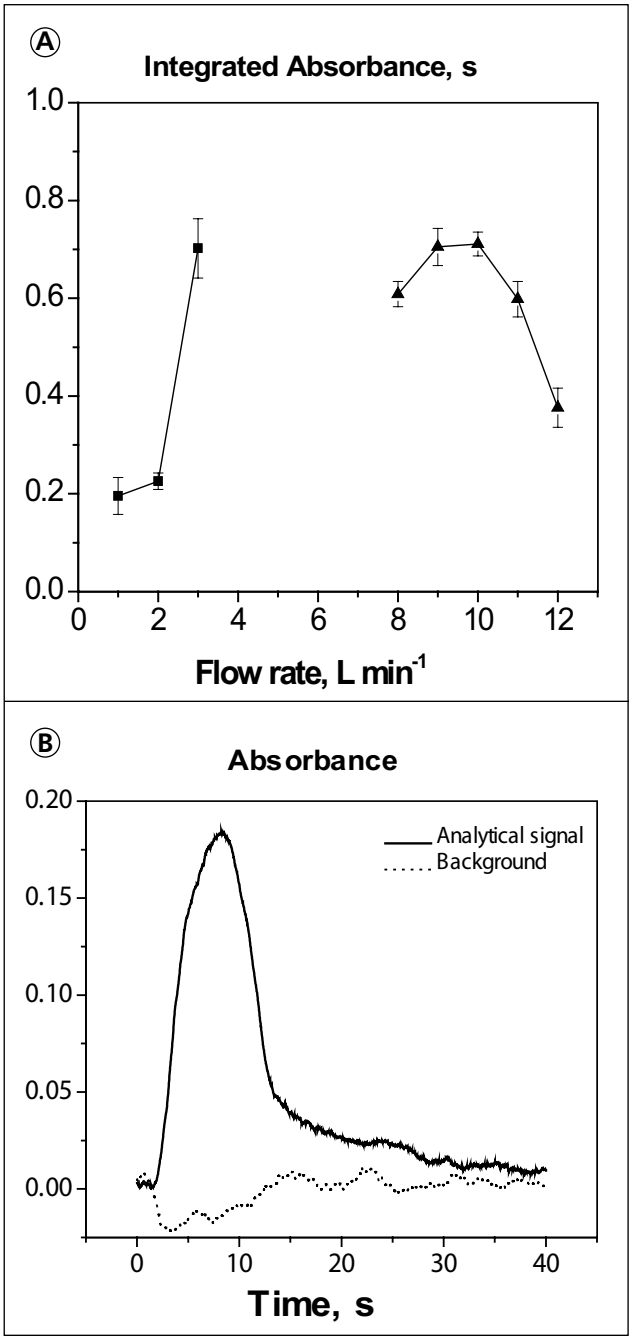

FIGURE 3. INFLUENCE OF THE AIR:ACETYLENE PROPORTION. (A) VARIATION OF ACETYLENE FLOW RATE (AIR FLOW RATE FIXED AT 10 L MIN^{-1}); AND VARIATION OF AIR FLOW RATE (ACETYLENE FLOW RATE FIXED AT 3 L MIN^{-1}); (B) ANALYTICAL SIGNAL AND BACKGROUND. 50 µG L^{-1} AS, 1 MOL L^{-1} HCL, 1% (W/V) $NaBH_4$ AND 0.8% (W/V) NAOH WERE THE CONDITIONS USED.

EFFECT OF THE INCONEL 600® TUBE TOTAL DRILLED HOLE AREA

Different metallic atomizer total drilled hole areas were evaluated (19, 43, 67, 91, 115 mm^2), as well as one atomizer without holes. The best result was obtained with a total area of 43 mm^2. The highest decrease (ca. 85%) was attained when the atomizer without holes was used, indicating that the arsine atomization is better when the flame is in contact with the hydride. From 67 mm^2 a 15% decrease in the integrated absorbance was noted. On the other hand, with larger total drilled hole areas, a more oxidizing atmosphere is attained, which tends to form As_2O_3 [16]. These results are in agreement with those presented in flame composition (see item 3.1.3).

EFFECT OF ACID AND TETRAHYDROBORATE CONCENTRATIONS

Hydrochloric acid and sodium tetrahydroborate are frequently recommended for hydride generation purposes [18]. Their concentrations were evaluated from 0.1 to 3.0 moL L^{-1} and from 0.2 to 1.5%, respectively. In terms of HCl concentration, the arsenic signal was not affected above 1.4 mol L^{-1} HCl. The effect of HNO_3 and H_2SO_4 was also verified, but a decrease in the As signal (*ca*. 24%) was observed for HNO_3, because the arsine release is hampered.

While lower $NaBH_4$ concentration may be not enough for hydride formation, higher ones can produces an excess of hydrogen, generating a turbulent reaction in the gas-liquid separator, hampering hydride release from the liquid phase. As a consequence, a decrease of As signal (ca. 16%) is also observed. Thus, a 7% (w/v) $NaBH_4$ concentration was used throughout this work.

FIGURES OF MERITS

The figures of merits of the method for As determination are presented in the Table I. The results are in agreement with those presented by Grinberg et al. [19], where the authors reported that the sensitivity using a flame atomizer is lower than with a quartz tube atomizer, but the linear range is larger.

TABLE I. ANALYTICAL PARAMETERS FOR AS DETERMINATION

Regression equation	Y=0.1379 + 0.03283X
R^2	0.9978
LOD* (µg L^{-1})	2.3
LOQ* (µg L^{-1})	7.7
Linear range (µg L^{-1})	7.7 – 80
Precision (%)**	< 5.8

* LOD and LOQ, [20];
** Based on As analytical responses from samples (as repeatability, n=6).

ACCURACY CHECK

The accuracy of the method for As determination was verified using certified materials. The results are shown in the Table II, and they are in agreement with the certified ones at 95% confidence intervals, according to *t* and *f* tests.

Table II. Arsenic determination (X ± error) in certified materials determined by the proposed method (PM) and compared with the certified value (CV).

Sample	CV (certified) ($\mu g\ g^{-1}$)	PM (determined) ($\mu g\ g^{-1}$)[a]
Cod muscle (BCR 422)	21 ± 1	21 ± 3
Oyster tissue (SRM 1566a)	14 ± 1	16 ± 2
River sediment (BCR 320)	77 ± 3	85 ± 14

[a] $X \pm t.\sigma/(n)^{1/2}$; n = 3 (number of sample decompositions); X = ($\mu g\ g^{-1}$), error= $t.\sigma/(n)^{1/2}$

Conclusions

The initial purpose in applying the metallic atomizer to hydride generation for As determination was successfully attained, since the performance of the metal furnace as well as the method for As determination can be compared to other methods already proposed in the literature (Table III).

Good figures of merit were obtained with the proposed method, such as wide linear range, good LOD and accuracy, pointing to the metallic atomizer as an effective alternative to the quartz tube when considering hydride generation. Besides these characteristics, low cost (*ca*. US$ 7/tube), long life-time (> 4000 h) and robustness are important points to be highlighted.

Table III. LOD and LOQ obtained for As determination by the proposed work when compared with some techniques reported in the literature.

Techniques	LOD ($\mu g\ L^{-1}$)	LOQ ($\mu g\ L^{-1}$)	References
HG-MF-AAS[a]	2.3	7.6	
QTA[b]	1.1*	-	21
MMQTA[c]	1.2*	-	21
SS/HG-AAS[d]		0.6	22
ETAAS[e]	2.9	-	23
QTAW[f]	1.5	-	13

a Hydride generation metal furnace atomic absorption spectrometry.
b Quartz tube atomizer; * LOD = ng.
c Multiple microflame quartz tube atomizer; * LOD = ng.
d Slurry sampling hydride generation atomic absorption spectrometry.
e Electrothermal atomic absorption spectrometry.
f Quartz tube atomizer with tungsten coil.

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Development of highly resistant fibers for use in solid phase micro extraction (SPME)

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Abstract: The commercially available fibers for solid-phase microextraction (SPME) made of optical fibers are fragile and can be easily broken. Thus, there is a clear need for the development of efficient, simple and highly resistant SPME fibers. This work describes the preparation of highly resistant fibers in which a polymer is coated onto an open tubular fused silica capillary tubing (0.360 mm O.D.) fitted with a stainless steel wire (0.200 mm O.D.) previously inserted and cemented into the capillary tube opening to guarantee higher mechanical resistance. The fiber was coated with 70-μm poly (dimethyl siloxane) (PDMS) and evaluated for the extraction of polycyclic aromatic hydrocarbons.

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Keywords: Gas Chromatography, Solid-phase microextraction, Fibers.

Introduction

Solid phase micro extraction (SPME) has several advantages including simplicity, easy of use (easy to handle) and rapid extraction when compared to other sample preparation techniques. In addition, this technique presents many advantages over traditional analytical techniques by combining sampling, preconcentration, and transfer of analytes into a system for analysis in a single step [1]. However, SPME with polymer-coated fibers still exhibits a significant drawback: fused-silica fibers are very fragile and can be easily broken, and therefore, extra care must be taken during their handling and use. The great majority of fibers that are commercially available at the moment are produced from fused silica fibers (optical fibers) that have the disadvantage of being extremely fragile. Thus, there is clearly a need to develop efficient, simple and highly resistant SPME fibers.

Two other investigations [2,3] have already described the development of resistant fibers for SPME. Wu and Pawliszyn [2] prepared a SPME fiber coated with poly--phenylpyrrole on the surface of stainless steel wires by electrochemical polymerization. The fiber was evaluated for the extraction of volatile organic compounds. Liu et al. [3] introduced an approach for solid-phase microextraction using porous layer-coated metal fibers. Porous silica particles were immobilized on stainless steel fiber using a high-temperature resistant epoxy. The authors reported the efficiency and selectivity of their SPME fiber, describing the extraction of polycyclic aromatic hydrocarbons (PAHs) from water. In the examples, described in these two papers, the SPME fiber coating was immobilized to the metal surface; the disadvantages of these procedures is that not all polymers can be immobilized onto a metal surface.

In the present work, a metal wire is inserted inside an open fused silica capillary tube, thus representing an increase in the mechanical resistance of the fiber, however without loss of the inertia presented by the fused silica capillary tube. In addition, the possibility of immobilization of various polymers types is maintained. To address the aforementioned concerns, SPME fibers showing high mechanical resistance were designed and prepared.

The present study describes the preparation of (highly resistant) fibers using a polymer coated on the external surface of a fused silica capillary tube (0.360 mm O.D., 0.250 mm I.D.). The SPME fiber was mounted in a holder, which was designed and built to be used with this fiber. To demonstrate the quality of the fibers being developed, PDMS was selected as the coating polymer due to its wide use in SPME. The produced fibers have been evaluated to extract PAHs that are ubiquitous environmental pollutants which present a potential health concern because of the toxicity,

mutagenicity, and carcinogenicity of these substances in animals [4,5].

Experimental

Chemicals and Materials

A solution containing the 16 PAHs listed by the US-EPA as priority pollutants at concentrations of 2000 µg mL^{-1} in methylene chloride-benzene (1:1, v/v) was purchased from Supelco (Bellefonte, PA, USA) whereas all the other chemicals were analytical grade products of Mallinckrodt (Xalostoc, Mexico). The standard solution was used for preparation of a working standard solution (100 µg mL^{-1} in methanol). Polydimethylsiloxane SPME fibers, 100 µm film, were purchased from Supelco (Bellefonte, PA, USA). Fused silica capillaries (0.250 mm I.D.) were purchased from Polymicro (Phoenix, AZ, USA) and cement (Epoxy MY 720) was obtained from CIBA (Manhattan, NY, USA). Stainless steel wire with 0.2 mm O.D. was purchased from Casa Nogueira (São Paulo, SP, Brazil). Poly(dimethylsiloxane) hydroxyl terminated and allyltriethoxysilane were obtained from Alldrich (Milwaukee, WI, USA).

Instrumentation

All measurements were made with a Shimadzu (Kyoto, Japan) model 2010 gas chromatograph equipped with a flame ionization detector (FID) and a split/splitless injector. The separations were performed on an CROMA-5 [poly(5% diphenyl/95% dimethyl siloxane)], 30 m x 0.250 mm x 0.30 µm homemade column. The initial column temperature was 90 ^{0}C for 1 min; then heated at 8 ^{0}C min^{-1} to 300 °C, for 3 min. The temperatures of the injector and detector were 280 °C and 300 °C, respectively. Hydrogen at a flow rate of 1.6 mL min^{-1} was used as carrier gas. All SPME desorptions were performed in the splitless mode using a narrow-bore injection port liner (2 mm). A LEO 440 (Oberkochen, Germany) scanning electron microscope was used for the investigation of the fiber surface.

SPME Procedure

The SPME fiber was conditioned at 280 °C in the GC injector for 30 min prior to use; daily the fiber was cleaned in the injector for 5–10 min. Aliquots of 5 mL of working standard solution or sample were extracted from a 6 ml glass vial with a screw top/teflon-lined silicone septum (Supelco, Bellefonte, PA, USA). The concentrations of polycyclic aromatic hydrocarbon compounds were 0.5 µg mL^{-1} (sample solution). The sample solution was stirred with a magnetic stirrer at 1120 rpm. The septum of the vial was pierced with the SPME needle guide and the SPME fiber was fully immersed in the stirred sample solution for 30 min at room temperature. Subsequently, the fiber was introduced in the injector (280 °C), and the split valve turned on after 3 min. The fiber desorption time in the injector was 5 min and no carryover was observed under these experimental conditions.

Fiber Preparation

The fused silica capillaries purchased were already externally coated with polyimide and had outer diameter of approximately 0.360 mm. One end of a 10 cm tube was closed in a flame while the other one was left open. The tip (1 cm) of the polyimide coating on the closed end of the fused silica tube was removed by gently burning it off. This was done carefully as fused silica becomes fragile when the polyimide is removed. The exposed tip was washed with excess of acetone and methanol, and then air-dried at room temperature. A mixture of PDMS, hydroxyl terminated, plus allyltriethoxysilane (adhesion promoter/catalyst), mass rate 10:1, was prepared for use in step 1 of the fiber coating procedure.

The SPME fiber coating was performed in two steps:

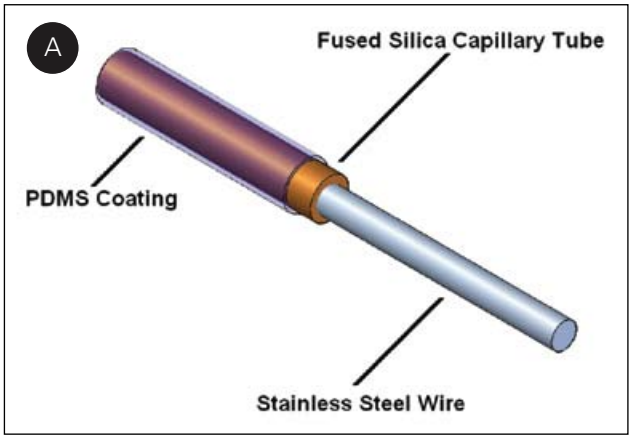
1. The fused silica capillary (the exposed part) was dipped for 1 minute vertically into the PDMS mixture. This mixture had been previously diluted with pentane (1 mg mL^{-1});
2. The capillary coated with PDMS was passed through a pre-perforated (0.500 mm diameter) PTFE disc (8 mm diameter and 1.5 mm thickness); this process is necessary for the determination of the film thickness.

Subsequently, the solvent was evaporated at room temperature and a uniform PMDS film coated the fused silica capillary. The fiber was placed in a vial saturated with vapor of azo-tert-butane under nitrogen for 2 hours. The azo-tert-butane was used as a free radical cross linking initiator to prepare a more resistant polymeric film. The curing process was done in an oven at 220 °C for 1 hour, and the fiber was then conditioned in a GC injector port at 250 °C for 3 hours. The produced fiber can be used at temperatures up to 300 °C without thermal decomposition. The final fiber thickness was 70-µm as measured by Scanning Electron Microscopy (SEM); two other fibers were also coated for comparison purposes. The reproducibility of the fiber preparation was good as the differences in the coating thickness were within 13% RSD, compared with a set of 3 commercial fibers (10% RSD).

A stainless steel wire was then dipped into the cement, and manually introduced into the open side of the capillary tubing. This extremity was left open from the beginning of the process to allow the excess of cement to be removed during the wire insertion.

The cement cure was carried out according to the following heat treatment: 100 °C for 1 h, followed by 1 h at 150 °C and finally by 1 h at 180 °C. Upon this

treatment the stainless steel wire was immobilized in the fused silica capillary tube (Figure 1A). Figure 1B shows the outside diameter measurement of the fiber. Afterwards, the fiber was mounted in a new home-made holder, and then conditioned for 1 hour in a GC injector at 280 °C.



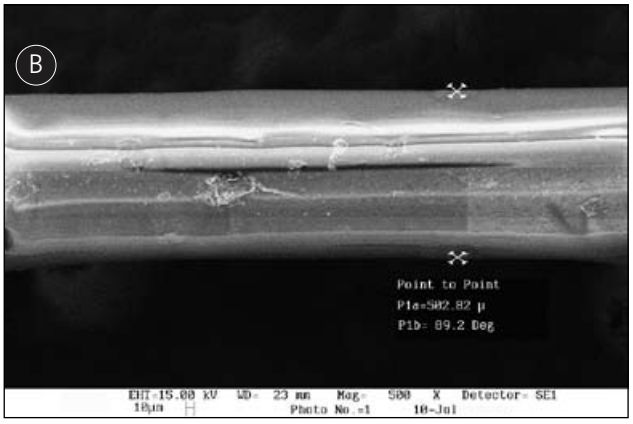


Figure 1. (A) Scheme of the assembled fiber apparatus showing the stainless steel wire inside the open fused silica capillary tube. (B) Measurement of the outside diameter of the fiber by Scanning Electron Micrography (SEM).

Results and Discussion

Fiber Resistance

The mechanical resistance of the fiber was tested by performing several GC fiber desorptions without retracting the fiber to its original position inside of the fiber holder needle. This was investigated considering the fact that, in routine SPME analyses, eventually the fiber is left unretracted before insertion into the GC injector, which results in easily visible fissures and/or loss of the fiber during the passage through the injector septum, since these commercially available fibers are very fragile.

The fibers produced and described in this paper were then tested being left unretracted after desorption into the GC injector. The fiber was mounted into a holder device initially designed to be used in a SPME heated interface [6]. In all cases after the exposure the fiber was subject to a Scanning Electron Microscopy (SEM) analysis and very small cracks were observed, without loss of fiber coating. Figure 2A shows the surface of the developed fiber before the 1st exposition and Figures 2B and 2C shows the surface of the fiber after the 3rd exposition, in two different positions, with the fiber unretracted, where cracks on the surface of the fiber can be observed.

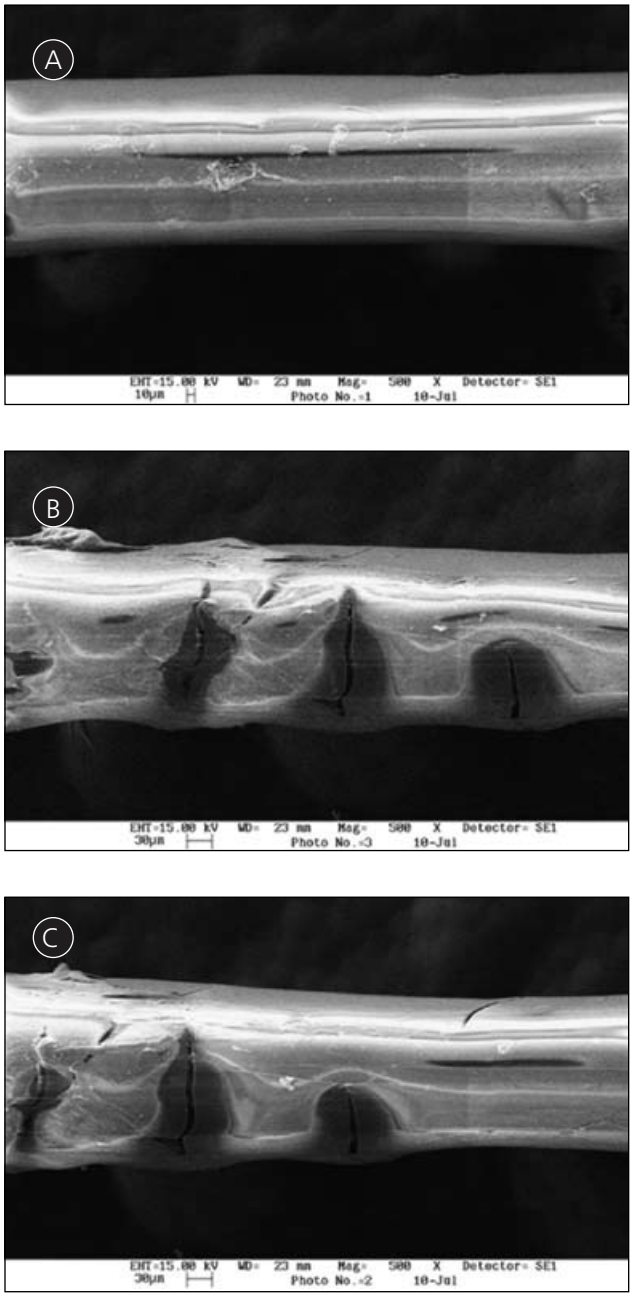


Figure 2. Surface structure of poly(dimethyl siloxane) (PDMS) fiber, as obtained by scanning electron microscopy (500-fold magnification). (A) Before assay; (B,C) After 3rd removal without retracting the fiber.

Figure 3 shows the chromatogram of a mixture containing 16 PAHs extracted using a commercial PDMS fiber (3A) and the developed fiber (3B-C). Before (Figure 3B) and after (Figure 3C) three exposures, the fiber was maintained unretracted after desorption.

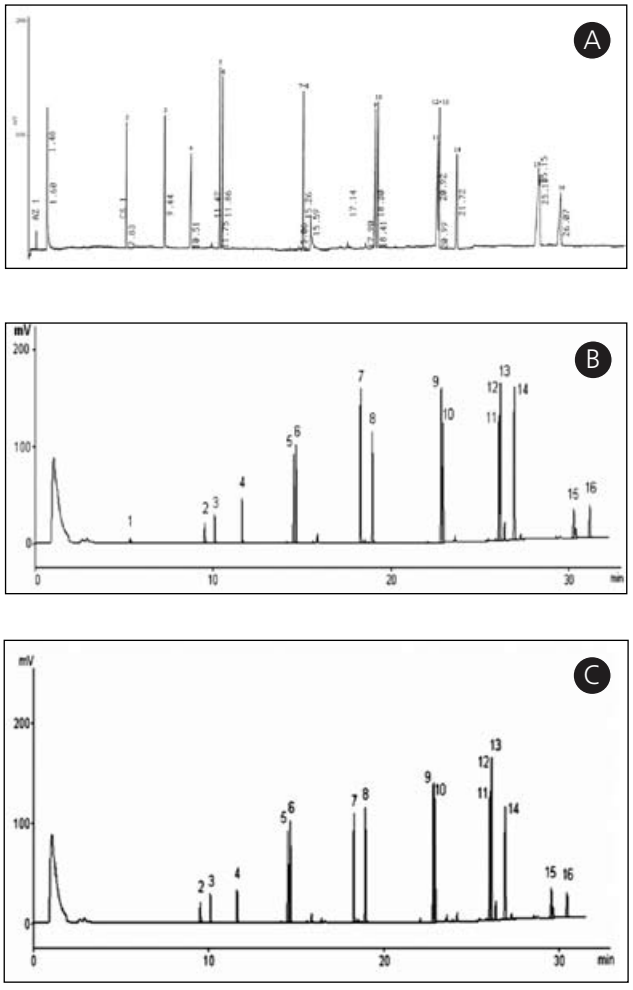


Figure 3. Gas chromatogram of PAHs extracted using (A) 100 μm polydimetthylsiloxane commercially coated fiber. (B) 70 μm highly resistant PDMS fiber, with fiber retraction before removal from GC injector; (C) After three expositions leaving the fiber unretracted during removal from GC injector. Columns: CROMA-5 and DB-5 [poly(5% diphenyl/95% dimethylsiloxane) 30 m X 0.250 mm I.D. X 0.30 μm]; Carrier gas, hydrogen; injection, splitless; detector , FID, 300 °C. Temperature programming: 90 °C/2 min – 8 °C/min^{-1} – 300 °C/5 min. (1-Naphthalene; 2-Acenaphthylene; 3-Acenaphthene; 4-Fluorene; 5-Phenanthrene; 6-Anthracene; 7-Fluoranthene; 8-Pyrene; 9-Benzo[a]antracene; 10-Chrysene; 11-Benzo[b]fluoranthene; 12-Benzo[k]fluoranthene; 13-Benzo[a]pyrene; 14-Indeno[1,2,3-cd]pyrene; 15-Dibenzo[a,h]anthracene; 16-Benzo[ghi]perylene).

The fiber tested in the unretracted position resisted up to five removals from the injector; loss of the polymeric layer occurred however, as it was noted that the extraction efficiency of the fiber decreased after the 3rd removal if left unretracted due to the presence of micro fissures on the capillary surface of the fused silica (Figures 2B and 2C), which modifies the original fiber surface. In addition, it has a deposition of a very thin rubber layer (originating from the injector septum) on the fiber polymer surface that might create an additional absorption effect on the rubber deposit. Thus, the proposed fibers need to be protected when being introduced into the GC, otherwise the stationary phase might be damaged.

Conclusion

The insertion of the stainless steel wire into the opening of the fused silica tubing after the fiber is prepared dramatically increases the SPME fiber mechanical resistance. In addition, the surface of fused silica has been maintained, which allows various other types of polymers to be used, since most polymers used in SPME cannot be directly immobilized onto a metal surface.

Finally, the proposed development is ideal in the training of new SPME users, as well as its use in a routine analysis, where more robust fibers are required. The system allows the easy in-house preparation of high quality highly resistant fibers for SPME.

Acknowledgments:

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Point of View

Universities and R&D Centers role as the foundation for the Brazilian Industrial and Economic Growth: Beyond Innovation

The past decades have shown well succeeded examples of industrial developments based on government-university-industry partnership programs denominated as Triple Helix model by Etzkowitz- Leydersdorff. Two real examples are the route 128 and Silicon Valley where the economical growth was based on scientific knowledge provided by universities, R&D Centers and supported by government programs. Two examples very well known are the Silicon Valley industry where the government implemented a program in conjunction with Stanford University to retain the students in the area, by creating incubated companies under university supervision. Similar action occurred around MIT geographical area, denominated route 128.

In Brazil we can mention many success cases, but I want to highlight one: the leadership in technology and production chain in ethanol, which efforts started in the 70´s as a result from the efforts of research centers as such CTA, universities, government and industry in order to find an alternative fuel source to replace the petroleum and its derivatives because the Petroleum Exporter Countries Organization (OPEP) decision to reduce the global petroleum production. Few decades later, the continued efforts resulted in the Brazil´s leadership in ethanol. Besides the current leadership, the future trends always challenge our researchers and our industry to drive the attention to integrated biomass studies over than just keep working in ethanol based developments.

The technological challenges to support the industrial and economy demands for growth and be expanded to other Brazilian markets as such energy, pharmaceutical, chemicals, minerals, agricultural and food processing, just to exemplify.

In addition to the Universities and R&D public Centers role, the installation of private R&D institutes shall become more frequent in order to support applied developments demanded by industries as the consequence of certain competitive environments. But, it does not mean that universities and public R&D Centers should lose space because they are complementary. All the institutions will need to work in conjunction on targeted projects because it requires multiple core competences available from different sources. Another attractive for global companies to invest in Brazil is because the country occupies the position number 13 in the scientific production worldwide, plus the fact that country delivered to the market 11,360 PhD degree professionals.

A good example comes from Petrobras that successfully implemented a Thematic Technology Networks Program with universities and research institutes in conjunction to their own Research Center as a way to move fast and anticipate the R&D demands. Global companies have been attracted by demand increase for High Tech products and services in order to serve science and industrial needs, as ThermoFisher . Other company that announced investments in Brazil is GE that will open a Research center in Rio de Janeiro neighbor to UFRJ and Petrobras R&D Center.

These events reinforce the universities and public research centers a key role in the actual economic development scenario, which consists in keeping and expands the network connections through a permanent dialogue with society, industry and government that goes beyond innovation by itself: It aligns the priorities and common actions on targeted interest areas. Innovation certainly evolves.

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A review of 11th Rio Symposium on Atomic Spectrometry

The Rio Symposium on Atomic Spectrometry was first organized by Dr Adilson Curtius and Dr Bernhard Welz at the Pontifical Catholic University of Rio the Janeiro, in 1988, with the idea that, by inviting world top researchers, it would help taking Latin America research to international level. Since then, the Rio Symposium has been successfully organized in different Latin American countries, showing steadily increase in the number of participants. Indeed, it has helped to launch Latin American research in Atomic Spectrometry to the state of the art level.

The 11th edition of Rio Symposium, in its second visit to Argentina, was organized by Dr Mabel Tudino (University of Buenos Aires) and Patricia Smichowski (Argentine Atomic Energy Commission), in Mar del Plata, from 24 to 28 October 2010. Sponsored by 9 companies and 19 expositors, and the presentation of 25 Invited Lectures, 28 Oral Presentations and a total of 224 posters were experienced there. Themes such as vapor generation, laser ablation-ICP-MS, microwave induced combustion, microscale separation with ionic liquids, non-metals determination by high resolution continuum source AAS, picoliter droplets sample introduction, atomic absorption in lasers, ancient ceramics analysis by m-X ray fluorescence, multielementar ET AAS, analysis of oily samples by FIA and ICP-MS, high resolution ICP OES and MS, direct solid sampling, field flow fractionation ICP-MS, LIBS in environmental and agricultural research, arsenic in the environment and selenium x mercury antagonism were addressed. Special attention was given to metalloproteins, with presentations dealing with accuracy in mass measurements, stroke biomarkers, comparative metalloproteomics and metal assignments for labile metalloprotein complexes. The event was perfectly organized where, alongside the high academic level, the social events included a splendid excursion to the Santa Isabel Ranch and a startling tango presentation that astonished the audience at the traditional Atomic Dinner. We all thank Mabel and Patricia for this marvelous meeting and invite all to the next edition, to be chaired by Dr Francisco Krug, in São Paulo, in 2012.

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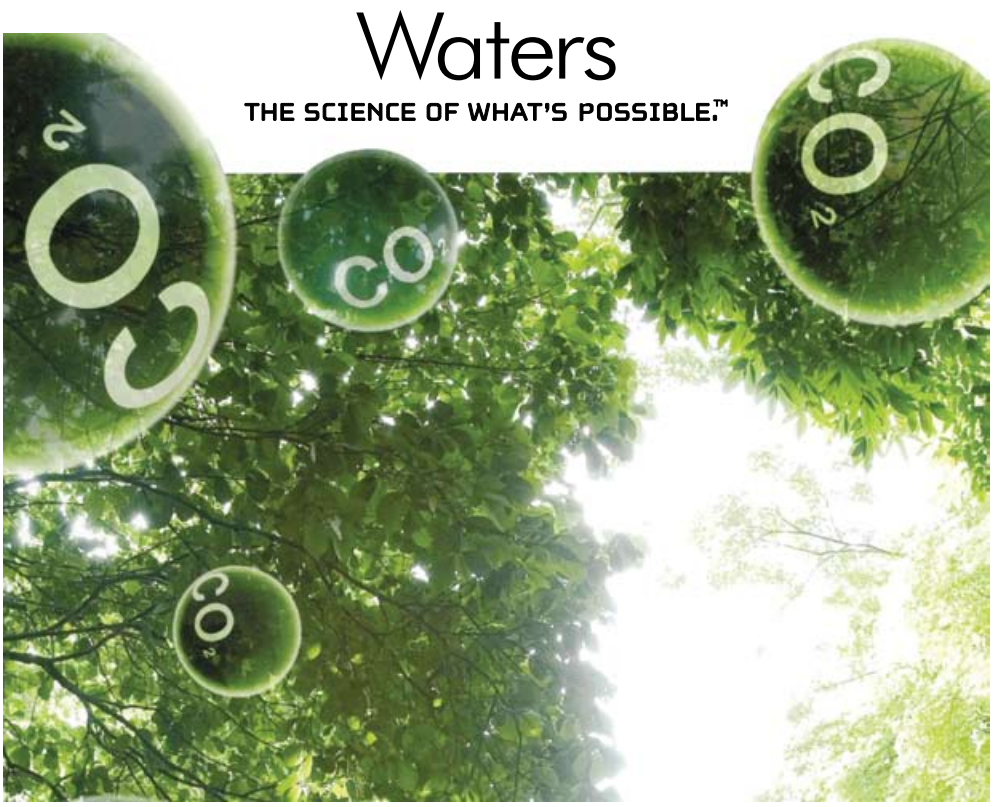

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The event has already begun to receive scientific papers and confirms the presence of international speakers.

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The event, simultaneously held with the 11th Analitica – International Trade Fair for Laboratory Technology, Analyses, Biotechnology and Quality Control, already has the confirmed participation of two renowned personalities: **Professor Ramon M. Barnes**, Director of the University Research Institute for Analytical Chemistry, and **Professor Anthony Wong**, PhD, Director of the CEATOX from the Instituto da Criança do Hospital das Clínicas,University of São Paulo.

In 2011, the **2nd Analitica Latin America Congress** will lead 3 major Analytical Chemistry themes to discussion: Metrology and Chemometrics, Analysis Methods and Sample Preparation and Instrumental Analysis Technologies. The congress program features 11 symposia, 2 of them international, in addition to round tables, mini courses, 84 oral presentations and a large display area for the papers.

Held by DKK and organized by NürnbergMesse, the event is scheduled to take place between September 20th and 22nd, at the Transamerica Expo Center, in São Paulo.

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Realização: Sindusfarma – Sindicato da Indústria de Produtos Farmacêuticos no Estado de São Paulo

Apoio: CRF SP; Instituto Racine; revista embanews; SINQFAR; SINFACOPE; Sindifar – Sindicato da Indústria de Produtos Farmacêuticos RS; FIP Farma; SBMF; SINFAR

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