BrJAC

Brazilian Journal of Analytical Chemistry

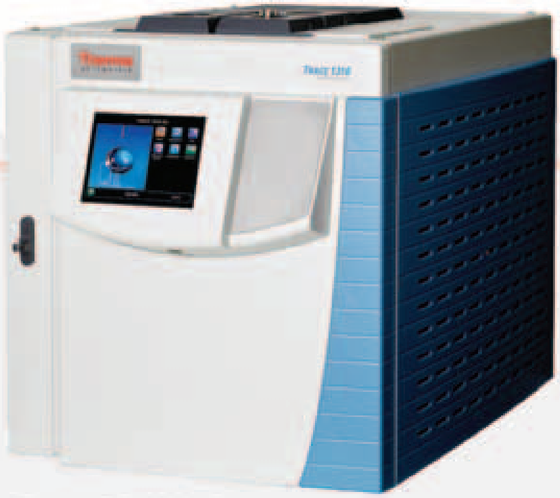
BrJAC 2012; 3 (10)
October / November/ December
ISSN 2179-3425

Novo Cromatógrafo a gás modular

O novo TRACE 1300 Series GC da Thermo Scientific é o primeiro e único cromatógrafo a gás com injetores e detectores que podem ser trocados pelo usuário através de conexões instantâneas. Esta característica exclusiva torna o TRACE 1300 um sistema capaz de satisfazer todas as necessidades do laboratório de forma simples e eficiente. Seu projeto modular facilita o acesso aos injetores e detectores, elimina a necessidade de interrupção das atividades para manutenção e permite ao usuário configurar rapidamente o instrumento conforme suas aplicações específicas e rotina diária. Conheça o desempenho e a produtividade incomparáveis do sistema TRACE 1300 Series GC

redefinindo a usabilidade do GC

- veja a melhor escolha para cromatografia a gás em: www.thermoscientific.com/trace1300



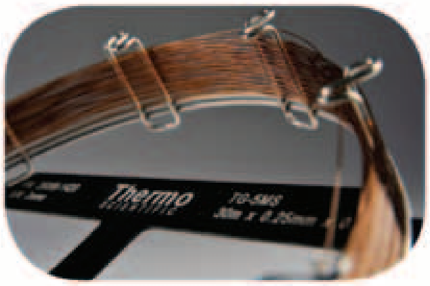
Trace GC 1300 Series

Eficiência incomparável para aplicações de rotina

- Injetores e detectores modulares, de conexão instantânea
- Fácil implementação de métodos
- Injetor com robustez inigualável
- Detector com sensibilidade excepcional
- Instrumento controlado por Chromeleon™ CDS

Exemplo de instalação de um módulo de conexão instantânea pelo usuário.

Com ferramentas extremamente funcionais e de fácil operação, o software Chromeleon 7.2 é simplesmente inteligente



As colunas e os demais consumíveis da Thermo Scientific garantem qualidade e a produtividade do seu novo GC

analitica
analiticaweb.com.br

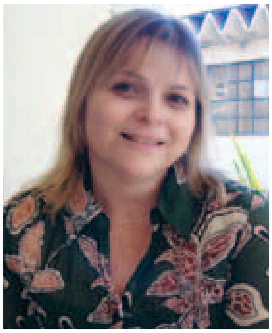
Thermo
SCIENTIFIC

BrJAC

Brazilian Journal of Analytical Chemistry

DKK Comunicação
October / November / December 2012

EDITORIAL



CREATING VALUE

We currently observe that at every technical and scientific forum, urgent issues that require the use of analytical chemistry as a science recurrently display the search for sustainable analytical solutions, resources optimization and for the use of modern methods of analysis, capable of detecting the total composition of a sample in a manner relevant to its market application.

Topics such as energy, renewable raw materials, green chemistry, life sciences, food safety and innovation permeate all economic sectors. We have reason to believe that never before has the interaction between industries and universities through cooperation programs, partnerships and academic exchanges been in such evidence.

However, the Brazilian indicators of expenditure on Research and Innovation reveal figures still not consistent with the urgent demand for competitiveness in products, processes and services.

It is therefore imperative that investments in qualified human resources with creativity and comprehensive vision of processes and products be made.

Indeed, the need for innovation, always mindful of environmental, social and economic aspects, through the pursuit of renewable raw materials and products, the preservation of intellectual property and the introduction of efficient biotechnological routes based on local realities push the innovative process forward in all sectors - industrial and academic- a boost that Brazil cannot take for granted.

Analytical chemistry supports the scientific basis of the findings and generates data for the optimization of the productive chain. One of the best ways to manage this in the corporate environment is through the organization of networks of local and global departments for sharing skills and competences. In this regard, the consolidation of BRJAC as a vehicle that embodies different views and portrays becomes a key factor in making the innovation process more active.

Taking part in this consolidation has become something that creates value.

So, I invite you to participate and contribute to BRJAC. We will bring forth a modern and transversal way to create value, as it is commonly required in our professions: using existing mechanisms, such as internal and global networks, qualified personnel, and BRJAC itself, as tools for market insight focused on the development needs. Enjoy it!

Cristina Maria Schuch
R&I Manager / Analytical Chemistry Department – Research Center of Paulinia
- Rhodia Solvay Group

EDITORIAL BOARD

EDITOR-IN-CHIEF

Lauro Tatsuo Kubota
Full Professor / Institute of Chemistry - State University of Campinas

EDITORS

Cristina Maria Schuch
R&I Manager / Analytical Chemistry Department - Research Center of Paulinia - Rhodia Solvay Group
Marco Aurélio Zezzi Arruda
Full Professor / Institute of Chemistry - State University of Campinas
Maura Moreira Gomes
Management of Engineering Projects - PETROBRAS
Pedro Vitoriano de Oliveira
Associate Professor / Institute of Chemistry - São Paulo University

ADVISORY BOARD

Adriano Otávio Maldaner
Criminal Expert / Forensic Chemistry Service - National Institute of Criminalistics - Brazilian Federal Police
Auro Tanaka
Full Professor / Department of Chemistry - Federal University of Maranhão
Carlos Roberto dos Santos
Engineering and Environmental Quality / Director - CETESB - Environmental Company of Sao Paulo State
Elcio Cruz de Oliveira
Engineering Management - Petrobras Transportes S.A.
Gisela de Aragão Umbuzeiro
Professor / Technology School - State University of Campinas
Helena Müller Queiroz
Federal Inspector / Inorganic Contaminants Unit - Ministry of Agriculture, Livestock and Food Supply
Janusz Pawliszyn
Department of Chemistry - University of Waterloo, Ontario, Canada
Joanna Szpunar
Laboratory of Analytical Chemistry and Environment Bio -CNRS, Pau, France
Joaquim de Araújo Nóbrega
Associate Professor II / Department of Chemistry - Federal University of São Carlos
Johanna Sabine Becker
Central Division of Analytical Chemistry - Head of Brain Met, Forschungszentrum Jülich, Jülich, Germany
Luiz Rogerio Silva
Laboratory Manager - U.S. Pharmacopeia
Márcio das Virgens Rebouças
Business Development / Basic Petrochemicals Unit - Braskem S.A.
Marcos N. Eberlin
Full Professor / Institute of Chemistry - State University of Campinas
Maria Luiza Tristão
Management of Chemistry / Reasearch and Development Center (CENPES) - PETROBRÁS
Mario Miyazawa
Researcher / Soil Chemistry - IAPAR - Agronomic Institute of Paraná
Renato Zanella
Associate Professor / Department of Chemistry - Federal University of Santa Maria
Ricardo Erthal Santelli
Full Professor / Analytical Chemistry - Federal University of Rio de Janeiro
Wokimar Teixeira Garcia
Research Laboratory Coordinator - CTC - Sugarcane Technology Center

SUMMARY

EXPEDIENT

BrJAC
Brazilian Journal of Analytical Chemistry

PUBLISHED BY
DKK Comunicação

PUBLISHER
Carlos Roberto Rodrigues
MTB 0060316 SP
crrodrigues@dkk.com.br

INTERVIEW
Patricia Logullo

TRANSLATOR
Patricia Logullo

TECHNICAL REVIEWER
Carol Hollingworth Collins

COORDINATOR
Regina Suga
reginasuga@dkk.com.br

ART DIRECTOR
Wagner G. Francisco

Letters to

DKK
COMUNICAÇÃO

Rua Embuaçu, 625 - Sala 2
Vila Mariana – São Paulo – Brazil
Zip Code 04118-080
Phone +55 11 5574-1010
BrJAC@BrJAC.com.br
www.BrJAC.com.br

BrJAC is a trade mark of DKK Comunicação. Quartely publication dedicated to Science, Technology and Innovation professionals of universities and industries of Brazil and abroad.

SPONSOR:
analitica
Thermo
SCIENTIFIC

SUPPORT:
Ministério da
Ciência e Tecnologia
GOVERNO FEDERAL
BRASIL
PAÍS RICO É PAÍS SEM POBREZA

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!

PEP TALK ON ANALYTICAL CHEMISTRY AND LUMINESCENCE

Since undergraduate school, I have been working in Analytical Chemistry and, fortunately, have learnt from different advisors that an analytical chemist must be curious and willing to work and learn from different analytical techniques, no matter how different they are from one's comfort zone.

Research in Analytical Chemistry is trilling because there is always the potential to solve important, real life problems. Analytical Chemistry is also a fast growing area of science, benefiting from the development of high technology (new materials, special optics and so on) and from the rapid advances in informatics and data processing. In fact, it is amazing how this area of Chemistry has evolved and how our peers are pushing us to pursuit higher levels of novelty in publications and in analytical applications. However, we must bear in mind that significant improvements in the performance of routine analysis or in the quality of the analytical results may arise from simple ideas and minor adjustments.

Although dealing with different techniques, the luminescence based ones are my favorites. Luminescence is a fascinating phenomenon that carries information, not only about the chemical species that emit light, but also about the environment around them. It is also important to remember that this spectroscopic phenomenon is inherently multidimensional since information can be gathered from spectral data, time profiles and also from the polarization of the emitted light. Therefore, it is also an excellent technique to apply multivariate approaches in chemometrics. Luminescence is widely used in medical diagnostics and imaging and the introduction of luminescent materials in nanoscale size have brought further importance as a number of nanoparticle materials (quantum dots, carbon-dots, fluorescent dye-doped silica nanoparticles and others) designed for analytical/bioanalytical utility has greatly increased. Quantum dots, in particular, are very interesting because of their size-dependent optical tuning capability, brightness needed for sensitive detection, high photo stability and nanoscale interface that enables biomolecular sensing. Planned modifications of the nanoparticle surface might bring selectivity in chemical sensing and biding, making them a very promising alternative in biomarking and bioimaging. In addition, such nanomaterials are very appropriate to develop assay kits and to be adapted to diagnosis platforms.

As it was done to me, I now challenge my students to find the excitement in chasing after the curve of knowledge and have fun in the process of learning. It is crucial for our Ph.D. candidates to be aware of the responsibilities of that degree title, to understand fundamentals, to be concerned with the quality of measurement by accessing and understanding the sources of uncertainty, to develop good writing skills and to quest for novelty and creativity to solve problems and to know that hard work pay off.

Ricardo Queiroz Aucelio
Chemistry Department, Pontifícia Universidade Católica do Rio de Janeiro

In May 1983, the Special Secretariat for Informatics (SEI), linked to National Security Council of the Brazilian government, through the Normative Act 024, determined the market reserve for domestic companies, restricting the manufacture of information technology equipment, such as digital instrumentation for test and measurement, biomedical equipment and analytical instrumentation. Until the reopening of the market in 1991, with the publication of the Federal Law No. 8248, Brazilian companies could thus operate with some freedom in the domestic market. This, however, also favored that companies producing outdated technology, of poor quality, high prices, poor service, lack of innovation could stay in the market. Of course, many were the consequences of this for the development of research in analytical chemistry in Brazil.

In this second interview for BRJAC, Professor Ivano G. R. Gutz, from the Chemistry Institute at USP (University of São Paulo), analyzes what this period represented for the scientific and commercial contexts for analytical chemistry. This is a detailed narrative from someone who personally faced (and still faces) the problems of lack of infrastructure for research in the country and who found creative solutions to the development of a brilliant scientific and didactic career. He shows that the market closure brought both positive and negative consequences to the research and development activities, and that there are good opportunities available.

What is the personal analysis you could do about the period of market reserve in Brazil?

The Normative Act 024/83, devoted to digital technical instruments, was part of a series of prior and subsequent acts that defined goods, ranging from electronic components up to medium-sized electronic equipment, whose domestic production would be allowed only to genuinely national companies. Locally known as "market reserve", these very tight trade barriers aimed to protect and nourish the development of a technologically independent domestic industry of informatics, from chips to mini- and microcomputers, peripherals, operational systems and software, as well as digital instrumentation for test and measurement in general, including modern analytical instrumentation and biomedical equipment. The enforcement of the Act was made by the Special Secretariat of Informatics (SEI), which set many working groups and subcommittees composed by representatives of the government, universities and manufacturers, among others. One of these was organized to deal specifically with Analytical Instrumentation.

This broadband protection plus the financial incentives from public resources gave impetus to the growth of the fledgling national industry of microcomputers and instrumentation, also encouraging many research groups to generate technology and prototypes aimed at industrialization. The production of analytical instrumentation by existing national manufacturers and startups was also cata-

Prof. Dr. Ivano G. R. Gutz at IQ-USP

lyzed, although to a smaller extent than the microcomputer industry and focusing mainly on instruments with favorable ratio between domestic demand and technological complexity. All industries and public and private institutions performing chemical analyzes had no choice than being precariously supplied by the domestic manufacturers and, in a later period, by some "nationalized" analytical instruments manufactured in Brazil by international companies in association with local ones.

Regrettably, most shortcomings of the local information technology and instrumentation industries were not overcome with the market reserve – and many still persist nowadays in variable degrees. For analytical instrumentation, a short list includes: lack of domestic production of most of the electronic components, mechanical parts, sensors and optical devices required by the supply chains; slow and expensive importation transactions of these goods; shortage of skilled human resources; scarcity of viable patented prototypes to absorb from the university; limited research and development (R&D) in the companies; inexperience in the conversion of prototypes into profitable commercial products; lack of economy of scale for more technologically sophisticated instrumentation due to small domestic demand and non-competitiveness in external markets; faster response to new challenges and greater technological evolution of major international competitors abroad. In summary, the positive results of the market reserve period have fallen short of expectations and in view of the reality, critics and internal and external pressures against the trade barriers intensified with time.

In a historical perspective, has the market reserve hold back Brazilian research in analytical chemistry for the production of knowledge and patents? What effect had the importation restrictions on research activities in analytical chemistry?

Research was most impaired in branches more in need of sophisticated and updated instrumentation during this period that was also plagued by the devaluation of research funds by inflation rates often exceeding 10% monthly, turning the importation transactions in a different currency into a risky adventure. Getting importation permits was difficult and, as a rule, computing resources coupled to the instrumentation were excluded from permits. Consequently time and effort was needed to adapt or develop interfaces and software to couple the imported equipment to the outdated and sometimes incompatible domestic microcomputers. This scarcity of means was thus an extra barrier to do research at the science frontier not only in analytical chemistry but also in all areas that are dependent on information technology or on chemical analysis performed with modern analytical instrumentation. Circumstances have led some researchers to upgrade old-fashioned analog instruments, especially those with excellent optics, by interfacing them with microprocessors or microcomputers. As a positive effect, the adversity period of market reserve induced the onset of groups aimed at creating and developing original analytical instrumentation. It also caused and expansion of the search for alternative techniques and methods that use simple accessible or improvised instruments. Both trends persist nowadays, as can be appreciated going through the abstracts of the recent 17º National Meeting of Analytical Chemistry (ENQA) and former editions.

How did the academic research recover after the market opening?

As of March 1990, legislation entered into force that exempted goods for scientific

> **"The positive results of the market reserve period have fallen short of expectations and in view of the reality, critics and internal and external pressures against the trade barriers intensified with time."**

and technological research from the restrictions due to similarity with domestic products, and from import taxes, within limits controlled by the National Council for Scientific and Technological Development (CNPq), obviously after fulfillment of lengthy bureaucratic requirements. The rate of modernization of the laboratories through the acquisition of new analytical instrumentation was dictated by the need and by the availability of resources.

Currently, the most competitive groups with multi-source funding work in world-class laboratories. This is, however, not the rule, especially for many of the newer or underfunded analytical laboratories in universities scattered around the country, sometimes installed and maintained solely with the little money that young PhDs obtain from the CNPq share from the Fund for Scientific and Technological Development (FNDCT), an extremely important but insufficient fund. This has nothing to do with the Normative Act 024/83 anymore, but the shortage of cutting-edge instrumentation restricts the research lines that scientists can develop in their laboratories. Expensive and lengthy servicing, frequently with unavailable stock of parts for repair of instruments, regardless if domestic or imported, are also chronic problems that persist and are aggravated in remote locations.

Nominal citations of instrumentation from domestic manufacturers seem to be concentrated in publications of scientific research performed in Brazil. How do the figures compare with imported equipment here and abroad?

By searching through publications with Google Scholar for citations of six Brazilian manufacturers of instrumentation, all well known and with distinctive names, one can retrieve nearly 10 thousand citations over the past 10 years in the experimental sections of the publications, the vast majority of them, indeed, in studies performed in Brazil. This approach somewhat underestimates the use researchers make of basic lab equipment made in Brazil, like stirrers, ovens, centrifuges, etc., because brands and models are customarily cited only if supposed to be relevant for the replication of published results by others. A similar search, now for six of the international producers of analytical instruments widely marketed in Brazil, results in about 380 thousand citations in the last decade, and Brazilians are among the authors of about 14% of them. These rough estimates suggest that instrumentation from Brazilian manufacturers plays a secondary role in most domestic research laboratories and is irrelevant abroad.

By visiting exhibitions of laboratory equipment and instrumentation, like Analitica Latin America in Brazil and the Pittsburgh Conference in the United States, the impression would not be different: in the first, instrumentation manufactured abroad predominates while products made in Brazil are mainly basic lab items and relatively simple analytical instruments; in the second, Brazilian analytical instrumentation, if any, is imperceptible among exhibitors from 28 countries.

The participation of Brazilian scientists in articles involving analytical instrumentation has grown in the last 10 years. However, it is still very low (about 3%). Is this due to low access to instrumentation/equipment here?

The Brazilian scientific production, evaluated by the number of indexed publications with at least one coauthor from a Brazilian institution, is close to 3% of the annual global production, the 14th largest one. Irrelevant until de 1970s, it has grown at high rates of about 10% per year since 1990, losing acceleration again in recent years. It is noteworthy, in parentheses, that our share of the world population is also 3%. The impact of the science made by Brazilians is however lower in terms of citations of the papers by other scientists. A closely similar growth trend happened with papers in chemistry as a whole, analytical chemistry in general and creation and development of analytical instrumentation in particular, all currently rounding up to 3% in the concert of nations. Since analytical chemistry follows the median scientific activity in Brazil, it is difficult to guess to what extent greater access to latest release instrumentation/equipment would improve the quality, quantity and the impact of the publications. However, in

> “Circumstances have led some researchers to upgrade old-fashioned analog instruments, especially those with excellent optics, by interfacing them with microprocessors or microcomputers. As a positive effect, the adversity period of market reserve induced the onset of groups aimed at creating and developing original analytical instrumentation.”

order to eliminate any "excuses" about this, it would be wonderful if the availability of analytical instrumentation could be as universal as the current access to the literature through the CAPES Portal, SciElo, etc.

Why are there little innovation and few Brazilian equipment prototypes? Is this a reflection of a timid scientific production compared to other countries?

I don't think that it is simply a reflection because our innovation capacity in terms of patents granted or new commercial products is very much lower in the concert of nations than our share of nearly 3% of the scientific production. It seems more that the emergent community devoted to science and technology in Brazil is still not large and qualified enough to do it all. The community has just learned how to generate lots of papers – mostly low impact incremental research and not many breakthroughs – and researchers are now being oriented to look at how to make technology and profit from research too. In other words, the introduction of a culture eager to generate innovations, prototypes and patents is relatively recent in universities, research institutes and domestic companies and not welcome by all academic researchers, afraid of brain draining from the still weak basic science frontier research. Specific financing is available, for example, from FINEP (Financiadora de Estudos e Projetos), Sectorial Funds for Science and Technology and programs such as PIPE- and PITE-FAPESP (programs for innovative research with university-industry cooperation co-financed by Fundação de Amparo à Pesquisa do Estado de São Paulo), or directly by companies like Petrobras. A law regulated in 2006 provides advantageous tax incentives for businesses that invest in innovative products and processes, aiming competitive advantages. A hundred National Institutes of Science and Technology were created in 2008 – including one in advanced analytical technologies and one in bioanalytics – and are expected to increment national cutting-edge science and technology. All these and other positive initiatives seem not to be sufficient yet or need a longer maturation. A look at the Global Innovation Index, who takes in account a broad matrix of influencing factors in addition to science output, reveals that other countries are improving faster, pushing Brazil from the 47th position on the ranking in 2011 to the 58th in 2012 and the 64th in 2013.

Answering the same question by focusing analytical instrumentation, there are interesting prototypes described every two years at the ENQAs, although in small number, like new electrochemical and photometric instruments and sensors, flow and microfluidic analytical devices and systems, etc. I would like to exemplify with three prototypes mentioned at the Analytical Instrumentation Workshop, during the 35º Annual Meeting of SBQ (Sociedade Brasileira de Química), all complete with hardware, software and real-world testing: a capillary electrophoresis equipment with contactless conductivity detection, from Claudimir do Lago's group at IQ-USP (Instituto de Química da Universidade de São Paulo), a near infrared spectrometer for at-process multicomponent monitoring in oil refineries, from Célio Pasquini´s group at IQ-Unicamp (Instituto de Química da Universidade Estadual de Campinas), and a patented electrochemical food additives flow analyzer from our group. A more complex prototype also developed by do Lago's group aimed for military use abroad, did also attract attention of local security force representatives looking for the World Cup or the Olympic Games. It is a motorized multiprocessor radio controlled robotic platform for remote chemical analysis integrating chemical sensing, microchips, capillary electrophoresis, and electrochemical detection.

What is the peculiarity of the instrumentation market oriented to analytical chemistry, compared to other markets, such as equipment for the health?

Brazil only has manufacturers of basic lab equipments and instrumentation with medium to high market demand and low or low-medium technology, like spectrophotometers and potentiometers and their sensors; the manufacture of chromatographs has ceased but certain chromato-

> **"Instrumentation from Brazilian manufacturers plays a secondary role in most domestic research laboratories and is irrelevant abroad."**

> **“It seems more that the emergent community devoted to science and technology in Brazil is still not large and qualified enough to do it all. The community has just learned how to generate lots of papers – mostly low impact incremental research and not many breakthroughs – and researchers are now being oriented to look at how to make technology and profit from research too.”**

graphic columns are in production. The development of medium to high technology instruments with patent protection or the acquisition of the know-how with payment of royalties is an extra barrier for domestic companies, but for corporations owning the technology abroad and with an established support and marketing network in Brazil, the creation of a supply chain for local production could be economically justifiable considering continuity of the existing high import taxes, although exportation would probably not be competitive. I guess that economies of scale could be acceptable, for example, for instruments like GC (gas chromatography) and HPLC (high-performance liquid chromatography), fast diode array or CCD (charge-coupled device) spectrophotometers and detectors, maybe for FTIR (Fourier transform infrared spectrophotometers). For equipment with higher technology and lower demand, e.g, nuclear magnetic resonance (NMR) and mass spectrometers, chances of a profitable local manufacture are lower but in the health business, where there is more investment and some items are sold in lots of many units, we have seen the recent start of manufacture of equipment for tomography and magnetic resonance imaging by more than one multinational company.

Instrumentation made in Brazil with competitive quality and price in comparison with taxed imports is attractive and frequently preferred by customers in general due to extra facilities such as legal compliance and appropriateness to local conditions and needs, lesser influence of currency fluctuations during acquisition, local training of staff, easier calibration, simpler acquisition of consumables, parts and accessories, faster maintenance and lower overall costs.

Regarding the competition, there is an ongoing international movement of acquisition and concentration of established instrumentation brands by a handful of multinational corporations, the components supply sources have moved to Asiatic countries, when not manufactured subsystems and even complete instruments. Chinese companies learned faster than Brazilian ones and although not yet leaders in innovation and quality standards, their own analytical instrumentation is now becoming available around the globe, including Brazil, with unbeatable prices.

How are the Brazilian analytical instrumentation companies evolving?

Good times of market reserve for the national analytical instrumentation companies were gone in 1992 with the liberation of imports, but most of them survived the transition and some new ones were founded because the high tariff barrier applied on imports still protects domestic manufacturers. Regrettably, one of the companies that closed doors was just the one that pioneered and dominated the manufacture of gas chromatographs, somewhat more complex systems than other analytical equipment produced here. Most companies evolved too slowly in their technology and products portfolio, compared to leading international manufacturers, some started to sell imported equipment under their own brand and eventually gave up local manufacturing, becoming service and reseller companies of instrumentation made abroad.

Despite the growing number of research laboratories in analytical chemistry with creative inventions and prototypes with potential like the examples I have already mentioned, by faults from both sides, few domestic companies interact effectively with universities aiming to convert prototypes into commercial products, although cases of success do exist. There is always a risky path, from what the academics considers a prototype of great potential to its transformation into a profitable commercial product by the private sector. It seems to be particularly difficult in the field of analytical instrumentation for reasons like cost, local demand, production scale, supply chain and other factors already mentioned, as well as concerns about the acceptance of new techniques and methods (welcome by the academy) in the larger but highly regulated and standardized markets of quality control laboratories like chemical, petrochemical and pharmaceutical industries, clinical and environmental analysis, thus inhibiting investments.

Noteworthy (although not common) is that one medium sized company which expanded its R&D team to more than 15 masters, engineers and technicians in different specialties, led by a PhD. By the way, the leader who undertook research for his thesis in analytical instrumentation and electroanalytical flow methods in our laboratory. Such teams may absorb part of the revenues of the company, but probably provide the best approach to expand the market share, not only by simply broadening the portfolio of common analytical instruments, frequently produced abroad at lower cost, but especially by introducing custom-made instruments for quite specific market niches, undetected or uninteresting for big corporations. These teams can also develop new alternatives or design full analytical systems for specific types of industries aiming at increased productivity, reliability and/or robustness, unattended operation, lower consumption of reagents and samples, lesser maintenance etc. Multinational corporations are obviously also aware of the effectiveness of selling solutions for analysis problems instead of instruments and, regardless of their intentions to produce or not certain goods in Brazil, some are strengthening their local support teams by absorbing an increasing number of masters and even PhDs in analytical chemistry, capable of supporting the marketing area properly in advising the customers about the best available choices and in developing solutions as needed.

After the development of a new product, by companies or by universities, what are the barriers to the generation of a new patent in Brazil?

We are very far from competing nations when the issue is patents of inventions, and I would like to exemplify with the Republic of Korea, a country with a quarter of our population that, in the 1970s, was nearly as irrelevant as Brazil in science and technology. In 2011, 72 thousand patents were granted to residents in Republic of Korea, with a predominance of high demand and/or high technology sectors. The number of patents granted to residents in Brazil in the same year of 2011 by the National Institute of Industrial Property (INPI) was 380. Yes, only three hundred and eighty patents of inventions, about 10 times less than the patents granted by INPI to non-residents.

The culture of generating innovative technology and protecting the resulting intellectual property with patents is still incipient among Brazilian scientists and entrepreneurs, although it is being promoted as I mentioned in a previous answer, but there is a long way to go. The process of filing patents in Brazil and in major world markets is expensive, slow, and laborious even through the Patent Cooperation Treaty, and it demands experts in the subject of the patent and the law of each country. Most Brazilian scientists and graduate students have read few patents, if any, and are not familiar with the preparation regulations. Few universities in Brazil are ready to assist their scientists efficiently in these matters, but this is gradually improving. However, issuing patents is an advisable starting point but far from sufficient to profitably manufacture a product, be it an analytical instrument or anything else.

Can the evolution of industrial automation courses (technologists and engineers) help in the development of patents in the area of analytical chemistry in Brazil?

The proportion of Brazilians with higher education is still small and oriented mainly to carriers in humanities. We have plenty of lawyers, shortage of engineers and too few scientists. Greater availability of experts in industrial automation is thus highly welcome. Positive synergy of the few PhDs in chemistry specialized in instrumentation with technologists and engineers is a way to foster innovation leading to patents and steps ahead, like the development of viable prototypes, their production and integration in larger systems with software adapted to the peculiarities of the customers, e.g., industries aiming to a "greener" production of chemicals or water and effluent treatment stations, where online analytical data can provide direct feedback for the optimization of the plants.

> **“Good times of market reserve for the national analytical instrumentation companies were gone in 1990 with the liberation of imports, but most of them survived the transition and some new ones were founded because the high tariff barrier applied on imports still protects domestic manufacturers.”**

> “Feel both pleased and rewarded with the extensive utilization achieved by CurTiPot, originally aimed for students and teachers, but now also widely used in research and routine analysis at universities and in chemical and pharmaceutical companies.”

In your presentation at the Workshop on Instrumentation, during the 35th RASBQ, you spoke about the development of the market of equipment in analytical instrumentation. How is the development of software to support this equipment and for analytical laboratories in general?

Software programmers experienced in team work are much needed during the creation of new instruments as well as after a prototype is transferred from the university to a company, to render the software functional, stable, friendly and intuitive for the final user. The software is typically tailored to the hardware of an analytical instrument with purposes of control, calibration, data acquisition and communication via USB or IP protocol. A quite variable package of standard and optional resources for data analysis and automation of operation is also embedded in the software. It is a challenging and unattractive task to modify or extend a more or less “black box” proprietary software of a commercial instrument without access to project details and documented software.

There are more opportunities in developing software to post process the data exported by one or various instruments providing complementary information in an external computer or network to assists in the interpretation and decision making. Some commercial LIMS (laboratory information management systems) softwares already combine information management, data mining, laboratory management, accounting, etc. LIMS eventually designed for large petrochemical or pharmaceutical industries are not optimized for all requirements that change from one lab to another, and they evolve with time. Due to the aforementioned difficulties in producing new instruments in Brazil, it may be more attractive to young entrepreneurs launch a startup focused on customized software, for example: specific niches not yet adequately addressed by available LIMS; deeper chemometric analysis of multidimensional data; simulation and planning of experiments; automation of specific instrumental analytical laboratories; better compliance with local legislation; faster decision feedback to production processes and environmental control.

Talk about the history of the development of CurTiPot; tell us why you decided to make it a freeware and what were the results of the online publication.

The original motivation to write CurTiPot was to assist students in understanding titrations of strong and weak acids and bases, including multiprotic systems and mixtures, and enabling them to perform virtual potentiometric titration on computers, after or before a real titration. Graphics of species distribution along the titration helped to reach the objective, and the random error simulation added realism to the curves, allowing evaluation of the effect of error sources. For superior data analysis of real and simulated curves, I added a cubic splines filter with smoothing and interpolation. The program was launched during my short course on “Chemometrics in Analytical Chemistry” in 1992, incidentally, the first course to be offered in an Annual Meeting of the SBQ (Brazilian Society of Chemistry). To amplify the educational outreach of CurTiPot I opted for the free distribution of the software over the Internet.

In 2006, I released the 3.0 version of CurTiPot running on Microsoft Excel with macros and, for the first time, also in English. Annual improvements included extra modules for pH calculation of any mixture, correction of the effect of ionic strength, pKa determination and analysis of difficult titration curves by nonlinear regression, generation of buffer capacity curves, pKa database, etc. The CurTiPot download site maintained by me received 700 thousand visits in 7 years, but this is just one of the 150 software sites that distribute the freeware. It has been downloaded in over 130 countries and applications have been described in 60 published papers and thesis indexed by Google Scholar. I feel both pleased and rewarded with the extensive utilization achieved by CurTiPot, originally aimed for students and teachers, but now also widely used in research and routine analysis at universities and in chemical and pharmaceutical companies.

Amostrador Automático de Headspace NOVO TriPlus 300

A Thermo Fisher Scientific, líder mundial a serviço da ciência, acaba de lançar o TriPlus 300, um sistema headspace de extração/injeção à base de válvula e loop, com bandeja para 120 vials e forno de incubação para 18 vials, que proporciona alta produtividade na determinação de compostos orgânicos voláteis.

As especificações técnicas do TriPlus 300 atendem integralmente as exigências dos métodos por GC e GC-MS dos laboratórios farmacêuticos, forenses e ambientais, tornando esse sistema de headspace o instrumento preferido para as determinações automáticas e confiáveis de compostos orgânicos voláteis.

TriPlus 300 Headspace autosampler oferece um sistema para economia de energia incorporado ao equipamento para operações eficientes e economia de recursos.

analítica
analiticaweb.com.br

Thermo
SCIENTIFIC

Correlation between chemical oxygen demand (COD) and the color of the wastewater in a cellulose industry

Carla F. Campos Ferreira[A], **Evandro S. dos Santos**[B], **Suzana T. Amaral**[A], **Emilse M. A. Martini**[A*]
A) Chemistry Institute, Federal University of Rio Grande do Sul, Av. Bento Gonçalves 9500, P.O.B. 15003, 91501-970, Porto Alegre, Brazil
B) CPMC, Rua São Geraldo 1680, 92500-000, Guaiba, Brazil

***Corresponding author:**
Phone: 55 51 3308 6274
Fax: 55 51 33087304
E-mail address:
emilse@iq.ufrgs.br

Abstract

Chemical oxygen demand (COD) analysis is widely employed in wastewater treatment stations in order to measure the amount of organic compounds in water and also to evaluate indirectly the pollution level of the generated aqueous waste to assign additional protocols before discharge, if necessary, to meet legal limits. This paper proposes a color determination method for evaluating the pollution level of waste generated by a cellulose industry as a replacement of COD analysis to reduce cost and laboratory waste. The samples were collected from three critical points in the treatment plant, and the waste color was determined colorimetrically. COD analysis was also performed, and a correlation between the two methods was verified. The results obtained showed that the correlation was satisfactory and that the color analysis developed is a feasible method for wastewater monitoring.

Keywords: cellulose waste; chemical oxygen demand (COD), photocolorimetry

1. Introduction

In environmental chemistry, the chemical oxygen demand (COD) test is commonly used to indirectly measure the amount of organic compounds in water. Most applications of COD determine the amount of organic pollutants found in surface water (e.g., lakes and rivers) or wastewater, making COD a useful measure of water quality. COD is expressed in milligrams per litre (mg L^{-1}), which indicates the mass of oxygen consumed per litre of solution [1]. The basis for the COD test is that nearly all organic compounds can be fully oxidised to carbon dioxide with a strong oxidising agent under acidic conditions [1-4]. Much work has gone into establishing a relationship between COD and specific classes of organic compounds [2-4].

Many governments have imposed strict regulations on the maximum chemical oxygen demand allowed in wastewater before it can be returned to the environment [5]. Therefore, industries are now attempting to reduce their environmental impact to comply with increasingly severe environmental regulations and to satisfy requirements for environmentally sound product manufacturing. Those industries that have successfully reduced their waste impact are now observing increased value for their products [6]. The regulations which establish the maximum COD allowed in an effluent before its disposal take into consideration the type of business activity, the degree of impact generated, the effluent flow, among other factors. In Rio Grande do Sul state, a cellulose industry with daily output of 1300 tons / day may dispose of up to 46,400 m^3/day of treated effluent with maximum COD of 145 mg O_2 L^{-1}.

The pulp and paper industries represent an important sector of the Rio Grande do Sul economy, but they also use large amounts of water during manufacturing [7,8] and generate industrial wastes characterised by high COD and color.

Chemical oxygen demand is one of the most popular methods for the evaluation of industrial wastewaters [9] from pulp and paper industries, but this method suffers from low throughput.

The cellulose production process is essentially the transformation of wood into a fibrous suspension named cellulosic pulp obtained through lignin dissolution. This process is achieved through the steps of wood selection, infusion or digestion, washing and depuration, oxygen delignification, whitening, drying and packing [10]. Methanol and reduced sulphur compounds are the main sources of water pollution during digestion and are rich in organic matter. These pollutants come from digesters, evaporators and recovery units [11]. The more polluted wastes are produced during the pulp whitening step. During this step, organochlorine compounds are also produced due to the use of whitening agents containing chlorine. Either these organochlorine compounds or the lignin fragments extracted from the pulp are responsible for the high levels of organic matter. Unfortunately, this type of waste is currently unmanageable [11].

Several parameters can be measured to ascertain the pollution level of a specific waste [5,12]. One of the most common techniques used to evaluate COD is the Close Reflux Titrimetric Method (SM 21th 5220-C). This method consists of hot oxidation using potassium dichromate ($K_2Cr_2O_7$) mixed with sulphuric acid (H_2SO_4) and silver sulphate (Ag_2SO_4), resulting in a powerful oxidant solution. The silver sulphate acts as a catalyst, allowing for effective oxidation of organic compounds. This method is successful because the COD is a measure of the amount of a strong oxidant, expressed as the oxygen necessary to oxidise organic or inorganic matter present in the wastewater. The wastewater generated by cellulose production contains a considerable amount of chloride (Cl^-). The chloride ion may be oxidised to gaseous chlorine (Cl_2) during analysis, generating COD. This ion can also react with the catalyst, precipitating as silver chloride (AgCl) and reducing catalyst loading. The interference caused by chloride ions may be minimised by the addition of mercury (II) sulphate ($HgSO_4$), which precipitates the chloride as mercury (II) chloride ($HgCl_2$), which is soluble in the acidic medium. Mercury (II) sulphate is thus used in the potassium dichromate ($K_2Cr_2O_7$) digestion solution.

The sample to be analyzed is submitted to digestion with a strong acid and an excess of potassium dichromate ($K_2Cr_2O_7$). After digestion, the potassium dichromate that was not reduced is titrated with an ammonium iron (II) sulphate solution and the amount of potassium dichromate consumed is determined. The level of oxidisable organic matter, obtained from the difference between the dichromate added and that remaining, is calculated as equivalents of oxygen. In an industrial setting, this analysis requires more than three hours and represents an impediment to necessary corrective action. Many studies have sought to reduce analysis times to avoid extended periods of wastewater retention [13-15]. Besides the time required for the analysis, many chemical reagents are needed, increasing the costs and generating toxic laboratory chemical residues.

Water color may be real (or genuine), observed after the removal of suspended species, which cause turbidity, or apparent color. The color is determined using spectrophotometry by comparing the sample with standard solutions having known concentrations of potassium chloroplatinate (K_2PtCl_6) between 450 and 465 nm. The color developed by a solution containing 1 mg L^{-1} of potassium chloroplatinate corresponds to one unit of color (UC).

The aim of this study is to propose an alternative method of analysis of effluents of the cellulose industry before disposal, where the COD analysis can be replaced with color analysis. The proposed method can be performed in a short time, with a faster response relevant to industrial processing. In addition, the cost is lower as well, as it generates less toxic chemicals than the currently employed analytical methods.

2. Materials and methods

The samples selected for this study were obtained from the wastewaters generated during the manufacturing of whitened cellulose. These samples were collected from three different points in a wastewater treatment unit. Analysis of COD, pH and determination of the real and apparent color of the samples was performed according to the methodology standardised in Standard Methods for the Examination of Water and Wastewater [16]. Plots of COD versus color were used to verify the correlation between these parameters.

The samples were named A, B, and C according to the point where they were collected in the treatment unit. Ten samples were collected at each point (A, B and C) in 500 mL plastic flasks with lid. Collection was always made at the same time, once a day. The analyses were carried out on the same day as collection.

Samples A: collected at the step immediately prior to the unox reactor entrance (closed aeration reactor where the effluent is subjected to biological activity). The wastewater was homogenised within the neutralisation tank after pH adjustment. The processed wastewater is first submitted to a primary treatment and passed through the stabilisation pool to incorporate oxygen and lower its temperature before entering the unox reactor. At this point, the wastewater has not yet been submitted to biological treatment.

Samples B: collected at the point where water is transferred from the secondary clarifier. At this point, the wastewater has already passed through pre-treatment, primary treatment and biological treatment.

Samples C: collected at the point where the water is transferred from the tertiary clarifier. At this point, the wastewater has already passed through the entire treatment and has been treated with the coagulating agent aluminium sulphate ($Al_2(SO_4)_3$). The wastewater treatment is finished and thus the water is ready to be discarded.

COD was analyzed by the method of closed reflux digestion, according to SM 21th5220-C, in an Oxylab model N1100 digestion block using a Metrohm model 765 automatic titrator, and the results are expressed in mg O_2 L^{-1}. The pH measurements were performed with a Mettler Toledo model Seven Easy pH meter, according SM21th4500-H^+ A and B. The sample color was determined using a Metrohm model 662 photocolorimeter, according SM 21th 2120, and the results are expressed in mg Pt Co L^{-1}. The sample pH values were measured because, according to the color determination methodology, the sample pH must be within the range 4 to 10. The sample pH must be adjusted to 7 with acidic or basic solution if the value is outside this range.

For the determination of COD, 10 mL of sample were mixed with 20 mL of solution containing $K_2Cr_2O_7$, Ag_2SO_4 and H_2SO_4 in a digestion tube. After homogenisation, the tubes were placed in the digestion block for two hours at 150°C ± 2ºC. After digestion, the samples were cooled to room temperature and transferred to a glass beaker. The digestion tubes were then washed with 100 mL of demineralised water. The samples were titrated with a 0.1 mol L^{-1} solution of ammonium iron (II) sulphate. A blank test (BT) was also included in the experiments using the same amount of reagents.

The COD results were calculated according to equation 1:

$$COD = \frac{(B\text{-}A) \times M \times 8000 \times F}{Vol} \quad (1)$$

where:
COD = chemical oxygen demand, expressed as mg O_2 L^{-1};
A = volume of ammonium iron (II) sulphate used in the BT titration, in mL;
B = volume of ammonium iron (II) sulphate used in the sample titration, in mL;
M = molar concentration of ammonium iron (II) sulphate;
Vol = sample volume, in mL;
F = dilution factor of the sample.

This procedure is applied when the COD values are greater than 40 mg O_2 L^{-1}. The factor 8000 is associated with the unit change in oxygen mass from grams to milligrams, and the number of transferred electrons (8) in the redox process.

For the color determination, the photocolorimeter was turned on 15 minutes prior to the experiments to stabilise the optical components. The wavelength selected was 410 nm. The zero was adjusted by filling the cuvette with demineralised water and adjusting to 100% transmittance. The sample was then transferred to the cuvette, and the transmittance was recorded. If the obtained value exceeded the detection limit of the photocolorimeter, the sample was diluted. The dilution must be considered in any color calculations. The results were calculated using equation 2:

$$C = \%T \times F \quad (2)$$

where:
C = real color or apparent color of the sample, in mg Pt Co L^{-1};
%T = transmittance obtained from the photocolorimeter;
F = calibration curve factor = dilution factor of the sample.

The analytical curve for color was built using six dilutions of the matrix solution of platinum-cobalt. The calibration curve equation obtained is shown in equation 3:

$$Color = 0.0679\,x^2 - 17.241\,x + 1049.5 \quad (R^2 = 0.9991) \quad (3)$$

The apparent color was measured directly without any treatment of the samples. For real color measurements, the samples were filtered using qualitative filter paper, and the pH and color of the filtrate were measured.

3. Results

The plots obtained for samples collected at point A of the effluent treatment, named "samples A", are shown in Figures 1a and 1b, respectively. The pH of samples A presented an average value of 7.4 ± 0.3 for the filtered samples and 7.4 ± 0.2 for unfiltered samples.

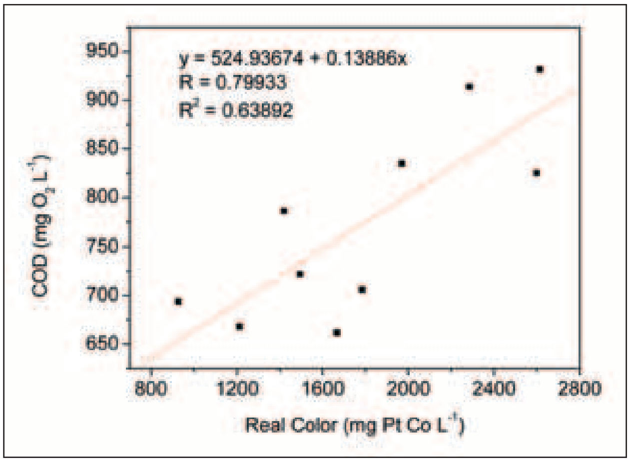


Figure 1a. COD x real color for samples A (pH_{medium} = 7.4 ± 0.3).

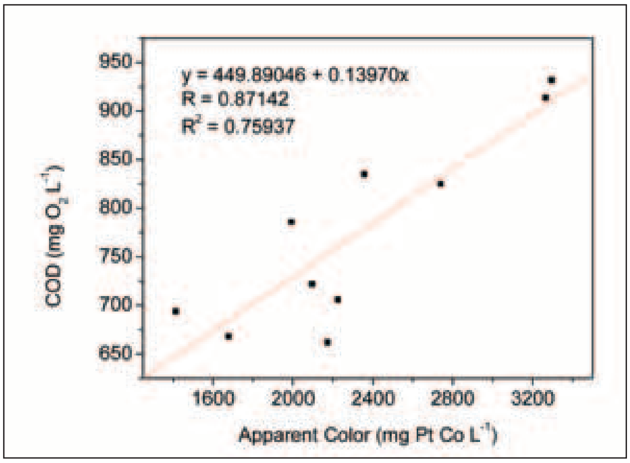


Figure 1b. COD x apparent color for samples A (pH_{medium} = 7.4 ± 0.2).

The plots of COD versus real color and apparent color were built for the samples collected at point B of the effluent treatment. These plots are shown in Figures 2a and 2b, respectively. The pH of samples B presented an average value of 7.0 ± 0.3 for the filtered sample and 6.9 ± 0.2 for the unfiltered sample.

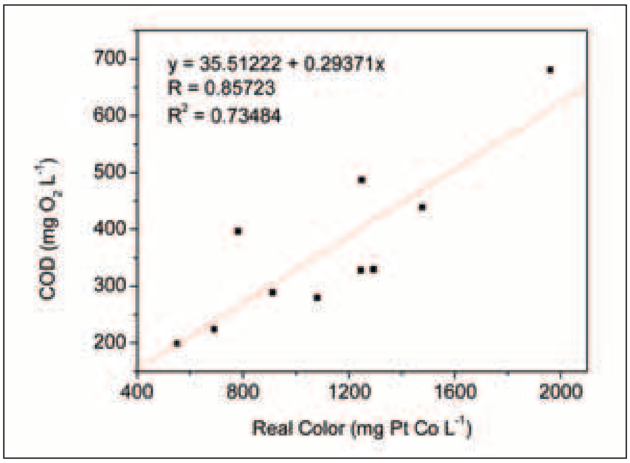


Figure 2a. COD x real color for samples B (pH_{medium} = 7.0 ± 0.3).

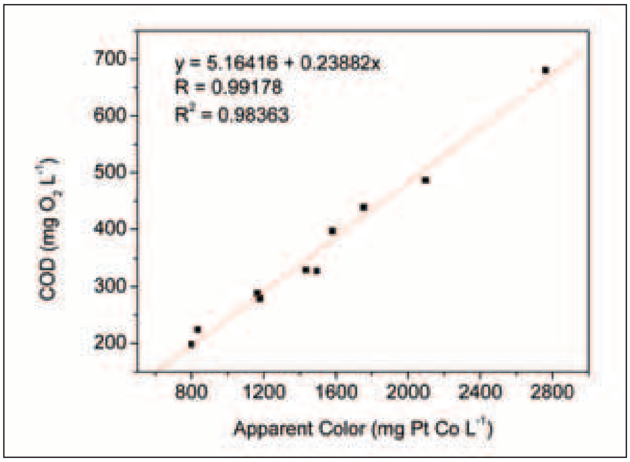


Figure 2b. COD x apparent color for samples B (pH$_{medium}$ = 6.9 ± 0.2).

The plots of COD versus real color and apparent color were built for the samples collected at point C of the effluent treatment. These plots are shown in Figures 3a and 3b, respectively. The pH of samples C presented an average value of 6.5 ± 0.3 for the filtered samples and 6.9 ± 0.2 for the unfiltered samples.

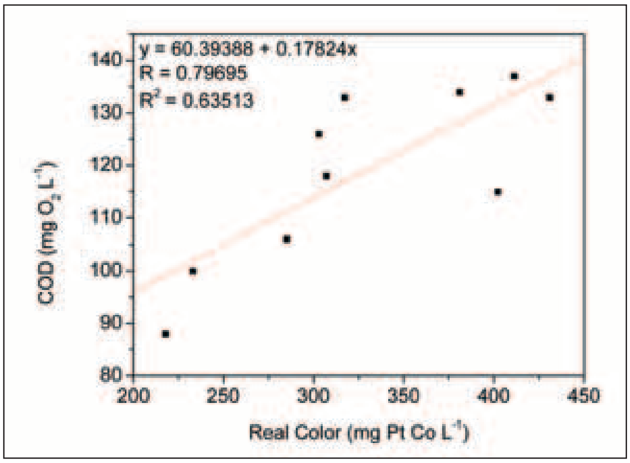


Figure 3a. COD x real color for samples C (pH$_{medium}$ = 6.5 ± 0.3).

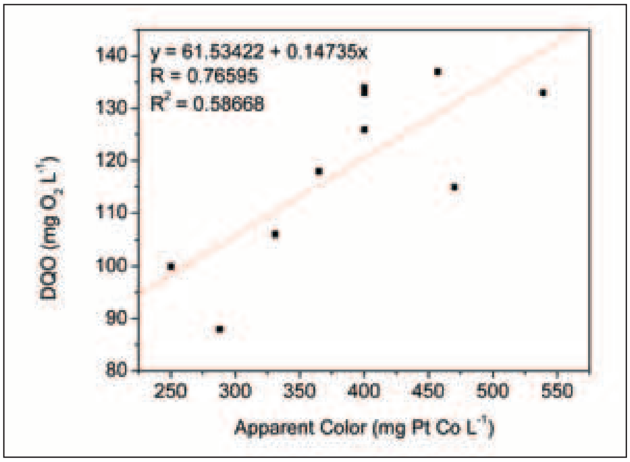


Figure 3b. COD x apparent color for samples C (pH$_{medium}$ = 6.5 ± 0.2).

4 - Discussion

The replacement of COD analysis with color analysis is possible only if a correlation between these parameters is verified. In other words, it is necessary to verify that changes in one variable affect related variables. For this reason, the data were evaluated by the correlation coefficient (R), which indicates how much two studied variables are linearly correlated, and the determination coefficient (R^2), which indicates the percentage of variance of one of the variables explained by another variable.

According to the results (Figures 1a and 1b), the best correlation for samples A was observed for COD and apparent color of the wastewater. This result was expected because the sample is filtered for real color determination, removing a large amount of organic matter. It is known that lignin primarily contributes to the color of wastewater from the cellulose industry, and thus, removing it decreases color intensity. The decreased intensity was not accompanied by a lower COD value because this analysis was performed on an unfiltered sample.

According to the literature [17], a correlation coefficient between $0.5 \leq R < 0.8$ is moderate and between $0.8 \leq R < 1$ is strong. The R determined for samples A was 0.871, and the R^2 value was 0.759, meaning 76% of the COD might have been predicted by the apparent color analysis of this sample.

For samples B, the best correlation was also with apparent color for the same reasons as samples A. An excellent correlation coefficient of 0.992 was obtained, indicating a strong correlation between the variables, and the determination coefficient indicated that approximately 99% of the COD results might have been predicted from the apparent color of samples B.

In the case of samples C, a slightly better correlation was obtained for COD and real color. The aluminium sulphate likely promoted coagulation and separation of particulate matter, making real and apparent samples similar in terms of color. The R determined was 0.797, and the R^2 was 0.635, indicating that only 64% of the COD results might have been predicted by the color.

For the other two collection points, a good correlation is an excellent result. This was particularly true for samples B, the point at which the water is transferred from the secondary clarifier, because at this point, something can still be performed to lower the COD. Improving the quality of the wastewater is possible by increasing the dose of coagulant at the next stage of treatment (tertiary treatment) or by reducing the amount of time needed to measure COD. It is also possible to divert the effluent to an emergency lagoon and gradually treat it to reduce the impact of a highly polluted load on the treatment facility.

Considering that the results of COD analysis are used for monitoring wastewater treatment, it is possible to state that all the evaluated samples presented satisfactory correlations. As a result, the chemical oxygen demand (COD) could be predicted from the color of the wastewater evaluated in this study.

5 – Conclusion

This work proposes an alternative method to analyze the effluent from a pulp industry. The method currently used is the determination of chemical oxygen demand, which is a time-consuming method that generates toxic chemicals. The effluent color was evaluated and correlated with the COD value. The correlation coefficients for the straight lines obtained from the experimental data indicated that the reported color evaluation method was appropriate to quantify chemical oxygen demand in real wastewater samples. The inherent advantages of this methodology should also be considered, including the elimination of mercury and toxic reagents, as well as the reduction in time required for the analysis from three hours to 30 minutes. Finally, it is possible to conclude that the color analysis proposed could be an alternative method which is acceptable, easy and fast and also very advantageous. It is now possible to implement monitoring directly in wastewater treatment facilities using an electronic spreadsheet that calculates the COD automatically from the measured color.

6 – References

1. Sawyer, C.N.; McCarty, P.L.; Parking, G.F.; *Chemistry for Environmental Engineering and Science*, 5th ed.; McGraww Hill: New York, 2003.
2. Baker, J.R.; Milke, M.W.; Mihelcic, J.R.. Water Res. 1999, 33, 327.
3. Vogel, F.; Harf, J.; Hug, A.; Rohr, P.R.. Water Res. 2000, 34, 2689.
4. Kylefors, K.; Ecke, H.; Lagerkvist, A.. Water, Air Soil Pollut. 2003, 146,153.
5. Clerescerl, L.S.; Greenberg, A.E.; Eaton, A.D.; *Standard Methods for Examination of Water and Wastewater*, 20th ed.; American Public Association: Washington DC, 2000.
6. Freire, R.S.; Pereira, W.S.. Quím. Nova 2005, 28,130.
7. Freire, R.S.; Pelegrini, R.; Kubota, L.T.; Durán, N.. Quim. Nova 2000, 23, 504.
8. Zamora, P.P.; Esposito, E.; Reyes, J.; Durán, N.. Quim. Nova 1997, 20, 186.
9. Pereira, R.S.. Rev. Eletrôn. de Recur. Hídr., 2004,1, 20. http://www.abrh.org.br/informacoes/rerh.pdf, accessed June 2012.
10. Lima, A.F.; *Processamento da Pasta Celulósica – Tecnologia de Fabricação da Pasta Celulósica – IPT*, 2th ed.; Instituto de Pesquisas Tecnológicas do Estado de São Paulo: São Paulo, 1988.
11. Silva, T.C.F.; *Processos oxidativos avançados para tratamento de efluentes de indústria de celulose Kraft branqueada,* Universidade Federal de Viçosa: Viçosa, 2007.
12. Bertazzoli, R; Pelegrini, R.. Quim. Nova 2001, 25, 477.
13. Pisarevsky, A.M.; Polozova, I.P.; Hockridge, P.M.. Russ. J. Appl. Chem. 2005, 78, 101.
14. Zheng Q.; Zhou, B.; Bai, J.; Li, L; Jin, Z; Zhang, J; Li, J.; Liu, Y; Cai, W.; Zhu, X.. Adv. Mater., 2008, 20, 1044.
15. Zhao, H.. Anal. Chem. 2004, 76, 155.
16. APHA/AWWA, *Standard Methods for the Examination of Water and Wastewater,* 21th ed.; American Public Health Association: Washington, 2005.
17. Santos, C.; *Estatística Descritiva – Manual de Auto-aprendizagem*, Edições Sílabo: Lisboa, 2007.

Spectrophotometric determination of platinum(IV) through the O-methylphenyl thiourea and iodide ternary complex after liquid-liquid extraction

Shashikant R. Kuchekar[A*], **Yogesh S. Shelar**[A], **Sung H. Han** [B]

A) *Analytical Chemistry laboratory, Department of Chemistry, Arts Commerce Science College, Satral 413711, Rahuri, Ahmednagar, Maharashtra state, India*

B) *Inborganic Nanomaterial Laboratory, Department of Chemistry, Hanyang University, Seoul 133-791, South Korea*

Abstract

A spectrophotometric method for the determination of platinum(IV) was developed using *o*-methylphenyl thiourea (OMPT). Platinum(IV) was determined as its ternary complex 1:1:2 (platinum(IV):OMPT:iodide) that is a yellow colored complex after liquid-liquid extraction from aqueous potassium iodide media (0.1 mol L^{-1}) using 4.5×10^{-3} mol L^{-1} OMPT in 10 mL chloroform and is measured at 362 nm. Beer's law was obeyed in the concentration range up to 15 µg mL^{-1}, with molar absorptivity and Sandell's sensitivity values of 1.25×10^{4} L mol^{-1} cm^{-1}and 0.016 µg cm^{-2} respectively. Repeatability of the method in terms of R.S.D was 0.34% (n=10). The stiochiometry of the platinum(IV)-OMPT-iodide complex was determined by the slope ratio method and verified by mole and Job's continuous variation methods. The complex was stable for more than 48 h. The influence of various factors such as potassium iodide and OMPT concentrations as well as the extraction solvent and the time of equilibration have been established. The interference potential of a number of different ions was evaluated and the use of masking agents is proposed. The developed method was successfully employed for the determination of platinum(IV) from binary synthetic mixtures, multicomponent synthetic mixtures, Adam's catalyst and pharmaceutical samples. The reliability of the method was assured by inter-comparison of experimental values with those obtained using atomic absorption spectrometry. A liquid-liquid extraction scheme is proposed for the separation of platinum(IV), palladium(II) and nickel(II) together.

***Corresponding author**
E-mail address:
shashi17@gmail.com

Keywords: Spectrophotometry, liquid-liquid extraction, platinum(IV), *o*-mehtylphenyl thiourea, ternary complex.

1. Introduction

Platinum is a rare and expensive metal widely used as a catalyst with abundance of 0.01 ppm in earth's crust [1]. It is the most important element from platinum group metals due to its applications in jewelry, catalysis, chemical, pharmaceutical, petroleum and electronic industries. Platinum is widely used in automotive catalytic converters. Nowadays the presence of platinum in the environment and its effects are widely discussed. A review of the effects of platinum and other platinum group metals on the environment is given by Jackowska and coworkers [2]. Articles are published on use of platinum in catalytic converters and their effect on the enviroment [3,4]. Platinum has major application for its use in anticancer drugs. A general approach of platinum complexes as anticancer agents is given Kostova [5]. The route of platinum containing drugs right from discovery is given by C. Monnert [6]. Many research papers are published with use of platinum in anticancer drugs [7-10]. Owing to its trace abundance and the wide variety of applications a large number of methods are reported for determination of platinum. Various instrumental techniques are reported for determination of platinum using flow injection and chemiluminescence [11], total reflection X-ray fluorescence spectrometry [12], graphite furnace atomic absorption spectrometry (GF-AAS) and inductively coupled plasma optical emission spectrometry (ICP-OES) [13]. To be able to use these techniques, a high initial investment in instrumentation is required, an expensive budget to maintain consumables and maintenance and highly specialized personnel. Spectrophotometric molecular absorption methods involve less expensive and easier to operate equipment and the detectivity achieved is enough to perform detection in most samples as long as interfering matrix components are eliminated. Spectrophotometric methods have simple operating procedures and selective determination possibilities.

Platinum has been determined by first derivative spectrophotometry using rhodamine 6G [14]. The method has a detection limit of 0.022 mg L^{-1} but suffers from serious interferences caused by palladium. Second derivative spectrophotometry was used for the determination of platinum using trioctylamine and diantipyrylmethane [15]. Platinum(IV) was separated from palladium(II) after extraction using triphenylphosphine oxide [16]. The method is limited by the use of extraction with hydrochloric acid at high concentration. Tri-iso-octylamine was used for liquid-liquid extraction of platinum(IV) [17] but the use of salting out agents impose difficulties in the application of the method. N,N'-dimethyl-N,N-diphenyltetradecyl malonamide (DMDPHTDMA) extracts platinum(IV) from chloride media but in the presence of tin [18]. Leuco xylene cyanol FF (LXCFF) was oxidized by platinum(IV) to form the blue dye xylene cyanol FF [19]. The method suffers from a narrow linear response range. Platinum was determined using, 4-[N,N-(diethyl)amino]benzaldehyde thiosemicarbazone (DEABT) [20]. This method suffers from a narrow Beer's range and the complex is sparingly soluble in water but completely soluble in water-ethanol-DMF medium. 5-[4-nitrophenylazo]-8-[p-toluenesulphonamido]-quinoline (NPTSQ) [21] has been used for determination of platinum but the narrow working range (0.0-1.0 mμg mL^{-1}) limits the method. Literature revision reveals the methods reported for separation and spectrophotometric determination of platinum are simple and precise but still have limitations such as narrow pH range for separation, poor dectetivity, require heating for complex formation, restricted applications, rarely available reagent and interferences from cations and anions.

Direct extraction spectrophotometric determination has merits over methods for separation followed by determination. Determination of platinum was performed using anisaldehyde-4-phenyl-3-thiosemicarbazone as a chromogenic ligand [22]. Restricted limit of pH for quantitative determination (1.0 – 3.0) limits the method. Platinum was determined using thiobenzhydrazide [23], narrow linear response range (0.49 – 4.92 µg mL^{-1}) and the need for heating limits its application. The phenanthroquinone monoximate complex of platinum was adsorbed on microcrystalline napthalalene and determined in chloroform [24]. Heating to temperatures as high as 90 °C and the need for 45 min stirring time are disadvantages of the procedure. Platinum-5-chloro-2-hydroxythiobenzhydrazide complex was adsorbed on naphthalene [25]. Extraction Spectrophotometric determination of platinum was studied using tetramethylthiuram disulfide [26] and N-(3,5-dihydroxyphenyl)-N'-(4-aminobenzenesulfortate)-thiourea [27]. In the present work a selective and sensitive chromogenic agent, *o*-methylphenyl thiourea, was used to enable trace level determination of Pt(IV). The method enabled selective quantitative and rapid analyte separation with a wide linear response range. The reagent *o*-methylphenyl thiourea (OMPT) has been used for extractive spectrophotometric determination of palladium(II) [28] and rhodium [29]. Drawbacks of reported methods are overcome by the developed method. Low reagent concentration, analysis at trace level (100 µg platinum(IV)), selective quantitative separation, and wide linear response range (up to 15.0 µg mL^{-1}) are the points of significance of the developed method.

2. Experimental

2.1. Apparatus and glasswares

Absorbance measurements were performed using an ELICO double beam digital spectrophotometer model SL-159 using 1 cm quartz cells. An electronic balance model Contech-CA-123 was used for weighing purpose. Calibrated glassware was used for volume measurements.

2.2. Solutions and standards

All the chemicals and reagents used were of analytical reagent grade. Glass distilled water was used throughout the work.

The stock solution of platinum(IV) was prepared after dissolving 1 g of chloroplatinic acid ($H_2PtCl_6.H_2O$) (Loba Chemie, Mumbai, India) in 20 mL HCl solution (1 mol L^{-1}) and adjusted to 250 mL in a volumetric flask. This solution was standardized following a standard procedure [30]. The working solutions of platinum(IV) (100 µg mL^{-1}) was prepared from the stock solution.

o-methylphenyl thiourea (OMPT) was synthesized as reported by Frank and Smith [31]. The stock solution of 0.01 mol L^{-1} OMPT was prepared after dissolving 0.830 g OMPT in 50 mL chloroform.

Solutions to test the effects of potential interferences from other chemical species were prepared by dissolving specific quantities of specific salts in doubly distilled water or in diluted hydrochloric acid solution. The proportions of Pt(IV) and the potential interferent chemical species were adjusted as required for the tests.

2.3. Recommended procedure

A 1.0 mL solution of platinum (100 µg mL^{-1}) was transferred into a 25 mL volumetric flask where potassium iodide stock solution was added with water in order to get a final concentration of 0.1 mol L^{-1} in the 25 mL volume. This solution was transferred into a 125 mL separatory funnel and vigorously mixed (1 min) with 10 mL OMPT solution (4.5×10^{-3} mol L^{-1}) in chloroform to extract the ternary complex. The two phases were allowed to separate and the yellow colored organic layer containing platinum(IV)-OMPT-iodide complex was dried over 1 g anhydrous Na_2SO_4, dissolved in chloroform and transferred to a 10 mL volumetric flask. The absorbance of the platinum ternary complex was measured at 362 nm (using chloroform as blank).

The absorbance of platinum(IV)-OMPT-iodide complex in chloroform was stable for more than 48 h.

3. Results and discussion

3.1. Optimization of conditions for the formation and extraction of Pt(IV)-OMPT complex

The absorption spectra of platinum(IV)-OMPT-iodide complex against the reagent blank in chloroform shows maximum absorbance at 362 nm. Thus further absorbance measurements of the complex were made at 362 nm against the reagent blank (Figure 1).

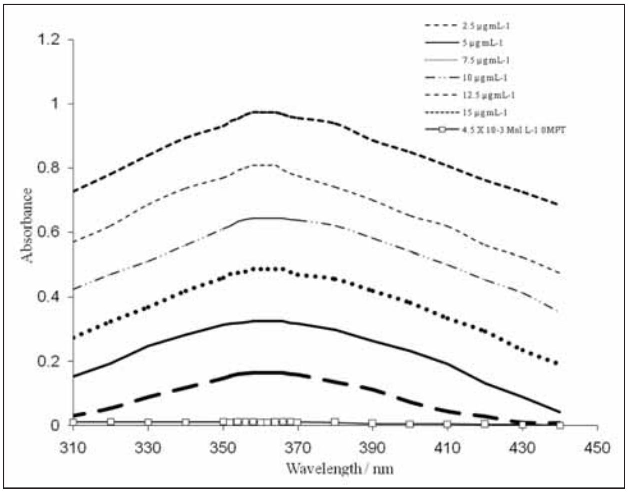


Figure 1. Absorption spectra of platinum(IV)-OMPT-iodide complex vs OMPT reagent blank

The extraction of platinum(IV) with *o*-methylphenyl thiourea in chloroform was carried out by the recommended procedure using platinum(IV) (100 µg mL^{-1}) and 10 mL OMPT (4.5 x 10^{-3} mol L^{-1}) in chloroform, with the KI aqueous solutions having concentrations varying from 0.1 to 3 mol L^{-1}. The absorbance value increases up to 0.1 mol L^{-1} KI concentration in aqueous phase and further increase in the KI concentration has no adverse effect on extraction of platinum(IV). Hence 0.1 mol L^{-1} KI was employed for further study.

The concentration of OMPT in chloroform was varied from 1x10^{-4} mol L^{-1} to 1x10^{-2} mol L^{-1} at 0.1 mol L^{-1} potassium iodide concentration. The extraction of platinum(IV) was quantitative and reproducible with 4.5x10^{-3} mol L^{-1} OMPT ensuring complete complexation. There is no significant change in the absorption with excess OMPT concentrations.

3.2. Effect of extraction solvent

Different organic solvents were studied for extractive spectrophotometric determination of platinum(IV) with OMPT. Among the solvents studied chloroform was the best solvent compared to other solvents studied and gave quantitative extraction and determination as seen in Table I. Hence chloroform was used for further extraction studies.

Table I. Effect of solvent on extraction of platinum(IV)-OMPT-iodide complex

Solvent	Dielectric constant	Percentage extraction (%E)	Distribution ratio (D)
Benzene	2.28	12.13	0.35
Toluene	2.38	14.15	0.41
Xylene	2.30	15.71	0.47
1,2-Dichloroethane	10.40	56.60	3.26
n-Butanol	17.10	73.25	6.84
n-Butyl acetate	5.00	74.65	7.36
Isoamyl alcohol	14.70	74.81	7.42
Methyl isobutyl ketone	13.10	78.17	8 .89
Chloroform	4.40	99.99	2497.5

3.3. Stiochiometry of the ternary complex

The stoichiometry of platinum(IV)-OMPT-iodide complex was determined by log-log plots [32]. Two plots were obtained as:

1) Log C (molar concentration) of OMPT in chloroform solvent versus Log D (distribution ratio) of platinum(IV) at fixed KI concentration in aqueous phase.

2) Log C (molar concentration) of KI versus log D (distribution ratio) of platinum(IV) at fixed OMPT concentration of 4.5x10^{-3} mol L^{-1} in chloroform solvent.

Potassium iodide concentration was fixed (0.1 mol L^{-1}). The amount of platinum(IV) taken was fixed (100 µg) and the OMPT concentration was varied from 1×10^{4} to 1×10^{2} mol l^{-1}. The recommended procedure was followed. As the concentration of OMPT increases percentage extraction and hence, the distribution ratio (D) was obtained.

The distribution of a platinum(IV) between two immiscible solvents is described by the term distribution ratio D.

$$D = \frac{\text{Total concentration of the platinum(IV) in organic phase}}{\text{Total concentration of the platinum(IV) in aqueous phase}}$$

when a two-phase system is of an analytical interest, as here where the chloroform solvent and aqueous phase are involved, D is given as:

$$D = \frac{[\text{Platinum(IV)}]_{\text{chloroform}}}{[\text{Platinum(IV)}]_{\text{aqueous}}}$$

where, [Platinum(IV)]$_{\text{chloroform}}$ and [Platinum(IV)]$_{\text{aqueous}}$ represents the stoichiometric or formal concentration of the platinum(IV) in the organic and aqueous phases, respectively. Further, the distribution ratio D can be simplified as:

$$D = \frac{\frac{V_{\text{aqueous}}}{V_{\text{chloroform}}} \times \%E}{(100 - \%E)}$$

where, % E = percentage extraction, V$_{\text{chloroform}}$ = volume of organic phase and V$_{\text{aqueous}}$ = volume of aqueous phase.

The log-log plot explains platinum(IV) at fixed 0.1

mol L^{-1} KI concentration gives the slope of 1.01, confirms the platinum(IV):OMPT ratio as 1:1. Similarly another log-log plot was plotted for log C (molar concentration) of potassium iodide versus log D (distribution ratio) of platinum(IV) at fixed reagent concentration of 4.5×10^{-3} mol L^{-1} gives the slope of 2.14 confirms platinum(IV):iodide ratio as 1:2, hence the probable stoichiometry of ternary complex (platinum(IV):OMPT:iodide) is 1:1:2 (Figure 2).

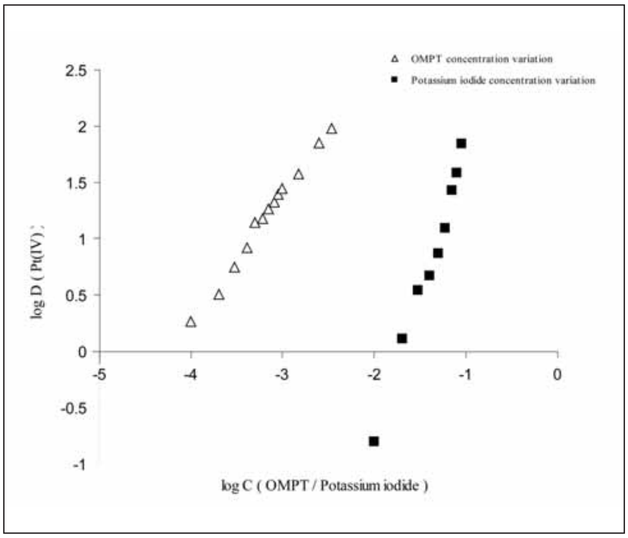


Figure 2. Plot of log C $_{(OMPT / Potassium iodide)}$ vs log D $_{(Pt (IV))}$

This composition of the complex was also verified by the mole ratio method (Figure 3) and by the method of continuous variation (Job's method) as seen in Figure 4. OMPT acts as a multidentate ligand, sulphur from the thio group (-C = S) and nitrogen from the amine group ($-NH_2$) coordinate with platinum to form a four membered chelate. Based on this investigation the recommended structure for the complex is given in Figure 5.

3.4. Analytical figures of merit

The platinum(IV)-OMPT-iodide complex obeys Beer's law over the concentration range of up to 15 µg mL^{-1} of platinum(IV) and by the Ringbom´s plot the range of linear response between absorbance and Pt (IV) concentration was from 3.09 µg mL^{-1} to 14.52 µg mL^{-1} (Figure 6). The relative standard deviation (R.S.D.) for this 10 determinations was 0.34%. The molar absorptivity was 1.25×10^4 L mol^{-1} cm^{-1}. The correlation coefficient was 0.99 and the analytical curve equation was Y= 0.0646 X + 0.0017. Repeatability of the method was evaluated by the determination of Pt (IV), at a 10 µg ml L^{-1}, in ten solutions (authentic replicates). The detection limit for platinum(IV) was 4.476×10^{-3} µg cm^{-3} and defined as the amount of analyte that enabled a signal equals to three times the standard deviation of the blank.

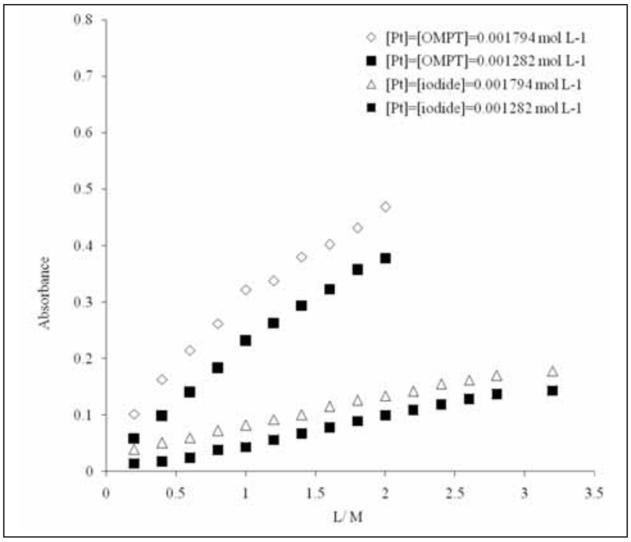


Figure 3. Mole ratio method: [Pt(IV)] = [OMPT] = 0.001794 mol L^{-1}, [Pt] = [OMPT] = 0.001282 mol L^{-1} when [iodide] = 0.1 mol L^{-1} and [Pt] = [iodide] = 0.001794 mol L^{-1}, [Pt] = [iodide] = 0.001282 M when [OMPT] = 4.5×10^{-3} mol L^{-1}.

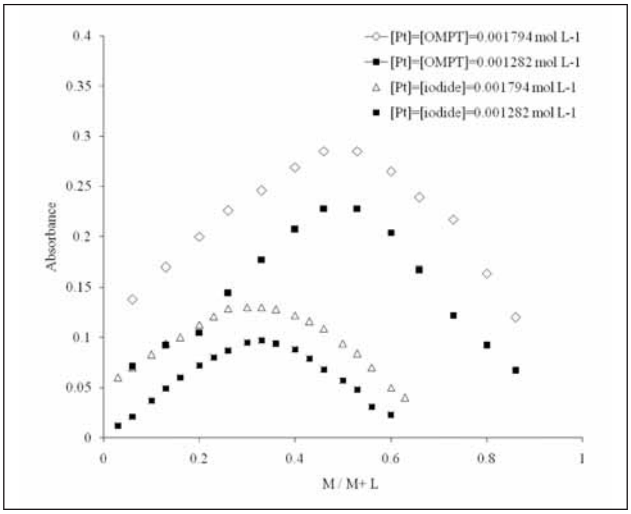


Figure 4. Job's continous variation method: [Pt(IV)] = [OMPT] = 0.001794 mol L^{-1}, [Pt] = [OMPT] = 0.001282 mol L^{-1} when [iodide] = 0.1 mol L^{-1} and [Pt] = [iodide] = 0.001794 mol L^{-1}, [Pt] = [iodide] =0.001282 mol L^{-1} when [OMPT] = 4.5×10^{-3} mol L^{-1}.

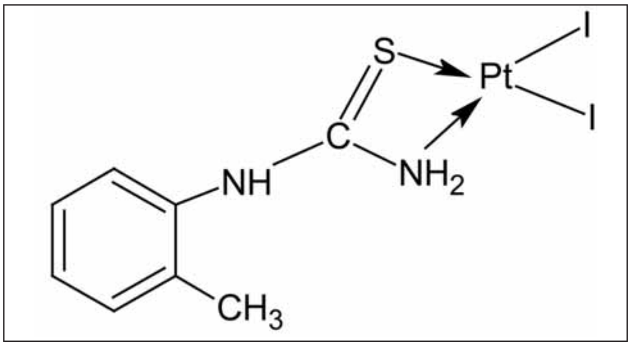


Figure 5. Probable structure of platinum(IV)-OMPT-iodide (1:1:2) ternary complex

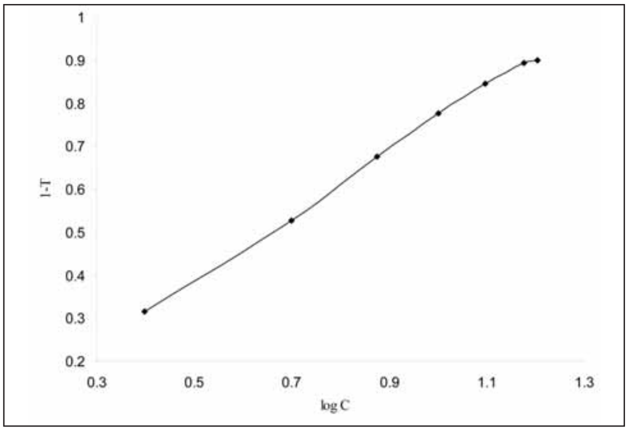


Figure 6. Ringbom's plot

3.5. Interference study

The effects of various foreign ions on determination of platinum(IV) were investigated. The tolerance limits of added ions which do not cause a deviation of more than ± 2 % in the determination of platinum(IV) was fixed. Selectivity of the method was enhanced by use of suitable masking agents (Table II).

Selective extraction of palladium(II) (60 µg) was carried out from 0.8 mol L^{-1} hydrochloric acid media [15]. Platinum (IV) (100 µg) and palladium (II) (60 µg) was taken in a 25 mL volumetric flask and hydrochloric acid was added so that the solution is 0.8 mol L^{-1} in the 25 mL volume. This mixture was equilibrated with 10 mL of 1×10^{-4} mol L^{-1} OMPT in chloroform for 10 s , which quantitatively extracts palladium(II) and platinum(IV) remains in the aqueous phase. The aqueous phase was evaporated to moist dryness and the residue was dissolved in water. Platinum(IV) content was determined as per the recommended procedure. The interference of osmium(VIII) was removed by prior extraction: to an aliquot containing 100 µg platinum(IV) and 200 µg osmium(VIII), 2 mL of 0.009 mol L^{-1} OMPT in ethanol and hydrochloric acid was added followed by dilution to 10 mL with water making the solution 0.8 mol L^{-1} in HCl. The pink colored Os(VIII)-OMPT complex that formed instantly was extracted into 10 ml chloroform after a single extraction. The aqueous phase was evaporated to moist dryness and platinum(IV) was determined as per the recommended procedure. Also the interference of iridium(III) was removed by prior extraction: to an aliquot of solution containing 100 µg platinum(IV) and 400 µg iridium(III), 4 mL of 0.004 mol L^{-1} OMPT in ethanol and HCl was added followed by dilution to 10 ml with water making the solution 0.6 mol L^{-1} in HCl. The pink colored Ir(III)-OMPT complex that formed instantly was extracted into 10 mL chloroform in a single step extraction. The aqueous phase was evaporated to moist dryness and platinum(IV) was determined as per the recommended procedure. Ruthenium(III) and rhodium(III) do not interfere in the determination. Interference limits for various foreign ions and their suitable masking agents is given in Table II.

Table II. Effect of foreign ions

Foreign Ion	Added as	Tolerance limit (mg)	Absorbance without masking agent at 362 nm	Absorbance with masking agent at 362 nm	Masking agent
Mn(II) [a]	$MnCl_2.6H_2O$	4.50	0.141	0.643	100 mg EDTA
Cd(II) [a]	$CdCl_2.2H_2O$	5.00	0.118	0.643	100 mg EDTA
Fe(III) [a]	$(NH_4)Fe(SO_4)_2.12H_2O$	3.50	0.178	0.643	100 mg EDTA
Hg(II)	$HgCl_2$	0.50	0.643		
Bi(III) [a]	$BiCl_3$	1.00	Ppt	0.643	100 mg EDTA
Ni(II) [a]	$NiCl_2.6H_2O$	3.00	1.069	0.643	100 mg EDTA
Cu(II) [a]	$CuSO_4.5H_2O$	1.00	1.116	0.643	100 mg EDTA
Al(III) [a]	$AlCl_3.6H_2O$	4.50	0.462	0.643	100 mg EDTA
Cr(III)	$CrCl_3$	25.0	0.643		
Zn(II) [a]	$ZnSO_4.7H_2O$	7.00	0.143	0.643	100 mg EDTA
Se(IV)	SeO_2	1.00	0.643		
La(III)	$LaCl_3.7H_2O$	1.50	0.643		
Li(I)	LiCl	25.0	0.643		
Ti(III) [a]	$Ti_2SO_4)_3$	2.60	0.084	0.643	100 mg EDTA
Pd(II) [c]	$Pdcl_2$	0.06	0.643		
Mg(II)[a]	$MgCl_2.6H_2O$	6.50	0.432	0.643	100 mg EDTA
Sn(II) [b]	$SnCl_2.2H_2O$	0.30	0.107	0.643	100 mg tartrate
Ga(III) [b]	$GaCl_3$	5.00	0.368	0.643	100 mg tartrate
Au(III)	$HAuClO_4.H_2O$	0.10	0.643		
Mo(VI)	$(NH_4)_5MO_7.2H_2O$	1.00	0.643		
Sb(III) [b]	Sb_2O_3	0.70	0.113	0.643	100 mg tartrate
Be(II)	$BeSO_4.4H_2O$	15.0	0.643		
Ce(IV) [a]	$Ce(SO_4)_2.4H_2O$	1.00	0.139	0.643	100 mg EDTA
Pb(II) [a]	$PbCl_2$	3.00	0.277	0.643	100 mg EDTA
V(V)	V_2O_5	10.0	0.643		
U(VI)	$UO_2(CH_3COO)_2.2H_2O$	25.0	0.643		
Co(II) [a]	$CoCl_2.6H_2O$	6.00	1.093	0.643	100 mg EDTA
Ba(II)	$BaCl_2.6H_2O$	35.0	0.643		
Ca(II)	$CaCl_2.2H_2O$	50.0	0.643		
Sr(III)	$SrCl_3.6H_2O$	40.0	0.643		
Tl(III) [b]	Tl_2O_3	6.00	0.304	0.643	100 mg tartrate
In(III) [b]	$InCl_3.4H_2O$	5.50	0.353	0.643	100 mg tartrate
Rh(III)	$RhCl_3$	0.80	0.643		
Ru(III)	$RuCl_3.3H_2O$	0.40	0.643		
Os(VIII) [d]	OsO_4	0.20	0.643		
Ir(III) [e]	$IrCl_3$	0.40	0.643		
Fluoride	NaF	100.0	0.643		
Phosphate	Na_3PO_4	100.0	0.643		
Sulfate	K_2SO_4	100.0	0.643		
Succinate	$(CH_3COONa)_2.6H_2O$	100.0	0.643		
Citrate	$C_6H_8O_7.H_2O$	100.0	0.643		
Malonate	$CH_2(COONa)_2$	100.0	0.643		
Tartrate	$(CHOH:COOH)_2$	100.0	0.643		
Acetate	$CH_3COONa.3H_2O$	100.0	0.643		
Oxalate	$(COOH)_2.2H_2O$	100.0	0.643		
E.D.T.A	Na_2EDTA	100.0	0.643		

Ppt: precipitate formation; [a] masked with 100 mg EDTA; [b] masked with 100 mg tartrate; [c] selective extraction of palladium (II)[15]; [d] prior extraction of osmium (VIII); [e] prior extraction of iridium (III)

3.6. Separation and determination of platinum from binary synthetic mixtures

The proposed method permits separation and determination of platinum(IV) from associated metal ions viz : nickel(II), cobalt(II), iron(III), manganese(II), zinc(II), copper(II), cadmium(II), aluminum(III) and magnesium(II). Each individual metal ion in solution was taken and a solution containing 100 mg of EDTA was added to mask these ions. 1 mL platinum(IV) solution (100 µg mL^{-1}) was added and after extraction of platinum(IV), the masked metal ions remained in the aqueous phase were treated with 5 mL HNO$_3$ and the mixture was boiled vigorously for 15 to 20 min with addition of water at intervals to maintain the volume. The mixture liberates violet vapors of iodine and after complete evolution of iodides in the form of iodine the raffinate was evaporated to moist dryness followed by treatment with HCl. The residue obtained was cooled dissolved in water and again evaporated to moist dryness. The residue was dissolved in water the added metal ions were determined by standard procedures [34] (Table III).

Table III. Separation of platinum from binary synthetic mixtures

Metal ion	Amount taken (µg)	Recovery[f] (%)	RSD (%)	Chromogenic ligand	Reference
Pt(IV)	100	99.3	0.28	OMPT	------
Ni(II)[a]	100	99.7	0.06	Dimethylglyoxime	33
Pt(IV)	100	99.6	0.20	OMPT	------
Co(II)[a]	300	99.1	0.50	Thiocynate	33
Pt(IV)	100	99.6	0.35	OMPT	------
Fe(III)[a]	75	99.4	0.58	1,10-Phenenthroline	3
Pt(IV)	100	99.8	0.23	OMPT	------
Mn(II)[a]	200	99.4	0.79	Potassium metaperiodate	33
Pt(IV)	100	99.6	0.48	OMPT	------
Zn(II)[a]	20	99.3	0.53	Dithizone	33
Pt(IV)	100	99.4	0.59	OMPT	------
Cu(II)[a]	30	99.2	0.80	Dithizone	33
Pt(IV)	100	99.6	0.49	OMPT	------
Cd(II)[a]	25	98.7	0.98	Dithizone	33
Pt(IV)	100	99.8	0.20	OMPT	------
Al(III)[a]	50	98.5	0.81	8-Hydroxyquinoline	33
Pt(IV)	100	99.5	0.20	OMPT	------
Mg(II)[a]	20	99.6	0.36	Titan yellow	33

[a] Masked with 100 mg EDTA; [f] average of four determinations

3.7. Separation and determination of platinum from multicomponent synthetic mixtures

A 1.0 mL (100 µg mL^{-1}) of platinum(IV) was taken and known amounts of different compositions of associated metal ions were added. Masking agent (100 mg EDTA) was added to mask the added metal ions. Recommended procedure was followed and the platinum(IV) content was separated from the ternary mixtures. The results obtained were in good agreement with the amount added (Table IV).

Table IV. Separation of platinum from multicomponent mixtures

Composition (µg)	Recovery [f] (%)	RSD (%)
Pt(IV) 100; Co(II)[a] 50; Fe(III)[a] 50; Mn(II)[a] 50	99.2	0.53
Pt(IV) 100; Zn(II)[a] 20; Cu(II)[a] 30; Cd(II)[a] 25	99.7	0.61
Pt(IV) 100; Mg(II)[a] 20; Zn(II)[a] 20; Cu(II)[a] 30	99.7	0.27
Pt(IV) 100; Fe(III)[a] 50; Mg(II)[a] 20; Bi(III)[a] 50	99.9	0.08
Pt(IV) 100; Cd(II)[a] 25; Al(III)[a] 50; Cu(II)[a] 30	99.8	0.20
Pt(IV) 100; Bi(III)[a] 50; Zn(II)[a] 20; Mn(II)[a] 50	99.8	0.23
Pt(IV) 100; Zn(II)[a] 20; Al(III)[a] 50; Cu(II)[a] 30	99.8	0.20
Pt(IV) 100; Mn(II)[a] 50; Zn(II)[a] 20; Fe(III)[a] 50	99.7	0.32

[a] Masked with 100 mg EDTA; [f] average of four determinations

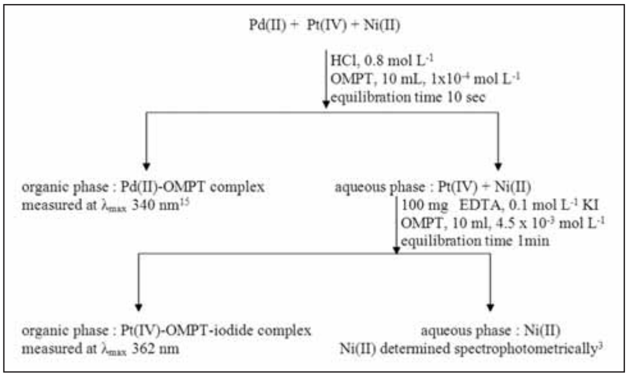
3.8. Mutual separation of palladium(II), platinum(II) and nickel(II)

Palladium(II), platinum(IV) and nickel are transition metals belonging to the VIIIth group. Three aqueous solutions containing palladium(II) (60 µg), platinum(IV) (100 µg, 75 µg, 50 µg) and nickel(II) (100 µg) were taken in a 25 ml volumetric flask. Extractive separation and determination of palladium(II) was carried out from 0.8 mol L^{-1} HCl media [15]. Aqueous phase containing platinum(IV) and nickel(II) was evaporated to moist dryness and the residue was dissolved in distilled water, 100 mg of EDTA was added to mask nickel(II) and the recommended procedure was followed separating the platinum(IV) into organic phase quantitatively and the nickel(II) remained in the aqueous phase. The aqueous phase containing nickel(II) was treated with 5 mL concentrated nitric acid and the mixture was boiled vigorously for 15 to 20 min with addition of water at intervals to maintain the volume. The mixture liberates violet vapors of iodine and after complete evolution of iodides in the form of iodine the raffinate was evaporated to moist dryness followed by treatment with HCl. The residue obtained was cooled and dissolved in water, which was evaporated to moist dryness. The residue was dissolved in water and nickel(II) determined spectrophotometrically by the dimethylglyoxime method [33] (Table V, Scheme 1).

Table V. Mutual separation of palladium(II), platinum(IV) and nickel

Mixture	Amount taken (µg)	Chromogenic ligand	Recovery[f] (%)	RSD %
Pd(II) + Pt(IV) + Ni(II)	Pd (60)	OMPT	99.42	0.67
	Pt (100)	OMPT	99.65	0.32
	Ni (100)	DMG	99.52	0.28
Pd(II) + Pt(IV) + Ni(II)	Pd (60)	OMPT	99.61	0.55
	Pt (75)	OMPT	99.69	0.36
	Ni (100)	DMG	99.42	0.14
Pd(II) + Pt(IV) + Ni(II)	Pd (60)	OMPT	99.71	0.37
	Pt (50)	OMPT	99.46	0.39
	Ni (100)	DMG	99.50	0.20

[f] Average of four determinations; DMG dimethylglyoxime[34]



Scheme 1. Mutual separation of palladium(II), platinum(IV) and nickel(II)

4. Applications

4.1. Separation and determination of platinum from pharmaceutical samples

The proposed method was successfully applied for extractive spectrophotometric determination of platinum(IV) from Cisplatin injection drug (Kemoplat, Dabur Pharma, New Delhi, India) and carboplatin injection drug (Carbopa-150, Intas Biopharma, Ahmedabad, India). A known volume of sample was treated with 5 mL aqua regia solution and then evaporated to moist dryness. The obtained residue was treated with two 5 mL portions of HCl and evaporated until all HNO_3 has been removed completely. The residue was dissolved in distilled water and filtered through Whatman filter paper No.1 and the filtrate was made up to mark in a 10 mL volumetric flask. The determination of platinum(IV) was carried out by employing the proposed procedure. The results obtained were compared with those obtained by atomic absorptions spectrometry (AAS) following the procedure indicated in reference 35 (Table VI).

Table VI. Separation and determination of platinum from pharmaceutical samples

Sample	Product name & manufacturer	Certified value of platinum(IV) taken (µg)	Platinum(IV) found by AAS (µg)	Platinum(IV) found by proposed method[f] (µg)	RSD (%)
Carboplatin	Carbopa-150,	52.6	52.2	52.2	0.28
	Intas Biopharma,	78.8	78.4	78.5	0.30
	Ahemdabad, (India)	105.1	105.0	104.8	0.31
Cisplatin	Kemoplat,	64.8	64.4	64.2	0.46
	Dabur Pharma,	97.2	96.9	96.9	0.37
	New Delhi, (India)	129.6	129.1	129.0	0.32

[f] Average of four determinations

4.2. Determination of platinum from Adam's catalyst

The composition of Adam's catalyst ($PtO_2.H_2O$) was prepared in the laboratory [36]. To the catalyst (50 mg), 5 mL aqua regia was added and the mixture was evaporated to moist dryness. Then, two 5 mL portions of HCl were added followed by the evaporation until all HNO_3 has been removed completely. The residue was dissolved in distilled water and filtered through Whatman filter paper No 1. The filtrate was transferred to a volumetric flask and the volume was adjusted to 250.00 mL. Aliquots of solutions containing 50 µg, 75 µg and 100 µg platinum(IV) were analyzed for their platinum(IV) content by the proposed method. The results obtained with the proposed method agreed with those obtained by AAS (Table VII).

Table VII. Determination of platinum from Adam's catalyst

Sample	Amount of Platinum(IV) taken (µg)	Platinum(IV) found by AAS[35] (µg)	Platinum(IV) found by proposed method[f] (µg)	RSD (%)
Adam's catalyst	50	49.75	49.70	0.66
	75	74.96	74.92	0.20
	100	99.72	99.72	0.43

[f] Average of four determinations

Conclusion

o-methylphenyl thiourea (OMPT) has been proven to be a selective reagent for the extraction of Pt (IV) allowing sensitive spectrophotometric determination through the formation of a ternary complex. The developed method is simple, reproducible and rapid. The selectivity of the determination has been enhanced by using suitable masking agents and prior extraction. The method was successfully applied to determine platinum(IV) from real samples and pharmaceutical preparations.

Acknowledgement

The authors are grateful to Management, Pravara Rural Education Society and the Principal Arts Science Commerce College Satral for providing necessary facilities in the department to carry out this research work.

References

1. Lee, J. D,; *Concise Inorganic Chemistry*, 5th ed., Blackwell science Ltd, oxford: UK, 1996.
2. Jackowska, A. D.; Polkowska,Z.; Namieśnik, J.; *Pol. J. Environ. Stud.* 2007,16, 329.
3. Eckard, H.; *Environ. Sci. Pollut. Res.* 1997, 4, 99.
4. Clare. L. S. W.; Fathi. Z.; *Sci. Total Environ*. 2009, 407, 2493.
5. Kostova. I.; *Recent Pate. Anti-Cancer Drug Discov*. 2006, 1, 1-22.
6. C. Monneret.; *Ann. Pharma. Fra.,* 2011, 69, 286.
7. Wang. D.; Lippard S. J.; *Nat Rev Drug Discov*, 2005, 4, 307.
8. Nial. J, W.; Shonagh. W.; Gemma E. C.; Rabbab. O.; *Dalton Trans*, 2010, 39, 8113.
9. Xiaoyong. W.; Zijian. G.; *Chem. Soc. Rev*, 2013, 42, 202.
10. Susanne. B.; Anton. IPM de Kroon.; *Future Medi. Chem.,* 2009, 1, 1467.
11. Malejko, J.; Milewska, R.; Leniewska, B.; Godlewska, B.; Kojo, A.; *Anal. Lett.* 2007, *40*, 961.
12. Suzuki, T.; Sasaki, A.; Nakai, I.; *J. Trace. Microprobe .Tech.* 2001, *19*, 547.
13. Canopavon, J. M.; Torres, G. D.; Rojas, F. S.; Ojeda, C. B.; *Spectrosc. Lett.* 2007, *40*, 27.
14. Beata, G. Z.; Barbara, L.; *J. Trace. Microprobe. Tech*. 2001, *19*, 345.

15. Balkcerzak, M.; Wyrzykowska, E.; *Analysis*. 1999, *27*, 829.
16. Mojski, M.; Kalinowski, K.; *Microchem. J.* 1980, *25*, 507.
17. Lee, J. Y.; Kumar, R. J.; Kim, J. S.; Park, H. K.; Yoon, H. S.; *J. Hazard. Mater*. 2009, *168*, 424.
18. Malik, P.; Paiva, A. P.; *Solvent. Extr. Ion Exch.* 2009, *27*, 36.
19. Revanasidappa, D.; Kumar, N. K.; *Anal. Bioanal. Chem.* 2003. *375*, 319.
20. Karthikeyan, J.; Shetty, A. N.; *Environ. Monit. Assess.* 2010, *171*, 639.
21. Zhao, J.; Xu, Q.; *Talanta.* 1991, *38*, 909.
22. Prakash, K. M. M. S.; Prabhakar, L. D.; Reddy, D. V.; *Anal. Lett.* 1987, *20*, 959.
23. Gangopadhyay, S.; Gangopadhyay, P. K.; Shome, S. C.; *Z. Anal. Chem*. 1976, *143*, 281.
24. Puri, B. K.; Wasey, A.; Katyal, M.; Satake, M.; *Analyst*. 1986, *111*, 743.
25. Sawant, S. S.; *Anal. Sci.* 2010, *26*, 95.
26. Uttarwar, R. M.; Joshi, A. P.; *Microchem. J.* 1978, *23*, 151.
27. Zhigang, Li.; Xuemei, Li.; Liya, Z.; Qiufen, H.; Jing, C.; Guangya, Y.; *Indian J. Chem. Sec. A.* 2006, *45*, 1852.
28. Shelar, Y. S.; Aher, H. R.; Kuchekar, S. R.; *Bulg. Chem. Commun.* 2012. *44*, 172.
29. Shelar, Y. S.; Kuchekar, S. R.; Han, S. H.; *J. Saudi Chem. Soc.* 2012 (in press) available online http://dx.doi.org/10.1016/j.jscs.
30. Furman, N. H.; *Standard Methods of Chemical Analysis,* 6th ed., Robert. E. Krieger Publishing Company: Malabar, FL, 1962, 874.
31. Frank, R. L.; Smith, P. V.; *Org. Synth*. 1995, *3*, 735.
32. Miller, J.C.; Miller, J.N.; *Statastics for Analytical Chemistry,* 3rd ed. Ellis Horwood PTR Prentice Hall, 1993.
33. Ringbom, A.; *Z. Anal. Chem*. 1939, *115*, 332.
34. Marckzenco, Z.; *Spectrophotometric Determination of Trace Elements,* John Wiley and Sons: Chichester, 1976.
35. Da-Costa, A. C.; Vieira, M. A.; Luna A. S.; De-Campos R. C.; *Talanta.* 2010, *82,* 1647.
36. Vogel, A. I.; *Vogel's Textbook of Practical Organic Chemistry*, 5th ed., Pearson Education Ltd: 2007, 459.

Br J Anal Chem 2013, 10, 429–435

Total and trivalent inorganic arsenic determination in water samples by selective hydride generation atomic absorption spectrometry

Patrícia Sueli Rezende, Letícia Malta Costa, Cláudia Carvalhinho Windmöller*
Department of Chemistry, ICEx, Federal University of Minas Gerais, Pampulha, Belo Horizonte, MG, Brazil, CEP 31270-901.

Abstract

The development of methodologies to quantify inorganic arsenic species is relevant due to the physical and chemical properties of each species, such as mobility and toxicity. In this study, a methodology was optimized and validated for inorganic arsenic speciation using selective hydride generation coupled to atomic absorption spectrometry (HGAAS). For As(III) determination, the samples were diluted in citrate buffer. 0.1 mol L^{-1} citric acid ($C_6H_8O_7$) was used as the acid carrier and $NaBH_4$ was used as the hydride generation reagent. For total As determination, the samples were pre-reduced using thiourea and were introduced into the HGAAS using 10% v/v HCl as the acid carrier. The As(V) concentration was estimated from the difference between the total and As(III) concentrations. After validation of the methodology, surface and ground water samples from an impacted gold mining area were analyzed. The total As concentration ranged from 1.6 µg L^{-1} to 113 µg L^{-1}. After speciation analysis, a predominance of pentavalent species was observed, ranging from 51% to 81%. After optimization of the instrumental conditions, arsenic quantification was performed using solutions with a lower concentration than those described in the literature.

***Corresponding author:**
Phone/Fax: 55 31 3409-5725
E-mail address:
claudiaufmg@hotmail.com

Keywords: arsenic, speciation, water samples, hydride generation.

1. Introduction

Arsenic (As) is widely distributed in the environment in both inorganic and organic forms [1]. In superficial water and ground water, the inorganic forms of As, As(III) and As(V), are predominant, and it is known that the toxicity of arsenic depends on its chemical form [2]. The inorganic forms of As are more toxic than the organic forms, and it has been reported that arsenite, As(III), is approximately 100-fold more toxic than arsenate, As(V) [3]. Furthermore, it is believed that arsenite is more mobile than arsenate in the environment, suggesting that arsenite can be easily transported in soil solutions in both horizontal and vertical directions [4].

In general, the concentration of arsenic in water is low; therefore, sensitive analytical methods are required for its determination [1,5-8]. Inductively coupled plasma mass spectrometry (ICP-MS) and atomic fluorescence spectrometry (AFS) are among the most sensitive techniques for As determination [5]. Other techniques, such as graphite furnace atomic absorption spectrometry (GFAAS) [1,7] and inductively coupled plasma optical emission spectrometry (ICPOES) [9], are extensively used; however, if no separation technique and/or derivatization method are applied, then only the total arsenic concentration is determined.

Methodologies for arsenic speciation analysis have been continuously developed in recent years [6]. The determination of chemical species is relevant to understanding toxicological effects, and separation techniques, such as gas chromatography (GC), high performance liquid chromatography (HPLC) and capillary electrophoresis (CE), are effective in speciation studies when they are coupled to spectrometric techniques [5].

Non-chromatographic methodologies for elemental speciation have occasionally been preferred because they can be less expensive, simpler, faster and sometimes more sensitive than chromatographic methods. Some methods for the determination of arsenic involve selective extractions or selective reduction since arsenic species depend on the pH of the medium [10,11].

The determination of arsenic using hydride generation coupled to atomic absorption spectrometry (HGAAS) has attracted interest due to its low cost and appropriate detectivity for environmental samples [2,12].

Tuzen *et al*. [3] determined As(III), As(V) and total arsenic in water, food and biological samples using HGAAS. The resin *Alternaria solani* coated with Diaion HP-2MG was used at pH 7, and As(III) was adsorbed on the column. Subsequently, As(III) was eluted with 1.0 mol L^{-1} HCl and quantified. The methodology was applied to tap water, seawater and hot springs water. The concentration of total arsenic varied from 0.73 µg L^{-1} to 2.30 µg L^{-1}. Trivalent arsenic was present in 25 % to 42 % of the total arsenic in these samples.

Maity *et al*. [2] employed citric acid and 0.1 mol L^{-1} HCl as the carrier acids for the selective hydride formation of As(III) and for total arsenic determination, respectively. The detection limit was 0.4 µg L^{-1}, and the procedure was applied to ground water samples from West Bengal, India. The concentration of trivalent arsenic ranged from 54 µg L^{-1} to 395 µg L^{-1}, representing 42 % to 99.6 % of the total arsenic in these samples.

This work describes a simple, sensitive and rapid methodology for the determination of As(III) in water samples using citric acid as the carrier and HGAAS for quantification. Total arsenic was determined using thiourea as the pre-reducing agent and HCl as the carrier acid. The methodology was optimized, validated and applied for the speciation of As(III) and As(V) in surface and ground water samples from an impacted gold mining area in the Paracatu River Basin, MG, Brazil.

2. Experimental

2.1. Standard solutions and reagents

All chemicals were of analytical grade. All solutions and dilutions were prepared using deionized water from a Milli-Q system (18.2 MΩ cm $^{-1}$, Millipore Direct-Q 3, France). Stock solutions (100 mg L^{-1}) of As(III) and As(V) were prepared from As_2O_3 (Merck, Darmstadt, Germany) and $Na_2HAsO_4.7H_2O$ (Quimibrás, Rio de Janeiro, Brazil), respectively. A mass of 0.1321 g of As_2O_3 was accurately weighed, dissolved in 40.0 mL of a 20% (w/v) NaOH solution, neutralized with 80.0 mL of a 10% (v/v) HCl solution, and diluted to 1 L. To prepare the As(V) solution, 0.2082 g of $Na_2HAsO_4.7H_2O$ was dissolved in 500 mL of deionized water. For total As, a certified standard solution (1000 mg L^{-1}) was obtained from Merck (Darmstadt, Germany). Working solutions were prepared by dilution of the stock solutions immediately before use.

Stock standard solutions containing Al^{3+}, Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, K^+, Mg^{2+}, Na^+, Ni^{2+}, Sb^{5+}, Cl^-, F^-, PO_3^{2-}, and SO_4^{2-} were prepared by dissolving their corresponding salts in high purity deionized water.

For HGAAS, $NaBH_4$ (Isofar, Duque de Caxias, Brazil), NaOH (Vetec, Rio de Janeiro, Brazil), HCl (Merck, Darmstadt, Germany), thiourea (Merck, Rio de Janeiro, Brazil), citric acid (Isofar, Duque de Caxias, Brazil), sodium citrate (Isofar, Duque de Caxias, Brazil), potassium iodide (Merck, Rio de Janeiro, Brazil), L-cysteine (Merck, Rio de Janeiro, Brazil), ascorbic acid (Isofar, Duque de Caxias, Brazil) and argon (99.997%, Linde, Contagem, Brazil) were used.

All glassware were kept in 10% v/v nitric acid for at least 24 h and then washed three times with deionized water before use.

2.2. Instrumentation

Inorganic arsenic determination was performed using a Perkin-Elmer AAnalyst FIAS 100 hydride generator system coupled to a Perkin-Elmer AAnalyst 200 flame atomic absorption spectrometer (FAAS) (Shelton, USA). A MHS-20 – Mantle Heating System was used for heating of the quartz cell and a deuterium lamp was used as background corrector. An electrodeless discharge lamp of As operated at 400 mA was used as the energy source and the arsenic signal was detected at 193.7 nm. A digital pH meter (Marte MB-10, Piracicaba, Brazil) and an analytical balance (Shimadzu AX200, São Paulo, Brazil) were also used.

2.3. Analytical procedures

2.3.1. Determination of total arsenic

For total As determination, the following instrumental conditions were applied: 0.2% w/v / 0.05% w/v $NaBH_4$/NaOH (4-6 mL min^{-1}) as the reducing agent;10% v/v HCl (8-12 mL min^{-1}) as the carrier acid; and Ar (50 mL min^{-1}) as the carrier gas.

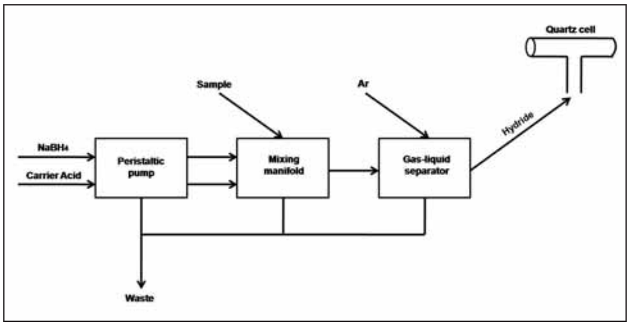


Figure 1. Schematic diagram of the FIAS 100 hydride generator.

Different arsenic species can produce hydrides at different speeds; arsine is formed more slowly from As(V) than from As(III), and this situation can produce errors in the results. Therefore, a pre-reduction step is extensively recommended to determine As(V) as As(III); consequently, all arsenic species will produce arsine under the same conditions and with the same kinetics. [2,7,8,12-17]. The concentration of the pre-reducing agent (L-cysteine, KI/ascorbic acid and thiourea) was investigated in a univariate manner at three levels (0.10% w/v, 0.55% w/v, and 1.0% w/v).

The proposed methodology was validated according to INMETRO (National Institute of Metrology, Standardization and Industrial Quality) [19], except for the limits of detection (LOD) and quantification (LOQ), which were determined according to IUPAC (International Union of Pure and Applied Chemistry) [20]. The selectivity was evaluated based on the effects of the interferent ions. The ions and concentrations were based on species that are commonly found in natural waters, as indicated in the literature [1,3,7,21]. For accuracy, the certified reference material SRM NIST 1643e (Trace Elements in Water) was used.

2.3.2. Determination of As(III)

Based on the kinetic mechanisms of formation of arsine from As(III) and As(V) ions, the measurements of the As(III) concentration in water samples were performed by selective hydride generation.

The methodology developed was based on previous work [2] using citric acid as the acid carrier for the quantitative analysis of As(III) in the presence of As(V). Due to the difficulty of working with $NaBH_4$ concentrations greater than 0.2% w/v (bubble generation), this concentration was kept. Tests using standard solutions of As(III) and As(V) were performed to obtain a high absorption signal for the first species and low signal for the second one. Then, the concentration carrier acid and pH of the sample were tested. The levels studied were 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 mol L^{-1} for citric acid and 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0 for pH.

The As(III) species was determined without a pre-reduction step. All samples were diluted by adding citrate buffer, pH 5, to a final dilution of 50% v/v. The operational conditions were as follows: reducing agent: 0.2% w/v / 0.05% w/v $NaBH_4$/NaOH w/vw/v (4-6 mL min^{-1}); carrier acid: 0.1 mol L^{-1} $C_6H_8O_7$ (citric acid) (8-12 mL min^{-1}); and carrier gas: Ar (50 mL min^{-1}).

Standard solutions of As(III) and As(V) were tested, and only the trivalent As species was determined under these conditions. Measurements were performed in triplicate for all calibration curve points, and the LOD was obtained from the analysis of 10 independent measurements of blank solutions.

2.3.3. Estimation of As(V)

The samples were divided into two parts: one part was diluted with citrate buffer, pH 5.0, for the determination of trivalent species; and on the other part, a thiourea solution was added for the determination of total arsenic. The As(V) concentration was estimated as the difference between total inorganic As and As(III).

2.4. Samples

First, synthetic water samples, 10 µg L^{-1} As(III) and 25 µg L^{-1} As(V) were analyzed. Ten samples of surface water and four samples of ground water were collected from an impacted gold mining area in Paracatu, MG, Brazil, in October/2010 (Table I and Figure 2). Immediately after sampling, all water samples were filtered through 0.45 µm nitrocellulose membranes. The samples were collected in plastic bottles, stored in high density polyethylene (HDPE) flasks and maintained under refrigeration until the analyses.

A 500 µL aliquot of 0.125 mol L^{-1} EDTA was added to the samples at collection to preserve the species for at least 3 months for speciation analysis [22,23].

Table I. Geographical coordinates of the sampling points.

Identification	Latitude	Longitude	Date
Natural Water			
Asup 01	S 17°12’698”	W 46°53’462”	15/10/2010
Asup 02	S 17°14’413”	W 46°51’697”	15/10/2010
Asup 03	S 17°13’868”	W 46°52’471”	15/10/2010
Asup 04	S 17°13’057”	W 46°53’427”	15/10/2010
Asup 05	S 17°12’081”	W 46°51’551”	15/10/2010
Asup 06	S 17°08’856”	W 46°49’443”	16/10/2010
Asup 07	S 17°15’360”	W 46°57’662”	16/10/2010
Asup 08	S 17°12’249”	W 46°52’500”	16/10/2010
Asup 09	S 17°18’15”	W 46°46’15”	16/10/2010
Abica	S 17°11’503”	W 46°55’355”	16/10/2010
Ground water			
Asub 01	S 17°14’160”	W 46°52’548”	15/10/2010
Asub 02	S 17°13’062”	W 46°53’306”	15/10/2010
Asub 03	S 17°12’08”	W 46°51’55”	15/10/2010
Asub 04	S 17°12’410”	W 46°54’029”	16/10/2010

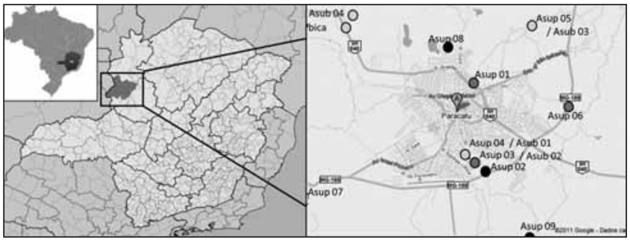


Figure 2. Paracatu city map.

3. Results and Discussion

3.1. Optimization of the methodology for total arsenic determination

Pre-reduction was chosen based on the absorbance signal for As(V) standard solutions at a concentration of 10 µg L^{-1}, as shown in Table II.

Table II. Evaluation of the 10 µg L^{-1} As(V) standard solution signal intensity under different conditions.

Pre-reducing agent Concentration	0.10% w/v	0.55% w/v	1.0% w/v
		Absorbance	
Without pre-reduction	0.920		
L-cysteine	1.200	1.524	1.564
Thiourea	1.848	1.983	2.003
KI/Ascorbic Acid	2.366	2.365	2.475

Table II reveals that the best signal was obtained using KI/ascorbic acid. However, these reagents were replaced by thiourea for further measurements because they caused blockage in the tubes of the flow injection system. Different concentrations of thiourea were tested, but the intensity of the signal was not drastically affected; therefore, the lowest concentration was chosen.

Total arsenic was determined using thiourea for preredution. An aliquot of thiourea prepared in 0.01 mol L^{-1} HCl was added to the samples at a final concentration of 0.10% w/v. This solution was kept in contact with the sample for at least for 1 hour before the analysis.

As a critical reagent for HG AAS measurements, the concentration of $NaBH_4$ was investigated over the range of 0.05 to 1.5% w/v. It was not possible to obtain a reproducible signal when the concentrations of $NaBH_4$ were greater than 0.4% w/v due to the intense generation of bubbles, which impedes the efficient separation of the liquid and gaseous phases because the bubbles wet the gas-liquid separator membrane and because droplets also were formed in the capillary that transports the gaseous phase. This caused a loss of signal and did not allow for continuous measuring. Therefore, the optimization of the $NaBH_4$ concentration was evaluated between 0.05 and 0.20% w/v using 10% v/v HCl. A concentration of 0.2% w/v $NaBH_4$ presented the best analytical signal, and this concentration was established for further quantifications. The instrumental conditions (0.2% w/v / 0.05% w/v $NaBH_4$/NaOH at a rate of 4-6 mL min^{-1} and 10% v/v HCl at a rate of 8-12 mL min^{-1}) established for the hydride generation measurements were less drastic than those used in previous papers described in the literature [2,13-17,22]. These experimental conditions are advantageous because they result in less consumption of reagents and low levels of blanks.

3.2. Optimization of the methodology for As(III) determination

Figures 3 and 4 show the effects of the pH in the sample and the concentration of citric acid on the determination of As(III) and As(V) in the standard solutions of 10 µg L^{-1} and 25 µg L^{-1}, respectively. As shown in these figures, the highest As(III) signals and the lowest As(V) signals were obtained using 0.1 mol L^{-1} citric acid and a pH of 5.0 without the addition of a pre-reducing agent.

First, the influence of the citric acid concentration was tested, and the pH selected for this test was 4.8 (2nd pKa value). After determining that 0.1 mol L^{-1} would be the most suitable concentration, the tests investigating the influence of the sample pH using 0.1 mol L^{-1} citric acid as the carrier acid were initiated. The other conditions employed in these tests (air flow rate, $NaBH_4$ concentration, reducing agent and acid flow rates, and sample volume) were those recommended by the manufacturer and were used for the determination of the total concentration.

As shown in Figure 5, the use of citric acid to replace the hydrochloric acid as the H^+ source in the hydride generation process was only efficient for detecting As(III).

Under these experimental conditions, the formation of hydride from pentavalent species was negligible. Changing the carrier acid to a weaker acid drastically reduces the availability of H^+, making the reduction of As(V) to As(III) more difficult and consequently reducing the formation of arsine. Notably, the reduction potential for the As(V) to As(III) reaction is pH dependent. Some studies [2,18]have shown that the formation of arsine from As(III) has low variation in detectivity over a wide pH range, whereas for As(V), a good response is only obtained at higher acid concentrations.

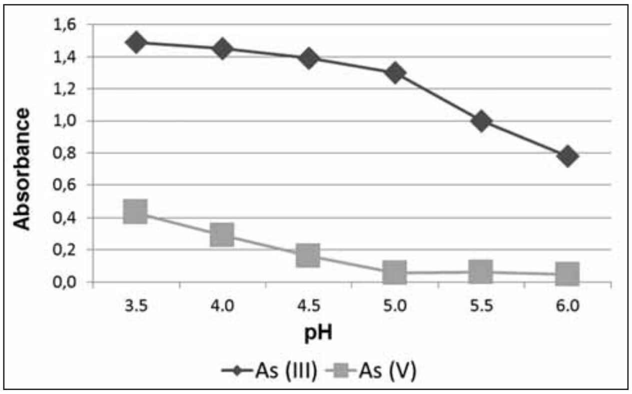


Figure 3. Effect of the sample pH on the selective hydride generation of As(III) and As(V) species.

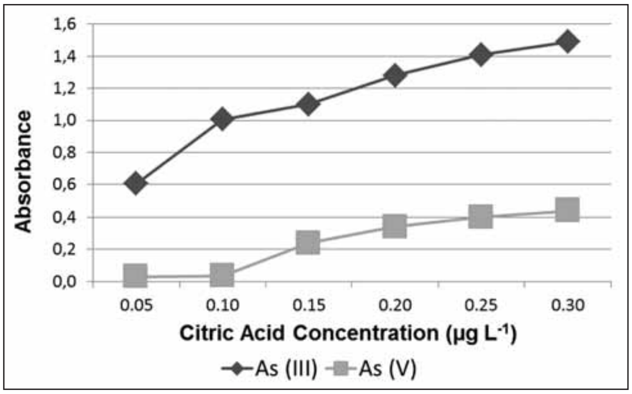


Figure 4. Effect of the citric acid concentration on the selective hydride generation of As(III) and As(V) species.

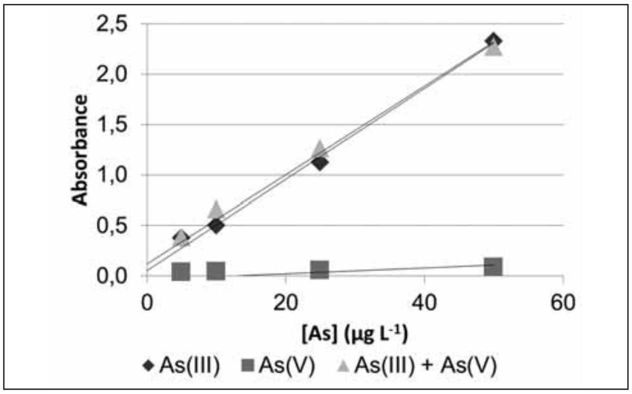


Figure 5. Analytical curves in 50% v/v citrate buffer, pH 5.0, for analyzing arsenic from As(III) and As(V) standard solutions and mixed in equal concentrations between the two species using 0.1 mol L^{-1} citric acid as the carrier acid. Points (5.0; 10.0; 25.0; and 50.0 µg L^{-1}).

3.3. Validation of the proposed methodology

The figures of merit were determined according to INMETRO recommendations [19], except for LOD and LOQ, which were determined according IUPAC recommendations [20]. The selectivity was evaluated using interference tests.

Table III presents the figures of merit that were evaluated for total As and As(III).

Table III. Figures of merit of the arsenic analysis methods (n=3).

	Determination of Total Arsenic	Determination of Trivalent Arsenic
Linear Range	0 – 20.0 μg L^{-1}	0 – 50.0 μg L^{-1}
Working Range	4.0 – 20.0 μg L^{-1}	3.0 – 50.0 μg L^{-1}
R	0.996	0.998
R^2	0.992	0.997
LOD	1.3 μg L^{-1}	2.0 μg L^{-1}
LOQ	4.0 μg L^{-1}	6.5 μg L^{-1}
Recovery	98.0%	96.0%
RSD (repeatability)	3.1%	4.0%
RSD (intermediate precision)	3.8%	4.9%
Sensitivity	0.198	0.045

The LOD and LOQ obtained differed for total As and As(III). This difference can be explained by the different experimental conditions, such as the pH and acid concentration used in the generation of hydride. The experimental conditions used for the determination of trivalent arsenic were milder than those used for the determination of total arsenic. Therefore, the proposed methodology is more sensitive for total arsenic, resulting in lower LOD and LOQ.

The total arsenic concentration in the certified reference material SRM NIST 1643e (Trace Elements in Water) is 60.45 ± 0.72 μg L^{-1}, and in this work, a concentration of 59.6 ± 1.9 μg L^{-1} was obtained using HG AAS, corresponding to a recovery of 98.5%. These values are in agreement by the Student-*t* test at the 95% confidence level. Measurements in water were also conducted using ICP-MS.The result for SRM NIST 1643e was 63.8 ± 3.7 μg L^{-1}. The samples were also analyzed using ICP-MS, and the results are consistent with the HG AAS results (Table V).

To evaluate the accuracy of the methodology for the determination of trivalent arsenic, recovery tests were performed for trivalent and pentavalent arsenic species. Aliquots from the standard solutions were added to real samples. The concentrations in the spiked samples were subtracted from the value of the sample without fortification and compared with the expected value at a 95% confidence level. In the determination of total As, fortified solutions were prepared with 10 μg L^{-1} of As(III) and As(V), which was followed by mixing the two species (10 μg L^{-1} each) in triplicate. The results were also compared with the certified reference material NIST 1643e. For the determination of As(III), the concentration added was 25 μg L^{-1}. The solutions were subjected to the proposed methodology for the determination of total and trivalent arsenic, and the mean recoveries were 98% and 96%, respectively.

The precision was tested by measuring the repeatability and intermediate precision. To determine the repeatability, the analysis was conducted under the same conditions and on same day in triplicate. To check the intermediate precision, the same solutions were analyzed by the same analyst under the same instrumental conditions and on different days. Both the repeatability and the intermediate precision were expressed as the percentage relative standard deviation of the readings (RSD). In both cases, the RSD values were less than 5% for total As and As(III).

The detectivity reflects the variation in the response as a function of the analyte concentration, and it is expressed by the slope of the regression curve. The detectivity values were 0.198 and 0.045 for total arsenic and trivalent arsenic, respectively. For trivalent arsenic, the detectivity was low, which is consistent with the formation of hydride under mild conditions.

To determine the selectivity, the effect of ions commonly found in water was evaluated. The results are presented in Table IV.

Table IV. Effect of foreign ions on the % recoveries of 10 μg L^{-1} total As and As(III).

Ion	Added Concentration (mg L^{-1})	% Recovery Total As	% Recovery As(III)
Na^+	1000	97	95
K^+	1000	98	96
Ca^{2+}	100	98	94
Mg^{2+}	100	97	105
Co^{2+}	10	102	108
Cu^{2+}	10	103	107
Fe^{3+}	10	92	91
Ni^{2+}	10	98	110
Al^{3+}	10	95	95
SO_4^{2-}	100	97	101
PO_4^{3-}	100	104	105
Cl^-	1000	95	95
F^-	1000	98	97

The ions Na^+, K^+, Ca^{2+}, Mg^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Al^{3+}, SO_4^{2-}, PO_4^{3-}, Cl^- and F^- were evaluated, and only Fe^{3+} caused a variation of greater than 5% in the total arsenic signal. However, for the trivalent arsenic measurements, Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+} and Ni^{2+} caused variations between 5-10% in the analytical signal. The proposed methodology showed good selectivity in the presence of the ions evaluated.

3.4. Total and trivalent arsenic analysis in water samples

The results obtained for water samples from an impacted gold mining area are presented in Table V.

Table V. Comparison of the total arsenic measurements using ICP-MS and HGAAS, As(III) concentrations using HGAAS and As(V) concentrations in water samples from an impacted gold mining area.

Samples	Total As (µg L^{-1})		HG AAS	
	ICP-MS	HGAAS	As(III) (µg L^{-1})	As(V) (µg L^{-1})
Asup 01	18.7 ± 0.5	17.3 ± 0.9	8.5 ± 0.4	8.8 ± 1
Asup 02	54.8 ± 0.2	54.4 ± 2.2	21.8 ± 1	32.6 ± 2.4
Asup 03	40 ± 0.1	37.9 ± 1.1	13.4 ± 0.7	24.6 ± 1.3
Asup 04	3.19 ± 0.03	1.6 ± 0.21	< LD	< LD
Asup 05	9.21 ± 0.13	8.2 ± 0.6	3.8 ± 1.1	4.4 ± 1.3
Asup 06	28.6 ± 0.9	28.5 ± 1	13.5 ± 0.6	15 ± 1.2
Asup 07	0.554 ± 0.007	< LD	< LD	< LD
Asup 08	110 ± 0.3	113 ± 4	53.7 ± 2.2	59.3 ± 4.6
Asup 09	73.7 ± 3.2	72.5 ± 2.7	13.6 ± 0.5	58.9 ± 2.7
Asub 01	0.379 ± 0.022	< LD	< LD	< LD
Asub 02	15.7 ± 1.2	14.1 ± 0.8	9.9 ± 0.5	4.2 ± 0.9
Asub 03	1.62 ± 0.03	< LD	< LD	< LD
Asub 04	1.11 ± 0.04	< LD	< LD	< LD
Abica	0.265 ± 0.031	< LD	< LD	< LD
1643e				
Certified	60.45 ± 0.72			
Obtained	63.8 ± 3.7	59.6 ± 1.9		

There is a predominance of As(V) in the analyzed samples. These results are consistent with other papers [3,21,24,25]. Tuzen *et al*. [3] and Liang *et al*. [21] analyzed superficial water, and Borba *et al*. [24] and Deschamps and Matschullat [25] analyzed superficial water and ground water. This information is relevant because As(V) is less toxic and less mobile than As(III).

A good correlation was observed in the results presented in Table V for total arsenic determined using ICP-MS and HGAAS. The results were consistent at the 95% confidence level when applying the Student- *t* test. It can be observed that all measurements using HGAAS were slightly lower than those obtained by ICP-MS, which is most likely due to matrix effects.

According to the quality limits of CONAMA resolutions 396/2008 and 397/2008, six samples of surface water (Asup 01, Asup 02, Asup 03, Asup 06, Asup 08 and Asup 09) exceeded the tolerable limit established for class 1 and 2 (10 µg L^{-1}), and four of these samples also exceeded the value for class 3 (33 µg L^{-1}). The Asup 02 ground water sample also exceeded the tolerable limit for the human consumption for arsenic (10 µg L^{-1}), but the concentration obtained was less than the standard for watering livestock (200 µg L^{-1}) and recreation (1000 µg L^{-1}). The water samples presented arsenic concentrations ranging from 1.6 µg L^{-1} to 113 µg L^{-1}, with a predominance of the pentavalent species, the less toxic species; however, five samples presented As(III) concentrations up to the established limit for human consumption.

The studied area has housed important gold mining for over two centuries, in which there are naturally high levels of arsenopyrite, FeAsS [26,27]. The concentrations are greater than the legal limits in some sites, this is more likely due to the availability of natural arsenopyrite in the region than from mining activities.

The proposed speciation methodology is simple and efficient for the determination of inorganic arsenic species in water, and it was successfully applied to real samples. From the arsenic concentration range in the analytical curve, it can be observed that there is no need for a more sensitive technique such as ICP-MS for detection. Also it highlights that the inductively coupled plasma optical emission spectrometry (ICP OES) technique has not sufficient detectivity for measuring both total and trivalent arsenic in this concentration range. Note that the conditions established here could be used in laboratories that are not equipped with an ICP-MS and or ICP OES.

4. Conclusions

The proposed methodology for arsenic speciation based on selective hydride generation is simple, inexpensive and efficient, and it presents satisfactory figures of merit, especially precision and accuracy. The optimization of the instrumental conditions enabled analyses using lower concentrations of reagents than those described in the literature (0.2% w/v $NaBH_4$ a rate of 4-6 mL min^{-1} and 10% v/v HCl at a rate of 8-12 mL min^{-1}).

Water samples from an impacted gold mining area presented dissolved arsenic concentrations ranging from 1.6 to 113 µg L^{-1}, with a predominance of the pentavalent species, which is less toxic and presents less mobility.

Acknowledgments

The authors would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and the PRPq (Pró Reitoria de Pesquisa da UFMG) for providing financial support.

References

1. Baig, J.A.; Kazi, T.G.; Shah, A.Q.; Arain, M.B.; Afridi, H.I.; Kandhro, G.A.; Khan, S.; *Anal. Chim. Acta*, 2009,*651*, 57.
2. Maity, S.; Chakravarty, S.; Takur, P.; Gupta, K.K.; Bhattacharjee, S.; Roy, B.C.; *Chemosphere,* 2004,54,1199.
3. Tuzen, M.; Saygi, K.O.; Karaman, I.; Soylak, M.; *Food Chem. Toxicol.*, 2010, 48, 41.
4. Liu, G.; Cai, Y.; *Developments in Environmental Science*, Vol. 5; Elsevier, Amsterdam 2007 Chapter 31.
5. Leemarkers, M.; Baeyens, W.; de Gieter, M.; Smedts, B.; Meert, C.; de Bisschop,H. C.; Morabito, R.; Quevauviller, Ph.; *Trends Anal. Chem.*, 2006, 25, 1.
6. Popp, M.; Hann, S.; Koellensperger, G.; *Anal. Chim. Acta*, 2010, 668,114.
7. Rivas, R.E.; López-García, I.; Hernández-Córdoba, M. *Spectrochim. Acta Part B*, 2009, 64, 329.
8. Pu, X.; Chen, B.; Hu, B.; *Spectrochim. Acta Part B*, 2009, 64, 679.
9. Pereira, J.C.; Guimarães-Silva, A.K.; Nalini Jr, H.A.; Pacheco-Silva, E.; Lena, J. C.; *Quim. Nova*, 2007, 30, 1249.
10. Gonzalvez, A.; Cervera, M.L.; Armenta, S.; de la Guardia, M.; *Anal. Chim. Acta*, 2009, 636, 129.
11. Vieira, M.A.; Grinberg, P.; Bobeda, C.R.R.; Reyes, M.N.M.; Campos, R.C.; *Spectrochim. Acta Part B*, 2009, 64,459.
12. Frentiu, T.; Vlad, S.N.; Ponta, M.; Baciu, C.; Kasler, I.; Cordos, E.; *Chem. Pap.*, 2007, 61, 186.
13. Quináia, S. P.; Rollemberg, M. C. E.; *J. Braz. Chem. Soc.*, 1997, 8, 349.
14. Quináia, S. P.; Rollemberg, M. C. E.; *J. Braz. Chem. Soc.*, 2001, 12, 37.
15. Moretto, A.; Cadore, S.; *Microchim. Acta*, 2004, 146, 239.
16. Bortoleto, G. G.; Cadore, S. *Talanta*, 2005, 67, 169.
17. Macarovscha, G. T.; Bortoleto, G. G.; Cadore, S. *Talanta*, 2007, 71, 1150.
18. Dedina, J.; Tsalev, D.; *Hydride Generation Atomic Absorption Spectrometry*, John Wiley & Sons: Chichester, 1995.
19. INMETRO. *Orientações sobre validação de métodos e ensaios químicos*, 2006.
20. IUPAC. *Pure Appl. Chem.*, 2002, 74, 835.
21. Liang, P.; Peng, L.; Yan, P.; *Microchim. Acta*, 2009, 166, 47.
22. Bednar, A.J.; Garbarino, J.R.; Ranville, J.F.; Wildeman, T.R.; *Environ. Sci. Technol.*, 2002, 36, 2213.
23. Huang, J.; Ilgen, G.; *Anal. Chim. Acta*, 2004, 512, 1.
24. Borba, R. P.; Coscione, A. R.; Figueiredo, B. R.; Zambello, F.; *Quim. Nova*, 2009, 32, 970.
25. Deschamps, E.; Matschullat,J.; *Arsênio antropogênico e natural: Um estudo em regiões do Quadrilátero Ferrífero*, FEAM – Fundação Estadual de Meio Ambiente: Belo Horizonte, 2007.
26. Mello, J.W.V.; Roy, W.R.; Talbott, J.L.; Scott, J.; Stucki,J.W.; *J. Soils Sediments*, 2006, 6, 9.
27. Andrade, R.P.; Figueiredo, B.R.; Mello, J.W.V.; Santos, J.C.Z.; Zandonadi,L.U.; *J. Soils Sediments*, 2008, 8, 123.

Br J Anal Chem 2013, 10, 436–440

Simultaneous determination of chromium and nickel in medicinal plants by slurry sampling graphite furnace atomic absorption spectrometry

Felipe Manfroi Fortunato[A], **Kelber Miranda**[A], **José Anchieta Gomes Neto**[A,*], **Gian Paulo Giovanni Freschi**[B]
A) *São Paulo State University – Unesp, Analytical Chemistry Department – P.O. Box 355, 14801-970 Araraquara – SP, Brazil*
B) *Alfenas Federal University – UNIFAL-MG, Institute of Science and Technology – 37715-400 Poços de Caldas – MG, Brazil*

Abstract

Slurry sampling graphite furnace atomic absorption spectrometry was employed for the simultaneous determination of Cr and Ni in medicinal plants. A chemical modifier was not required and the optimum pyrolysis and atomization temperatures were 1600 °C and 2300 °C, respectively. Calibration using aqueous standards allowed accurate and precise results. Characteristic masses for Cr and Ni were 5.8 and 31 pg, respectively. The limits of detection obtained were 0.04 $\mu g\ g^{-1}$ for Cr and 0.3 $\mu g\ g^{-1}$ for Ni using 0.5% (m/v) slurries (100 mg/20 mL). Relative standard deviations of 3 measurements for Cr and Ni in medicinal plant slurries were below 10%. Accuracy was evaluated after analyzing the standard reference materials Tomato Leaves (1573a), Apple Leaves (1515), Pine Needles (1575a) and Peach Leaves (1547). The results obtained were in agreement with certified values at a 95% confidence level (unpaired *t*-test). The proposed method was applied to slurries of ten medicinal plant samples and the concentrations varied from 1.76 to 11.6 $\mu g\ g^{-1}$ for Cr and from 1.2 to 5.5 $\mu g\ g^{-1}$ for Ni. The lifetime of the graphite atomizer was around 600 firings.

Keywords: Chromium; Nickel; Medicinal plants; Slurry sampling; Graphite furnace atomic absorption spectrometry

***Corresponding Author**
Phone: 55 16 33019611
Fax: 55 16 33019692
E-mail Address:
anchieta@iq.unesp.br

1. Introduction

The consumption of medicinal plants for the treatment of several diseases or other health benefits has increased worldwide in the last decades due to lower risk of side-effects than those caused by synthetic pharmaceutical formulations as well as higher costs of conventional drugs [1,2]. From the cultivation to the final product, many factors may influence the metallic composition of herbal medicines, such as the conditions in which the medicinal plants are grown or collected, the storage and transport circumstances, and the processing of raw materials for the finished products [2,3]. Considering that both deficiencies and excesses of essential micronutrients and traces of toxic metals may cause undesirable effects on human health, it is crucial to control the inorganic composition in medicinal raw materials in order to assure quality, safety and efficacy of herbal medicines [2,3].

Chromium is an essential nutrient with functions in carbohydrate, lipid and nucleic metabolisms. High Cr levels, particularly as hexavalent chromium, are associated with gastrointestinal and lung cancer [4,5,6]. Nickel is an essential trace metal for several animal species but its deficiency state in humans has not been described in the literature. Nickel is a moderately toxic element and when present in high concentration causes a skin disorder known as “nickel-eczema” [4,5,6]. Taking into consideration the toxic effects of Cr and Ni on human health, the development of simple, fast and accurate analytical methods to assess the quality of medicinal plants is relevant [7]. The low concentrations of Cr and Ni usually found in most plants [6] require sensitive analytical techniques such as inductively coupled plasma mass spectrometry (ICP-MS) or graphite furnace atomic absorption spectrometry (GF AAS). GF AAS stands out due to good detectivity, selectivity, simplicity and capability for slurry analysis of solid samples [8]. Slurry sampling GF AAS does not require hazardous reagents for sample preparation, reduces the overall time of analysis, minimizes the risk of sample contamination, improves analytical blanks and generates minimum waste [8-12]. The applicability of GF AAS has been further extended with the development of simultaneous multi-element instruments [9].

The current literature reports several papers about slurry sampling for the determination of Cr or Ni in different matrices such as airborne particulate matter [13], biological [14], and composted materials [15], fish feces [16], plant [10], sediment [17-19], soil [17,18,20], titanium dioxide [21], tungsten trioxide [22] and wheat flour [23]. However, these methodologies are limited to a single-element determination. There are a few publications dealing with the simultaneous determination of Cr and Ni by GF AAS [9,24-26] but none of them employs slurry sampling.

This paper reports the development of a simple and fast method for the simultaneous determination of Cr and Ni in medicinal plants employing slurry sampling GF AAS with a transversely heated graphite atomizer and longitudinal Zeeman-effect background correction. The performance of the proposed method was evaluated by analyzing standard reference materials. The method was then applied for the determination of Cr and Ni in medicinal plant samples.

2. Experimental

2.1. Reagents and analytical solutions

High purity water (resistivity 18.2 MΩ cm) was obtained using a Millipore® Rios 5 Reverse Osmosis™ and a Millipore Milli-Q™ Academic™ system (Bedford, MA, USA). Suprapur® nitric acid (Merck, Darmstadt, Germany) was employed to prepare all solutions. Analytical solutions used for calibration (5.0, 15.0, 25.0, 40.0 and 50.0 µg L^{-1} Cr and Ni) were prepared in 0.14 mol L^{-1} HNO_3 after successive dilutions of the metal standard stock solutions (1000 mg L^{-1}). The autosampler washing solution was 0.1% (v/v) Triton®X-100 in 0.14 mol L^{-1} HNO_3. All solutions were stored in Nalgene® high-density polypropylene bottles (Rochester, NY, USA). All glassware and polypropylene flasks were soaked in 10% (v/v) HNO_3 for 24 h and rinsed with deionized water prior to use.

2.2. Instrumentation

A PerkinElmer SIMAA™ 6000 simultaneous multi-element atomic absorption spectrometer equipped with a transversely heated graphite atomizer (THGA), longitudinal Zeeman-effect background (BG) correction, and an AS-72 autosampler (PerkinElmer Life and Analytical Instruments, Shelton, CT, USA) were used. Absorbance was measured at 357.9 nm (Cr) and 232.0 nm (Ni) using PerkinElmer Lumina™ hollow cathode lamps (HCL) with lamp currents of 25 mA for both elements. High-purity argon (99.99%, White Martins, Sertãozinho, Brazil) was used as the protective and purge gas with a 250 mL min^{-1} flow rate. It should be pointed out that the experiments were carried out using the stabilized temperature platform furnace (STPF) conditions [27]. A PerkinElmer USS-100™ ultrasonic slurry sampler with titanium tip was used to promote homogenization of particles during sampling.

2.3. Sample preparation and standard reference materials

Medicinal plant samples of *Annona muricata* (graviola), *Baccharis trimera* (carqueja), *Bauhinia forticata* (pata de vaca), *Echinodorus grandiflorus* (chapéu de couro), *Equisetum arvense* (cavalinha), *Matricaria chamomilla* (camomila), *Melissa officinalis* (erva-cidreira), *Peumus boldus* (boldo), *Pimpinela anisun* (erva-doce) *and Mikania glomerata* (guaco) were purchased at a local market in Araraquara, São Paulo, Brazil.

All samples were dried at 50 °C for 48 h in a forced air oven and ground in a cutting mill fitted with a 20 mesh screen at the bottom of the cutting chamber. Thereafter samples were powdered in a Spex 6750 cryogenic mill (Metuchen, NJ, USA) using a program developed for plant materials [28]. Samples of 100 mg were accurately weighed and dispersed in 20 mL of a 0.2% (v/v) HNO_3 / 0.05% (v/v) Triton®X-100 solution. Slurries of 1515 Apple Leaves, 1547 Peach Leaves, 1575a Pine Needles and 1573a Tomato Leaves standard reference materials (SRMs) from National Institute of Standards and Technology (Gaithersburg, MD, USA) were similarly prepared.

2.4. Analytical Method

All atomic absorption measurements were made in triplicate and based on peak area mode. Experiments involving permanent modifiers were carried out by coating the platform surface of the graphite atomizer with 500 µg Ir and Rh as previously described [29]. For absorbance measurements, 20 µL of analytical solutions, slurry samples and blanks were dispensed onto the graphite platform. Samples and SRM slurries were manually homogenized and transferred to autosampler cups. An ultrasound probe operated at 40% amplitude was automatically introduced into the slurry sample for homogenization during 10 s immediately before sampling by the autosampler arm. The thermal behavior of Cr and Ni was evaluated by means of pyrolysis and atomization temperature curves in order to select the optimum pyrolysis and atomization temperatures. Pyrolysis and atomization curves were constructed in the following media: a) 0.2% (v/v) HNO_3 aqueous solution containing 10 µg L^{-1} Cr + 50 µg L^{-1} Ni; and b) 0.5% (m/v) plant slurries of 1573a Tomato Leaves in 0.2% (v/v) HNO_3 / 0.05% (v/v) Triton®X-100 spiked with 50 µg L^{-1} Ni. The optimized heating program of the graphite tube (temperature, °C; ramp time, s; hold time, s) was: drying, step 1 (110; 1; 30); drying, step 2 (130; 10; 30); air-assisted pyrolysis, step 3 (600; 10; 40); purge gas, step 4 (40; 5; 35); argon-assisted pyrolysis, step 5 (1600; 10; 10); atomization, step 6 (2300; 0; 5); and cleaning, step 7 (2500; 1; 5). The argon flow-rate was 250 mL min^{-1} at steps 1, 2, 4, 5 and 7. For atomization (reading), the Ar flow-rate was stopped. Air flow-rate was 250 ml min^{-1} (step 3).

Matrix effects were evaluated by comparing the slopes of the analytical curves (5.0 - 50 µg L^{-1}) prepared in 0.2% (v/v) HNO_3 aqueous solution, and 0.5% (m/v) slurries in 0.2% (v/v) HNO_3 / 0.05% (v/v) Triton®X-100. The maximum concentration of powdered samples in the slurries was evaluated by analyzing slurries prepared in the 0.5 – 2.0% (m/v) concentration range.

After optimization, the accuracy of the proposed method was evaluated by using four SRMs. Then, the method was applied to ten medicinal plant samples. The limit of detection (LOD) was defined as the concentration corresponding to 3 times the standard deviation of 10 measurements of the blank divided by the slope of the calibration curve [30].

3. Results and discussion

Conventional modifiers usually do not improve the thermal stability of refractory elements and may be dispensable [15]. On the other hand, permanent coatings over the integrated platform may increase the useful lifetime of the graphite tube and promote catalytic decomposition of the sample [31]. Preliminary tests were carried out using Ir- and Rh-coated platforms. However, these modifiers inhibited release of the analytes from the platform even at the 2600 °C atomization temperature (the maximum temperature available in the equipment software), resulting in poor atomization and loss of detectivity. Thus, the method was developed without any modifier. In order to help the complete removal of the organic matrices, an air-assisted pyrolysis at 600 ºC (step 3) was added as the first pyrolysis step. It should be noted that temperatures above 600 °C should not be used in the presence of air, otherwise the atomizer is ashed. Therefore, another step was included to exchange the gases (from air to Ar), obtaining an inert environment (step 4). The use of the ultrasonic probe was important to ensure adequate homogeneity of the slurry. Preliminary tests revealed that sonication for 10 s was sufficient to generate reproducible analytical signals.

3.1. Electrothermal behavior

In simultaneous GF AAS determinations, the atomizer heating program is the same for all analytes, and the optimized pyrolysis and atomization temperatures may not coincide with those observed in single-element conditions. In most cases, the pyrolysis and atomization temperatures are selected taking into consideration the required detectivity, accuracy and precision. The thermochemical behaviors of Cr and Ni in aqueous and slurry media are illustrated in Figure 1. According to Figure 1, Cr and Ni were stabilized in both media up to 1600 °C, the temperature selected for further studies.

The better atomization temperature was observed at 2300 °C since it provided the highest absorbance, the narrowest transient peaks and the lowest relative standard deviation (RSD) for Cr (< 3%) and Ni (< 1%). Analysis of Figure 1 reveals a decrease in absorbance of Ni and Cr at temperatures higher than 2200 °C due to diffusional loss of analytes that was more pronounced in slurries than for aqueous standards. The sample matrices contributed to more atoms or molecular species inside the atomizer, increasing the probability of collisions and further diffusion.

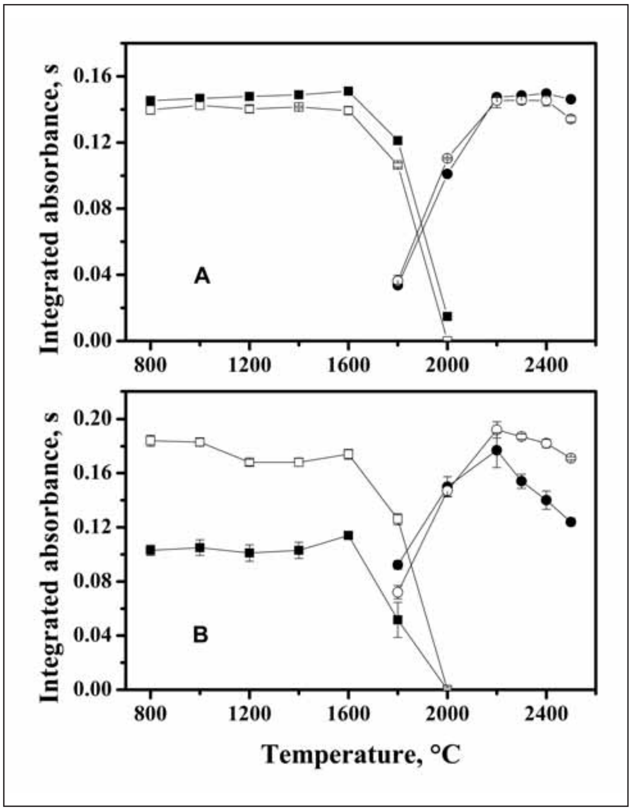


Figure 1. Pyrolysis (■: Cr; □: Ni) and atomization (●: Cr; ○: Ni) temperature curves in aqueous solution (A) and slurry (B) media. Curves in A correspond to 10 µg L^{-1} Cr + 50 µg L^{-1} Ni in 0.2% (v/v) HNO_3. Curves in B correspond to 0.5% (m/v) slurries of Tomato Leaves enriched with 50 µg L^{-1} Ni in 0.05% (v/v) Triton®X-100 + 0.2% (v/v) HNO_3.

3.2. Matrix effects

In order to evaluate matrix effects, the slope of the calibration curve obtained with aqueous solutions was compared with those acquired from slurries of *Baccharis trimera* and *Matricaria chamomilla*. Analytical curves with slurries were established by spiking both samples with increasing concentrations of Cr and Ni. Slopes corresponding to these three media were 0.0130 ± 0.0005, 0.0131 ± 0.0004 and 0.0132 ± 0.0007 for Cr and 0.0029 ± 0.0001, 0.0030 ± 0.0001 and 0.00284 ± 0.00006 for Ni, respectively. A paired t-test (*n*=3) showed no differences between the slope of the analytical curve obtained with aqueous solutions when compared with those acquired with slurries at a 95% confidence level. Taking into consideration the benefits of slurry sampling, external calibration with aqueous standards was adopted for further studies.

3.3. Effect of slurry concentration

The influence of the concentration of powdered samples in the slurries was evaluated by analyzing slurries prepared in the 0.5 – 2.0% (m/v) concentration range. Results for Cr obtained for all concentrations were in agreement at 95% confidence level (*t*-Student test) with the certified value (1.99 ± 0.06) of SRM Tomato Leaves (Figure 2A). With respect to Ni, slurries with concentrations higher than 0.5% (m/v) improved the precision of measurements but the accuracy deteriorated. Results for Ni with 0.5% (m/v) slurries were in agreement with the certified value (1.51 ± 0.10) of SRM Tomato Leaves (Figure 2B) at 95% confidence level. The characteristic masses for Ni and Cr in aqueous media are 20 pg and 7 pg, respectively [32]. For slurries, these values we 31 and 5.8 pg, respectively. The precision increased with slurry concentration due to the increased amount of Ni atoms inside atomizer, but the accuracy deteriorated due to increased matrix effects. Besides poorer detectivity, the high matrix effect contributed to inhibit Ni atomization. Hence, the maximum tolerant concentration of powdered samples in the slurries was 0.5% (100 mg/20 mL) for the simultaneous determination of Cr and Ni.

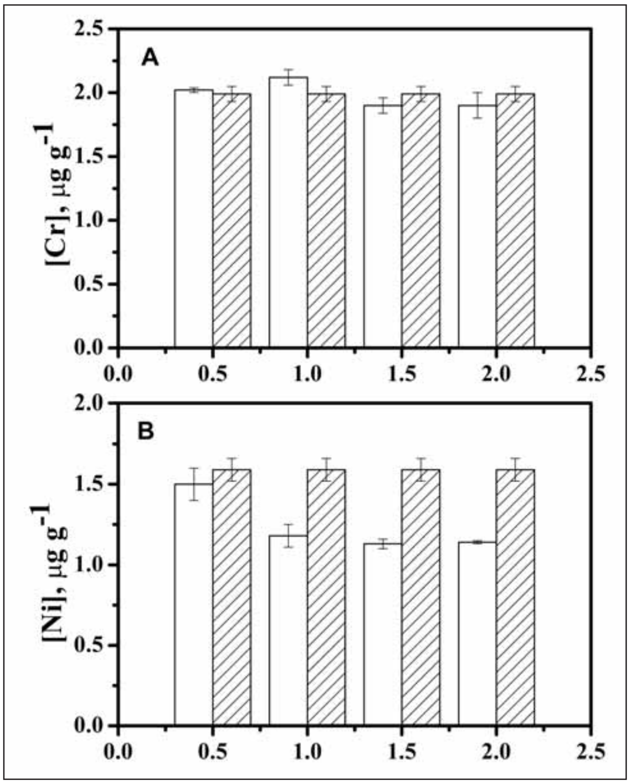


Figure 2. Influence of slurry concentration on simultaneous determination of Cr (A) and Ni (B) by GF AAS (▨ certified; ☐ found).

3.4. Validation studies

For 20 µL aliquots of 0.5 % m/v slurries (100 µg sample mass) delivered into the platform, working ranges (dry basis) for Cr and Ni were respectively 0.13 - 10 µg g^{-1} (r=0.9992) and 1.0 - 10 µg g^{-1} (r=0.9995). The limits of detection/limits of quantification were 0.04/0.13 µg g^{-1} and 0.3/1.0 µg g^{-1} for Cr and Ni, respectively. The calculated characteristic masses were 5.8 pg Cr and 31 pg Ni. Relative standard deviations of 3 measurements for both Cr and Ni in medicinal plant slurries were below 10%. The useful lifetime of the graphite tube was equivalent to 600 firings. The RSD for Cr and Ni varied in 1.9 - 8.4% and 2.2 - 9.9% intervals, respectively. It is a good practice to check the quality of the atomizer by monitoring the RSD values: when RSD is typically > 10%, the atomizer must be checked and changed. Accuracy of the proposed method for the simultaneous determination of Cr and Ni was checked after analyzing four plant SRMs. The results obtained can be seen in Table I. A unpaired-*t* test showed that the results were in agreement with certified values at a 95% confidence level. It should be mentioned that SRM Tomato Leaves was employed to optimize the procedure. The chemical composition varies from Tomato Leaves to the selected SRMs and medicinal plants. Analysis of four plant SRMs presenting different matrices showed matrix effects were not observed since accurate results were achieved. The matrix of Tomato Leaves may match the matrices of selected SRMs and samples, so that variations in extraction efficiency of analytes to the aqueous medium and in atomization efficiency were unimportant.

Table I. Results (n=3[a]) obtained for Cr and Ni in SRMs using the proposed method.

SRM	[Cr], µg g^{-1}		[Ni], µg g^{-1}	
	Found	Certified	Found	Certified
Tomato Leaves	2.02 ± 0.03	1.99 ± 0.06	1.59 ± 0.07	1.51 ± 0.10
Apple Leaves	0.38 ± 0.01	0.3[b]	< 1.0[c]	0.91 ± 0.12
Pine Needles	0.47 ± 0.03	(0.3-0.5)[b]	1.2 ± 0.2	1.47 ± 0.10
Peach Leaves	1.17 ± 0.02	1.0[b]	< 1.0[c]	0.69 ± 0.09

[a] SRM prepared in triplicate; [b] Non certified; [c] Limit of quantification.

3.5. Application of the method

The method was applied to ten medicinal plant samples. All the results are summarized in Table II. The concentrations ranged from 1.76 to 11.6 µg g^{-1} for Cr and from 1.2 to 5.5 µg g^{-1} for Ni.

Table II. Results (*n*=3[a]) obtained for Cr and Ni in medicinal plant samples.

Samples	[Cr], µg g^{-1}	[Ni], µg g^{-1}
Annona muricata	3.58 ± 0.30	2.67 ± 0.07
Baccharis trimera	2.5 ± 0.2	< 1.0[b]
Bauhinia forticata	8.8 ± 0.4	3.26 ± 0.08
Echinodorus grandiflorus	4.3 ± 0.3	2.12 ± 0.08
Equisetum arvense	8.8 ± 0.4	3.92 ± 0.05
Matricaria chamomilla	1.76 ± 0.08	1.7 ± 0.2
Melissa officinalis	11.6 ± 0.2	4.6 ± 0.2
Mikania glomerata	3.36 ± 0.09	1.2 ± 0.1
Peumus boldus	3.5 ± 0.1	1.7 ± 0.1
Pimpinela anisun	5.7 ± 0.2	5.5 ± 0.1

[a] Samples prepared in triplicate. [b] Limit of quantification

4. Conclusion

This work describes a simple and fast method for the simultaneous determination of Cr and Ni in medicinal plants by slurry sampling GF AAS. A chemical modifier was not necessary and matrix effects were unimportant. Calibration using aqueous standards allowed accurate and precise results. The limit of quantification for Ni (1.0 μg g^{-1}) was higher than that obtained for Cr (0.13 μg g^{-1}) but the proposed method was adequate to determine both analytes in medicinal plant samples. Besides the reduced risk of sample contamination and reduced analysis time, slurry sampling GF AAS may be considered a clean analytical method, since hazardous reagents are not employed and the generation of waste is minimum.

Acknowledgements

The authors thank Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (Grant 2009/52480-0) for financial support. Authors are also grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the scholarship and research grants to F.M.F. and J.A.G.N., respectively.

References

1. Chan, K. Chemosphere, 2003, 52, 1361.
2. Zhang, J.; Wider, B.; Shang, H.; Li, X.; Ernst, E. *Complement. Ther. Med.,* 2012, 20, 100.
3. Rao, M.M.; KumarMeena, A.; Galib *Environ. Monit. Assess.,* 2011, 181, 267.
4. Fariñas, M.V.; Garcías, J.B.; Martíns, S.G.; Crecente, R.M.P.; Latorre, C.H. *Food Chem.,* 2008, 110, 177.
5. Azevedo, F.A.; Chasin, A.A.M. Metais - *Gerenciamento da toxicidade,* Rio de Janeiro: Atheneu, 2003.554p.
6. Farago, M.E. *Plants and the Chemical Elements,* Weinheim: Wiley-VHC, 1994. 292p.
7. World Health Organization, *Quality control methods for medicinal plant materials,* Geneva, 1998. 122p.
8. Cal-Prieto, A.M.J.; Felipe-Sotelo, M.; Carlosena, A.; Andrade, J.M.; López-Mahía, P.; Muniategui, S.; Pradas D. *Talanta*, 2002, 56, 1.
9. Amorin Filho, V.R.; Fernandes, K.G.; Moraes, M.; Gomes Neto, J.A. *J. Braz. Chem. Soc.,* 2004, 15, 28.
10. Sánchez-Moreno, R.A.; Gismera, M.J.; Sevilla, M.T.; Procopio, J.R. *Phytochem. Anal.,* 2010, 21, 340.
11. Tsalev, D.L., Ivanova, E. *J. Anal. At. Spectrom.,* 2012, 27, 1645.
12. Resano, M.; Vanhaecke, F.; de Loos-Vollebregt, M. T. C. *J. Anal. At. Spectrom.*, 2008, 23, 1450.
13. Pancras, J.P.; Ondov, J.M.; Zeisler, R. *Anal. Chim. Acta,* 2005, 538, 303.
14. Stupar, J.; Dolingek, F. *Spectrochim. Acta Part B,* 1996, 51, 665.
15. Laborda, F.; Górriz, M.P.; Castillo, J.R. *Talanta,* 2004, 64, 631.
16. Silva, F.A.; Padilha, C.C.F.; Pezzato, L.E.; Barros, M.M.; Padilha, P.M. *Talanta,* 2006, 69, 1025.
17. Mierzwa, J.; Sun, Y.C.; Yang, M.H. *Anal. Chim. Acta,* 1997, 355, 277.
18. Felipe-Sotelo, M.; Carlosena, A.; Andrade, J.M.; Cal-Prieto, M.J.; Fernández, E.; Prada, D. *Microchem. J.*, 2005, 81, 217.
19. Pazos-Capeáns, P.; Barciela-Alonso, M.C.; Bermejo-Barrera, A.; Bermejo-Barrera, P. *Anal. Chim. Acta,* 2004, 524, 121.
20. Dobrowolski, R. *Spectrochim. Acta Part B,* 1996, 51, 221.
21. Zhu, X.; Hu, B.; Wang, L.; Li, S.; Jiang, Z. Fresenius *J. Anal. Chem.*, 2001, 371, 497.
22. Krivan, V.; Heger, A.; Hauptkorn, S. Fresenius *J. Anal. Chem.,* 1998, 360, 167.
23. González, M.; Gallego, M.; Valcárcel, M. *Talanta,* 1999, 48, 1051.
24. Lehndorff, E.; Schwark , L. *Atmos. Environ.,* 2010 , 44, 2822.
25. Anselmi , A.; Tittarelli, P.; Katskov, D.A. *Spectrochim. Acta Part B*, 2002, 57, 403.
26. Álvarez, M.A.; Hermida, J. *Quim. Nova,* 2011, 34, 940.
27. Slavin, W.; Manning, D.C.; Carnrick, G.R. *At. Spectrosc.,* 1981, 2, 137.
28. Ambrozini, B.; Amorin Filho, V.R.; Oliveira, S.R.; Sacramento, L.V.S.; Gomes Neto, J.A. *Food Chem.,* 2009, 116, 1024.
29. Freschi, G.P.G.; Freschi, C.D.; Oliveira, S.R.; Gomes Neto, J.A. *At. Spectrosc.*, 2006, 27, 179.
30. Analytical Methods Committee, *Analyst*, 1987,112, 199.
31. Volynsky, A.B. *Spectrochim. Acta, Part B*, 1998, 53, 509.
32. Perkin Elmer; *Recommended Conditions for THGA Furnaces*, Perkin Elmer Publication N. B3110.06: Überlingen, 1991.

Different methods for extracting oil from the microalga *Scenedesmus accuminatus* for biodiesel production

Tatiana Rodrigues da Silva Baumgärtner[B*], **Jorge Augusto Mendes Burak**[A], **Gisella Maria Zanin**[A], **Dirceu Baumgärtner**[B], **Nyamien Yahaut Sébastien**[B], **Pedro Augusto Arroyo**[A]

A) Departamento de Engenharia Química, Universidade Estadual de Maringá – UEM, Av. Colombo, 5790, Bloco D90, 87020-900 Maringá, PR, Brazil.

B) Centro de Engenharias e Ciências Exatas, Universidade Estadual do Oeste do Paraná – Unioeste, Rua da Faculdade 645, 85903-000 Toledo, PR, Brazil.

***Correspoding author:**
Phone: 55 45 99155218
Email Address:
tati.rb@hotmail.com

Abstract

The present study aimed at applying three different methods for total lipid extraction from the microalga *Scenedesmus acuminatus*, in order to produce biodiesel. The oil was extracted and characterized. We tested the following extraction methods: Bligh and Dyer, extraction with Soxhlet, and successive extraction. For the last two methods, the tested solvents were hexane, ethyl ether, and dichloromethane. Oil characterization was carried out through analysis of fatty acid profiles, acidities, and molar masses. We observed that solvent polarity affects the quality and the quantity of the total lipids extracted. Analysis of the fatty acid profile of the oil was essential to determine the best extraction method. Successive extractions, using hexane as a solvent, was better than the other methods.

Keywords: extraction, oil, microalgae, *Scenedesmus acuminatus,* method.

1. Introduction

Recently a lot of interest has been drawn to the biotechnological potential of microalgae, mainly due to the identification of several substances synthesized by these organisms. The huge biodiversity of algae, associated with genetic improvement and the establishment of large-scale cultivation technologies has allowed the commercial use of some species [1]. The biomass produced has several uses [2]. Among the compounds with commercial potential, polyunsaturated fatty acids, carotenoids, phycobilins, polysaccharides, vitamins, sterols, and several natural bioactive compounds stand out [1].

Fatty acids and lipids draw great interest due to their potential to generate bioenergy, for instance in biodiesel production. The high lipid content of algae, associated with high productivity per unit area compared to traditional oilseed crops, also draws interest.

The extraction of the lipid fraction is very important for a broad variety of feedstocks, hence, it must be performed with extreme accuracy. Some samples require special care to obtain the lipid fraction, since factors such as the co-extraction of non-lipid components and undesired oxidation may affect the final quality of the oil [3].

For extracting oil from algae, their cell membrane needs to be broken. The oil may be extracted through chemical processes, osmotic shock, use of enzymes, and with supercritical fluids [4].

Lipid extraction from microalgae is relatively easy to perform, the methods used in the analysis of lipids from algae are based on extraction with solvents and gravimetric analysis of the extracted oil. However, the methods described in the literature for total lipid extration from microalgae are not very clear, although the extraction of oil from microalgae has developed considerably in recent years. However, most studies on the productivity of oil extraction from microalgae do not make any distinction between total and neutral lipids [5].

Hence, our study aimed at analyzing three different methods for extracting total lipids from the microalga *Scenedesmus acuminatus* for biodiesel production. The oil was extracted, its fatty acid profile and acidity were characterized, and its molar mass was determined.

2. Material and methods

The microalga *Scenedesmus acuminatus*, the model for the present study, was cultivated in a controlled environment at a temperature of 25 °C, with a photoperiod of 24 h, and aeration from an air compressor. Recovery of biomass from the culture was made with the addition of the cationic flocculant, in order to promote the decantation of biomass, which was then filtered and dried in an oven at 60 °C for 24 h. The dry biomass was ground and stored at -8 °C until use.

2.1. Oil extraction

In the process of lipid extraction from dry biomass two methods were analyzed, they are described in the literature aimed at oil extraction from animals and microorganisms: method 1, proposed by Bligh & Dyer (1959) [6], and method 2, a system that uses a Soxhlet extractor [5]. An alternative method was also used: method 3, which is based on successive extraction (washing) of the sample using a hot solvent. The methods are described below.

2.1.1. Method 1 - Bligh & Dyer

In a beaker containing 100 g of dry biomass, 300 mL of a mixture of chloroform and methanol was added in the proportion 1:2 (v/v). The suspension was homogenized for 2 min to obtain a monophasic system. Next, 100 mL of chloroform were added to the mixture, which was stirred for 30 s. Then, 100 mL of distilled water were added, stirring the beaker continuously for 30 s. The resulting mixture was vacuum-filtered in a Büchner funnel, and the filtrate was transferred to a 500 mL separatory funnel.

After separation, the lower phase was drained to a 500 mL flat-bottomed flask, which was previously weighed (denominated P_1). The solvent was evaporated in a rotaevaporator. The flat-bottomed flask with oil was dried under vacuum until obtaining a constant mass (denominated P_2). The lipid content was determined gravimetrically, based on the mass difference between P_2 and P_1, i.e., (P_2-P_1).

2.1.2. Method 2 - Extraction with Soxhlet

The dry microalgae sample (30 g) was placed into a cartridge made of filter paper, which was transferred to a Soxhlet extractor. A flat-bottomed flask (P_1) was accurately weighed and the solvent was added (150 mL). Next, the flat-bottomed flask was connected to the extractor and the system was heated in a silicone bath at the boiling point of the solvent for 10 h. When the extraction process was completed, the flat-bottomed flask containing the extract was taken to a rotaevaporator for the evaporation of the solvent, at a temperature of 50 °C. Next, the flat-bottomed flask containing the fatty matter was placed in an oven at 60 °C until obtaining a constant mass (P_2). The total lipid content was gravimetrically determined, based on the difference in mass between P_2 and P_1. Three solvents were tested: hexane, ethyl ether, and dichloromethane.

2.1.3. Method 3 - Successive extraction

Ten mL of solvent were added to a Falcon tube containing 1 g of biomass. Next the tube was placed in water bath, and stirred at 60 °C for 30 min. Then, the sample was centrifuged for 5 min at 4,000 rpm, and the supernatant was transferred to a previously weighed flat-bottomed flask (P_1). To the tube containing the residual biomass, 10 mL of solvent were added and the tube was taken to the water bath for an additional 30 min. The process was repeated six times. In the end, the solvent was evaporated in a rotaevaporator and the flat-bottomed flask containing the fatty matter was dried in an oven at 60 °C, until obtaining a constant mass (P_2). The total lipid content was determined based on the difference in mass between P_2 and P_1. Three solvents were tested: hexane, ethyl ether, and dichloromethane.

2.2. Oil characterization

2.2.1. Determination of the index of acidity

The determination of the index of acidity followed that of the Instituto Adolfo Lutz (1985) [7]. Thus, 2 g of the oil sample were weighed in an 125 mL Erlenmeyer, 25 mL of an ether:alcohol solution in the proportion 2:1 (v/v) were added, and the sample was stirred until total dissolution. Next, 2 drops of 1% phenolphthalein were added, and the sample was titrated with a standardized solution of sodium hydroxide 0.01 mol L^{-1}, until it reached a pinkish color. Equation 1 provides the index of acidity in mg KOH g^{-1} and equation 2 provides the acidity as oleic acid (%) in (m/m), where *V* is the volume of the solution of 0.01 mol L^{-1} NaOH used in titration (mL), *f* is the correction factor of the solution of 0.01 mol L^{-1} NaOH, *P* is the mass of the sample (g), and C is the concentration of the solution of NaOH (mol L^{-1}).

$$\text{Index of acidity} = \frac{V f\ 5.61}{P}\ (\text{mg KOH g}^{-1}) \quad (1)$$

$$\text{Acidity in oleic acid (\%)} = \frac{V f\ \text{C}\ 28.2}{P}\ (\text{m/m}) \quad (2)$$

2.2.2. Determination of the fatty acid profile of the oil

Since the microalga oil had high acidity (above 0.5%), we followed Hartmann & Lago (1973) [8] to convert the oil into components of higher volatility, such as methyl esters, in order to identify and quantify the fatty acids through gas chromatography.

In a test tube 100 mg of oil were weighted and 4 mL of a NaOH solution in methanol (0.5 mol L^{-1}) were added. The tube was closed and heated in a water bath until the oil globules were dissolved and the solution became transparent. Then, the tube was cooled and 5 mL of the esterifying reagent (methanol) were added. Next, the tube was heated again in water bath and 4 mL of saturated solution of sodium chloride and 5 mL of solvent (n-heptane) were added. The tube was stirred for 30 s and then it was left to stand for approximately 90 min at 10 °C. The supernatant was used for the analysis of the oil through gas chromatography.

The fatty acid profile was determined using a gas chromatograph (Varian, model CP-3800) with a flame ionization detector (FID), containing a capillary column BP-X70-SGE of 30 m x 0.25 mm, and using helium as carrier gas, at a split ratio of 1:10. The temperature in the detector was 220 °C and in the injector it was 260 °C. The initial temperature of the column was 140 °C, and it was heated at a rate of 5 °C min^{-1} until reaching 250 °C.

The fatty acids were identified by comparing the retention times with the FAME (*fatty acid methyl ester*) Mix pattern, and quantified using as an internal standard (IS) tricosanoic acid methyl ester 99% [9].

2.3. Data analysis

To determine the best conditions for the extraction of total lipids a two-way factorial analysis was used, considering as factors: (a) the extraction method (Soxhlet or successive extraction) and (b) the solvent (hexane, ethyl ether or dichloromethane), followed by a post hoc test (Tukey test). We did not consider method 1 as a level of the factor 'extraction method' due to the difficulty in lipid recovery and, consequently, the difficulty in the analysis of the fatty acid profile, as described in the results and discussion. All analyses were carried out in Statistica 7.0^{TM} [10].

3. Results and discussion

3.1. Oil extraction

The results of oil extraction from the dry biomass are presented in Table I. In method 1, the values observed were consistent with the values found in the literature for this species of microalga [2]. However, one disadvantage of this method for the microalga tested was that the separation of phases did not occur clearly after adding water to the system, which hindered the total recovery of the lipid phase. In addition, the recovery of the solvent for reuse in another extraction was also hindered, because this method uses a mixture of two solvents (chloroform and methanol). This problem was not observed in the other methods tested in the present study, in which the recovery and reuse of the solvent used in the process were easily done.

Due to the difficulties found in method 1 it is possible to infer that the costs of oil extraction would be higher than in other methods, which could make large-scale production unfeasible. Hence, method 1 was not considered in the analysis of the oil profile.

Table I. Lipid mass obtained from the oil of the microalga *Scenedesmus acuminatus*.

Method	Solvent	Result*
1	Chloroform/Methanol	8.3
2	Hexane	5.96
2	Ethyl ether	7.39
2	Dichloromethane	10.19
3	Hexane	5.14
3	Ethyl ether	8.51
3	Dichloromethane	17.46

*:gram of total lipids per gram of dry biomass (%).

Methods 2 and 3 resulted in higher amounts of total lipids per gram of biomass in the extraction with the solvent dichloromethane, followed by ethyl ether, and hexane (Table I). There was broad variation in the total lipid yield per gram of dry biomass. Several factors may have influenced this variation, such as the characteristic of the solvent, the feedstocks, the extraction technique, and the ratio between solvents [11-13].

The amount of lipids extracted increased with an increase in solvent polarity. This result suggests that most lipids in the biomass of the microalga *Scenedesmus acuminatus* are polar, which corroborates the results obtained with other species of microalgae [14].

Table I shows that the highest amount of oil was obtained with the polar solvent dichloromethane. However, it is important to notice that, together with neutral lipids, a significant amount of pigment, characteristic of extraction with this type of solvent, was also obtained, and this may lead to false conclusions.

Several studies reported broad variation in the efficiency of lipid extraction with different techniques and even with the same technique [11-13]. Hence, we believe that solvent polarity may have caused the variation in the mass of total lipids extracted per gram of microalga, i.e., the solvents dichloromethane and ethyl ether removed a high amount of polar components, such as pigments, phospholipids, and glycolipids.

Due to this wide variety of yields in lipid extraction from this feedstock, a careful choice of the extraction method is recommended [13]. Hence, the determination of the best extraction method still requires oil characterization through analysis of the fatty acid profile.

3.2. Characterization of the oil extracted

3.2.1. Acidity and molar mass

Table II presents the average molar mass of the microalga oil and the index of acidity, which is defined as the amount in mg of potassium hydroxide needed to neutralize the free fatty acids in one g of oil.

Table II. Characteristics the oil extracted from the microalga *Scenedesmus acuminatus*.

Characterization	Result
Average molar mass of the fatty acids	263 g mol^{-1}
Average molar mass of the oil	827 g mol^{-1}
Acidity	4.9 mg KOH g^{-1}

The average molar mass of oil extracted from the microalga *Scenedesmus acuminatus* was within the expected range for this species [2]. In addition, oil acidity was higher than 0.5%. This value suggests that the best way to produce esters for biodiesel production is through acid esterification and transesterification [13].

3.2.2. Fatty acid profile in microalgae oil

As the extraction efficiency of each lipid class depends on the solvent, the determination of the lipid composition is essential to the validation and development of extraction methods [12]. The results obtained in the fatty acid profile of the oil extracted from the microalga *Scenedesmus acuminatus* are presented in Table III.

Table III. Fatty acid profile of the oil extracted from the microalga *Scenedesmus acuminatus*.

Fatty acids	Hexane		Ethyl ether		Dichloromethane	
	Successive extraction	Soxhlet	Successive extraction	Soxhlet	Successive extraction	Soxhlet
C 4:0	10.5*a	3.44b	5.91b	2.36b	5.19b	2.84b
C 6:0	2.54a	0.84b	1.08b	0.44b	1.01b	0.70b
C 8:0	2.94a	2.97a	1.86a	2.15a	1.55a	1.17a
C 10:0	1.11a	1.22a	0.73b	0.97ab	0.57b	1.04ab
C 12:0	0.00c	0.83a	0.20b	0.22b	0.12bc	0.23b
C 13:0	0.00c	0.00c	1.70b	11.8a	0.00c	0.00c
C 14:0	0.75a	0.79a	0.75a	0.71a	0.71a	0.84a
C 14:1	0.63a	0.71a	0.56a	0.60a	0.54a	0.7a
C 15:0	0.00c	0.14bc	0.37b	1.21a	0.00c	0.15bc
C 16:0	12.1ab	13.4a	10.3b	10.3b	10.7b	11.8ab
C 16:1	1.83a	2.14a	2.34a	2.07a	2.33a	2.47a
C 17:0	0.54b	0.54b	1.31a	0.85ab	1.25a	1.23a
C 17:1	0.00b	0.15ab	0.21a	0.22a	0.13ab	0.21a
C 18:0	2.71c	2.42c	5.34a	3.66bc	6.21a	4.99ab
C 18:1	21.1a	19.8ab	15.07b	15.1b	16.0b	16.3b
C 18:2	10.1bc	8.87c	11.3b	7.9c	14.0a	10.2bc
C 18:3	30.5c	37.8ab	35.3b	25.5d	33.9bc	41.4a
C 20:1	0.81d	2.23c	2.53bc	12.8a	3.29b	2.91bc
C 20:3	0.00b	0.00b	0.00b	0.30a	0.00b	0.36a
C 20:4	0.00b	0.00b	0.92a	0.00b	0.47a	0.00b
C 20:5	0.00c	0.00c	0.87a	0.00b	0.43b	0.00c
C 22:0	0.00b	0.22a	0.00b	0.26a	0.00b	0.00b
C 22:1	1.85a	0.00c	0.40b	0.00c	0.74b	0.00c
C 22:2	0.00b	0.34a	0.00b	0.20a	0.00b	0.20a
C 24:0	0.00b	0.68a	0.58a	0.43a	0.53a	0.00b
C 22:6	0.00b	0.49a	0.39a	0.00b	0.26ab	0.25ab

*Oil composition in mass percentage.
a,b,c,d: The same letters in the same row did not differ significantly at the level of 5%.

According to the fatty acid profile of the microalga, the highest proportions of fatty acids extracted (more than 70%), in descending order, were C 18:3, C 18:1, C 16:0, and C 18:2. Four fatty acids (C 8:0, C 14:0, C 14:1, and C 16:1) did not differ between extraction methods (method 2 or 3). Ten fatty acids (C 4:0, C 6:0, C 8:0, C 14:0, C 14:1, C 16:0, C 16:1, C 18:1, C 18:2, and C 18:3) had, on average, higher mass concentration among all 27 fatty acids identified in the analysis, as presented in Figure 1. In general, there was a predominance of unsaturated fatty acids, among which a high concentration of poly-unsaturated fatty acids (C 18:3) stood out. Hence, we expect the biodiesel produced from the oil of this microalga to present low oxidative stability [15]. On the other hand, there would be a gain in cold flow and viscosity properties, due to a not negligible contribution to the fatty acid profile from short-chain components (C 4:0 to C 8:0).

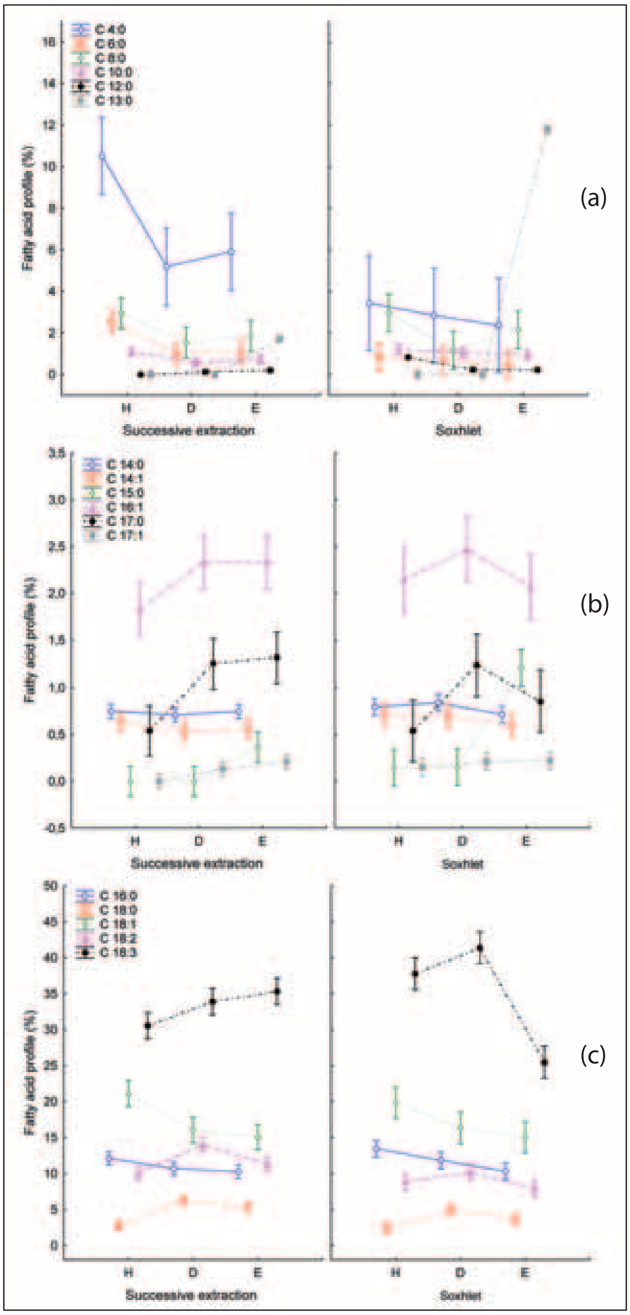


Figure 1. Fatty acid profile, in % mass, of the oil extracted from the microalga *Scenedesmus acuminatus*: a) C 4:0, C 6:0, C 8:0, C 10:0, C 12:0, and C 13:0; b) C 14:0, C 14:1, C 15:0, C 16:1, C 17:0, and C 17:1; and c) C 16:0, C 18:0, C 18:1, C 18:2, and C 18:3. The solvents used were: H-hexane, D-dichloromethane, and E-ethyl ether.

To determine the best method to be used to extract oil from microalgae, an analysis of the fatty acid profile in relation to the solvent was required. When Soxhlet extraction (method 2) was used with hexane as a solvent, the fatty acids that had the highest proportions were C 18:3 (37.8%), C 18:1 (19.8%), and C 16:0 (13.4%). When Soxhlet extraction (method 2) was used with ethyl ether, the fatty acids that had the highest proportions were C 18:3 (25.5%), C 18:1 (15.1%), C 20:1 (12.2%), C 13:0 (11.8%), and C 16:0 (10.3%). With Soxhlet extraction (method 2) and dichloromethane, the fatty acids that had the highest proportions were C 18:3 (41.4%), C 18:1 (16.3%), C 16:0 (11.8%), and C 18:2 (10.2%).

For method 3, when hexane was used as solvent, the fatty acids that had the highest proportions were C 18:3 (30.5%), C 18:1 (21.1%), C 16:0 (12.1%), C 4:0 (10.5%), and C 18:2 (10.0%). When ethyl ether was used, the fatty acids that had the highest proportions were C 18:3 (35.3%), C 18:1 (15.1%), C 18:2 (11.3%), and C 16:0 (10.3%). When dichloromethane was used, the fatty acids that had the highest proportions were C 18:3 (33.9%), C 18:1 (16.0%), C 18:2 (14.0%), and C 16:0 (10.7%).

The post hoc Tukey test carried out with the results presented above (Table III) showed an influence of solvent type on the relative extracted amount of each fatty acid that composed the oil profile of *Scenedesmus acuminatus*. Hence, the method of successive extraction (method 3) led to a profile in which there were both short-chain fatty acids and a predominance of fatty acids in the range of C 16 to C 18. The latter type is characteristic of most oilseeds traditionally used for biodiesel production. This balance in oil composition may lead to significant gain in the final quality of the biodiesel. Hence, we suggest that the best method used is successive extraction (method 3) with hexane as a solvent.

4. Conclusions

The oil obtained from the microalga *Scenedesmus acuminatus* is acidic, which is characteristic of algae oil. During oil extraction, the polarity of the solvent might have caused a variation in the amount of total lipids extracted per gram of microalga sample. Hence, the determination of the fatty acid profile of the oil from microalgae was necessary to validate and develop the extraction method. For *Scenedesmus acuminatus* the method 3 (successive extraction) was the best, and the solvent hexane led to a fatty acid profile that may bring significant gain to the final quality of the biodiesel.

Acknowledgments

We thank CNPq for scholarships and grants.

References

1. Derner, R. B.; Ohse, S.; Villela, M.; Carvalho, S. M.; Fett, R.; *Ciênc. Rural* 2006, 36(6), 1959.
2. Becker, E. W.; *Microalgae: Biotechnology and Microbiology*. Cambridge University Press, Cambridge 1994.
3. Brum, A. A. S.; Arruda, L. F.; Regitano-D´Arce, M. A. B.; *Quim. Nova* 2009, 32, 4, 849.
4. Pereira, C. M. P.; Hobuss, C. B.; Maciel, J. V.; Ferreira, L. R.; Del Pino, F. B.; Mesko, M. F.; Jacob-Lopes, E.; Neto, P. C.; *Quim. Nova* 2012, 35, 10, 2013.
5. Spector C.; Gobbi, C. N.; Conceição, R. C.; Araújo O. Q. F.; Chaloub, R. M.*; Anais do XVII Congresso Brasileiro de Engenharia Química*. Recife – PE, 2008.
6. Bligh E. G.; Dyer W J.; *J. Biochem. Physiol*. 1959, 37, 911.
7. Instituto Adolfo Lutz; *Métodos Físico-químicos para Análise de Alimentos*. Câmara Brasileira do Livro-SP, Vol. 1, 3ª Edição, São Paulo, 1985.
8. Hartmann, L.; Lago, R. C. A;. *Laboratory Practices*, 1973, 22, 475.
9. Visentainer, J. V.; Franco, M. R. B.; *Ácidos Graxos em Óleos e Gorduras: Identificação e Quantificação,* Ed. Varela, São Paulo, 2006.
10. STATSOFT INC. 2005. Statistica, version 7.1. Avaiable at: www.statsoft.inc.
11. Brian, J. G.; *Renew. Energy* 2011, 36, 158.
12. Smedes, F.; Askland, T. K.; *Mar. Pollut. Bull.* 1999, 38, 3, 193.
13. Lewis, T.; Nicholsb, P. D.; Mcmeekina, T. A.; *J Microbiol. Methods* 2000, 43, 107.
14. D’oca, M. G. M.; Viêgas, C. V.; Lemões J. S.; Miyasaki E. K.; Moron-Villarreyes, J. A.; Primel, E. G.; Abreu, P. C.; *Biomass Bioenergy* 2011, 35, 1533.
15. Ramos, M. J.; Fernández, C. M.; Casas, A.; Rodríguez, L.; Pérez, A.; *Bioresour. Technol.* 2009, 100, 261.

Spectrophotometric determination of New Fuchsin dye in water samples after cloud point extraction

Ardeshir Shokrollahi*; Zeinab Fatemi
Department of Chemistry, Yasouj University, Yasouj 75918-74831, Iran

***Corresponding author:**
Phone: 98 741 2223048
Fax: 98 741 2242167
E-mail address:
ashokrollahi@mail.yu.ac.ir

Abstract

Cloud point extraction methodology was successfully employed for preconcentration of trace amounts of New Fuchsin prior to its quantification by spectrophotometry. The method was based on the cloud point extraction of New Fuchsin from an aqueous solution using Triton X-114. The extracted surfactant rich phase was diluted with ethanol and its absorbance was measured at 554 nm with a spectophotometer. Under optimum conditions a linear calibration graph in the range of 0.013-0.560 mg L^{-1} of New Fuchsin in the initial solution with $r^2 = 0.996$ ($n = 7$) and detection limit 5.64 µg L^{-1} ($n = 5$) was obtained. The method was applied to determine New Fuchsin in tap, mineral and factory water samples.

Keywords: New Fuchsin; Cloud point; Triton X-114; Spectrophotometry

1. Introduction

Synthetic dyes are used in the textile, paper, leather, rubber, plastics, cosmetics, pharmaceuticals and food industries. Generally, synthetic dyes have complex aromatic structures that make them stable and difficult to biodegrade [1]. New Fuchsin is a dye with the chemical structure shown in Figure 1 and has been used to determine sulfide ions by a kinetic spectrophotometric method [2], as a copper corrosion inhibitor [3], photosensitive reagent [4], kit for immunehistochemical and in situ hybridisation staining [5], and in a graphene-New Fuchsin modified electrode for determination of DNA bases [6]. In addition it is a hazardous material with potential chronic health effects, which may cause eye and skin irritation. Also, inhalation of it is dangerous. Due to both the risks and the many applications of this dye there is the probable existence of it in laboratory and medical wastewaters, so it is very important to determine it.

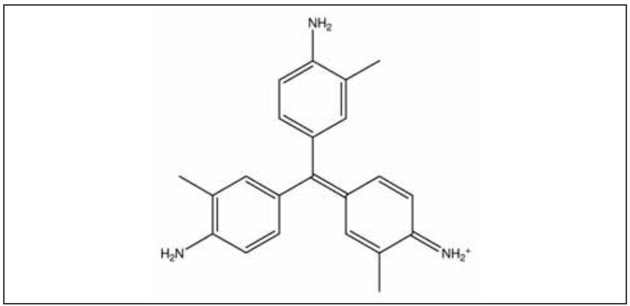


Figure 1. Chemical structure of New Fuchsin

In the two last decades, increasing interest in the use of aqueous micellar solutions has been found in the field of separation science [7]. Aqueous solutions of many nonionic surfactant micellar systems become turbid over a narrow temperature range, when the experimental conditions (i.e., temperature or pressure, addition of salt or other additive, etc.) have been changed. This temperature is named the cloud point temperature. Above the cloud point, the aqueous surfactant micellar solution separates into a concentrated phase containing most of the surfactant (termed surfactant-rich phase) and a dilute aqueous phase containing low concentration of surfactant corresponding to the critical micellar concentration (cmc). Any components originally present that bind to the micellar aggregate in solution can thus be extracted from the original solution and concentrated in the small volume of the surfactant- rich phase. In other words, cloud point extraction (CPE) is based on the property that a solute present in aqueous solution of surfactant is distributed between two phases. CPE has been successfully employed for the purification, determination and preconcentration of different species of biological interest [8], organic compounds such as phenols, polycyclic aromatic hydrocarbons [9], inorganic compounds [10], polychlorinated compounds [11], radionuclides [12] and dyes [13, 14] from water and wastewater. This simple technique enables us to avoid hazardous organic solvents and allows us achieve a much higher concentration of analyte than in the case of liquid-liquid extraction, because the micellar phase volume is about 10–100-fold less than the volume of an aqueous phase [15]. Cloud point extraction can be easily combined with several analytical methods such as spectrophotometry [16], spectrofluorimetry [17], inductively coupled plasma [18], liquid chromatography [19], flow injection [20], graphite furnace atomic absorption [21] and flame atomic absorption spectrometry [22-24], cold vapor atomic absorption spectrometry [25] and CPE-paptode as a new method for determination of dyes [26]. The paptode method is based on scanning of a simple paper or other ordinary porous material such as clay, cotton, filter paper, or even TLC as a substrate support for the reagent [27]. Thus extraction with nonionic surfactant phases can be possible for elaborating on highly sensitive and convenient hybrid analytical methods. The indication of pK_a of dyes can be helpful to describe the effect of pH on the CPE process. Potentiometric pH-pK_a titration is a powerful technique for determination of the pK_a of ligands and dyes. In the present work, a simple and sensitive cloud point extraction procedure has been developed for the spectrophotometric determination of New Fuchsin as a dye.

2. Experimental

2.1. Materials and apparatus

All chemicals were of analytical grade and doubly distilled water was used throughout. A 1% (w/v) Triton X-114 (Fluka, Buchs, Switzerland) solution was prepared by dissolving 1.0 g of TX-114 in a 100 mL volumetric flask. All the dyes were purchased from Merck (E. Merck, Darmstadt, Germany). A stock solution of 100 mg L^{-1} of New Fuchsin was prepared by dissolving 0.0100 g of New Fuchsin in water and diluting to 100 mL in a volumetric flask. All the other chemicals were analytical grade from Merck (Darmstadt, Germany). The surfactant-rich phase was diluted with ethanol.

A Jasco V-530 UV–visible spectrophotometer (Hachioji, Tokyo, Japan) was used for recording absorption spectra and absorbance measurements using 1 cm glass cells. A Metrohm (Herisau, Switzerland) digital pH-meter, model 827, with combined glass electrode was used for adjusting pH. A F.A.G. (Iran) thermostat bath maintained at the desired temperature was used for the cloud point temperature experiments. A model 794 Metrohm Basic Titrino was attached to an extended combined glass-calomel electrode mounted in an air-protected, sealed, thermostated jacketed cell maintained at 25.0±0.1 °C by circulating water, from a constant temperature bath, Fisherbrand model FBH604, Lauda, Germany, equipped with a stirrer and a 10.000 mL capacity Metrohm piston buret. The pH meter-electrode system was calibrated to read -log (H^+).

2.2. General procedure

A typical CPE experiment was carried out according to the following procedure. An aliquot of 15 ml of an aqueous solution containing 0.33 mg L^{-1} of New Fuchsin and 0.153 % (w/v) of TX-114 at pH 4 was prepared in a centrifuge tube. The mixture was left to stand for 5 min in a thermostat bath at 40° C. Separation of the phases was achieved by centrifugation for 15 min at 3500 rpm. After the separation of the two phases, the mixture was cooled in an ice bath to increase the viscosity of the surfactant-rich phase, and the aqueous phase was removed by a Pasteur pipette. The surfactant-rich phase was dissolved and diluted with ethanol to 1.0 mL, and then the absorption spectrum of this solution was recorded by the spectrophotometer.

2.3. Potentiometric equilibrium measurements

The procedure employed for the potentiometric pH measurements has been described in detail elsewhere [28-31]. In general, an experimental run involves collecting equilibrium data points throughout the entire pH range, between 2.0 and 11.5 as a function of milliliters of standard NaOH that was added by using the piston burette through a fine capillary tip immersed in the solution. In titration, after each addition, the required time was allowed to reach chemical equilibrium. All potentiometric pH measurements were made on solutions in a 75-mL double-walled glass vessel using a Model 794 Metrohm Basic Titrino using a thermo-stated cell and an extended combined glass-calomel electrode. Atmospheric CO_2 was excluded from the titration cell with a purging steam of purified nitrogen gas. The concentration of New Fuchsin was 2.5×10^{-3} mol L^{-1} for the potentiometric pH titration. A standard carbonate-free NaOH solution (0.09351 mol L^{-1}) was used in the titration. The ionic strength was adjusted to 0.1 mol L^{-1} with the $NaNO_3$. Protonation constants of New Fuchsin were evaluated using the BEST program [32], and the corresponding distribution diagrams were depicted using the new version of the Hyss2009 program [33]. The value of $K_W = [H^+][OH^-]$ used in the study was the same as that of our previous works [32].

3. Results and discussion

Figure 2 shows the absorption spectra of New Fuchsin in aqueous solution and in ethanol before and after CPE, respectively. A peak with high intensity at 554 nm is seen after cloud point extraction of this dye. Therefore, all the absorbance measurements were performed at this wavelength. As the extent of cloud point extraction is influenced by the presence of additives, the surfactant concentration, the pH of the medium, the temperature, the time in the bath, and the centrifuge time, these parameters were optimized in order to achieve the highest sensitivity.

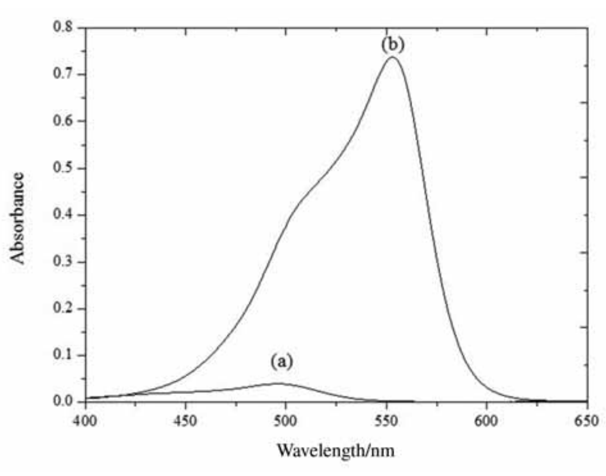


Figure 2. The absorption spectrum of 0.5 mg L^{-1} of New Fuchsin in bidistillated water without cloud point extraction (a) and in ethanol after cloud point extraction (b)

3.1. Protonation studies

In these experiments, the fully protonated forms of New Fuchsin were titrated with a standard NaOH solution (Figure 3a) to obtain the protonation constants. The protonation constants were calculated by fitting the pH/volume data to the BEST program. The calculated amounts of $\log k_1$ and $\log k_2$ were 12.91± 0.10 and 2.33± 0.15, respectively. The first amine group is protonated at $\log k_1 = 12.91$ and the second group at $\log k_2 = 2.33$. The corresponding species distribution diagram is shown in Figure 3b.

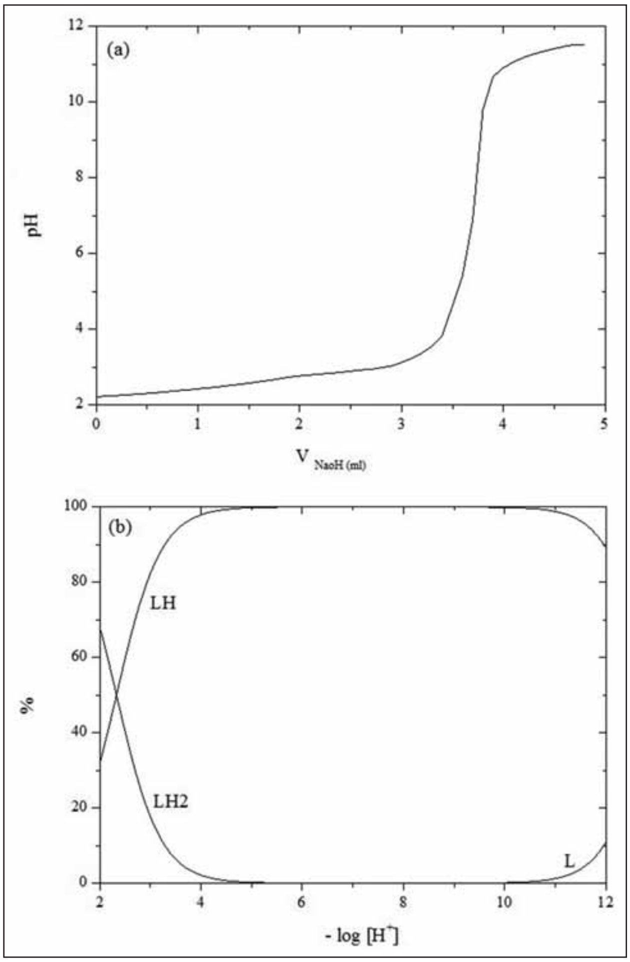


Figure 3. Potentiometric titration curve of New Fuchsin (a) with 0.09351 mol L⁻¹ NaOH solution at 25± 0.1 °C and μ=0.1 mol L⁻¹ $NaNO_3$ and (b) distribution diagram of New Fuchsin (L)

3.2. Effect of pH

For organic molecules, pH is perhaps the most critical factor regulating the partitioning of the target analyte in the micellar phase. Especially for ionizable species, maximum extraction efficiency is achieved at pH values where the uncharged form of the target analyte prevails. The ionic form of analyte does not interact with the micellar aggregate as strongly as its neutral form does.

Cloud-point extraction of 0.33 mg L^{-1} New Fuchsin in the presence of 0.153% (w/v) TX-114 was performed at different pH values adjusted by adding appropriate amounts of HCl (0.01- 0.5 mol L^{-1}) or NaOH (0.01- 0.1 mol L^{-1}). As shown in Figure 4, the efficiency of extraction of the New Fuchsin to the concentrated micellar phase is strongly pH-dependent. A maximum preconcentration of New Fuchsin was achieved at pH 4, which was selected as the optimum pH in further studies. As is obvious from Figure 4, within the pH range from 4 to 2, the CPE efficiency decreased slowly and in the range 2 to 1, this sharply decreased, most probably this observation is due to the protonation of New Fuchsin in acidic media. As it is seen from the distribution diagram (Figure 3b) at acidic pH, the LH_2^{2+} species is the most abundant and this species could not be attracted to the nonaqueous phase. At pH around 4 LH^+Cl^- as a stable ion pair species could not be extracted to the enrichment phase with high efficiency. At higher pH, the presence of OH^- anion can cause some interference in the cloud point formation process.

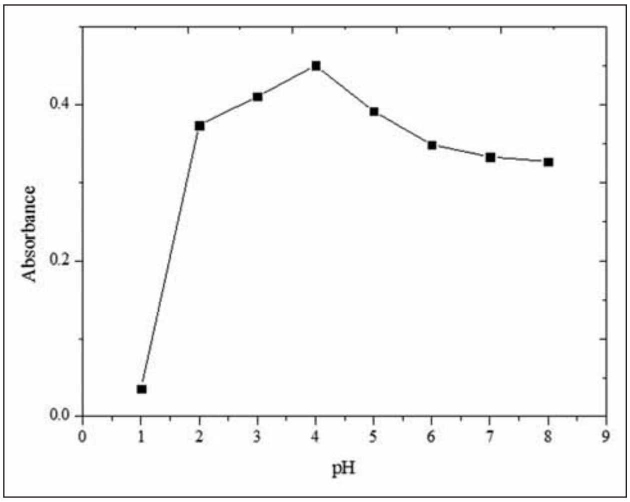


Figure 4. Effect of pH on the CPE of New Fuchsin, (conditions: New Fuchsin: 0.33 mg L⁻¹; Triton X-114: 0.153% (w/v) and pH: and 45 °C).

3.3. Effect of Triton X-114 concentration

For a successful cloud point extraction, it is desirable to obtain a minimum amount of surfactant for maximum extraction of dye. Therefore, the effect of TX-114 concentration on the performance of the extraction system was studied. The preconcentration efficiency for 0.33 mg L^{-1} New Fuchsin at pH, 4 was evaluated using TX-114 concentrations ranging from 0.1% to 0.186% (w/v). The results are demonstrated in Figure 5. The highest signal was obtained with 0.153% (w/v) TX-114. At lower concentrations the recovery was reduced, because formation of assemblies between dye and surfactant is not complete. At higher concentrations, the probable presence of excess of TX-114 in the aqueous phase causes some of the analyte to remain in the aqueous solution. Therefore this phase can compete with the surfactant-rich phase in containing analyte. Also, at high concentrations of surfactant, the viscosity of the enrichment phase was increased and the signal was decreased**.** Thus the concentration of 0.153% (w/v) for TX-114 was selected for all subsequent experiments.

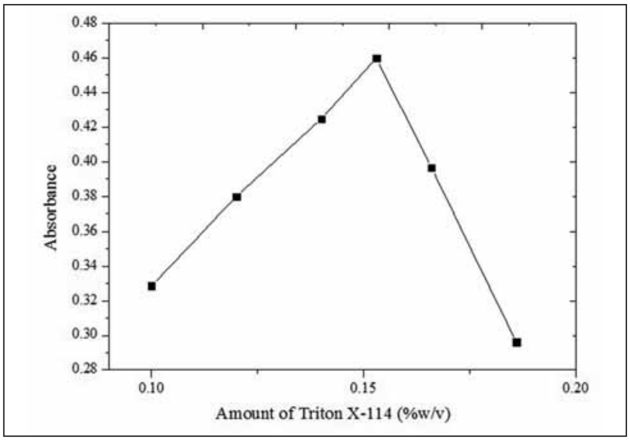


Figure 5. Effect of Triton X-114 concentration on the CPE of New Fuchsin, (conditions: New Fuchsin: 0.33 mg L^{-1}; pH: 4.0 and 45 °C).

3.4. Effects of equilibration temperature and time

Optimal incubation time and equilibration temperature are necessary to complete the reaction, and to achieve easy phase separation and preconcentration as efficient as possible. The greatest analyte preconcentration factors are thus expected under conditions where the CPE is conducted using equilibration temperatures that are well above the cloud point temperature of the surfactant. As is shown in Figure 6, the effect of equilibrium temperature in the range of 25–55°C was studied. It was found that 40 °C is adequate for the quantitative analysis. When the temperature is lower than 40 °C the cloud point is not complete. However, higher temperatures cause the dissociation of the surfactant–dye assembly while much higher temperatures may lead to decomposition of the analyte. The dependence of absorbance upon equilibration time was also studied within the range of 5–35 min. Five minutes was chosen as the optimal time for equilibration and incubation. The short time is a significant advantage for analysis of an analyte.

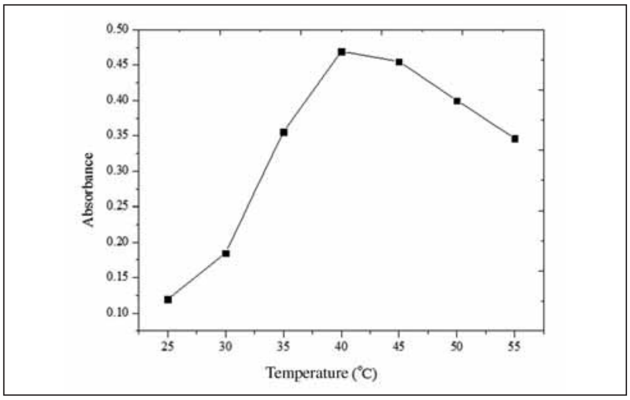


Figure 6. Effect of temperature of bath on the CPE of New Fuchsin, (conditions: New Fuchsin: 0.33 mg L^{-1}, Triton X-114: 0.153% (w/v) and pH: 4.0).

3.5. Effect of centrifuge rate and time

It is necessary to preconcentration trace amounts of New Fuchsin with high efficiency within a short time. Therefore, for obtaining the optimum centrifuge rate and time, the CPE on a set of experiments using 15 mL samples under optimum conditions was carried out with centrifuging at various rates and times. It was found that centrifugation at 3500 rpm for 15 min separates the two phases completely, at lower rate and time, the aggregation, extraction and separation was not complete.

3.6. Effect of electrolyte

The CP of micellar solutions can be affected in the presence of salts, alcohols, nonionic surfactant and some organic compounds. In order to study the effect of the addition of electrolyte on micellar solutions of the studied dye, $NaNO_3$ solution was tested. It was observed that the addition of $NaNO_3$ within the interval of 0.01–0.30 mol L^{-1} had no significant effect on the cloud point extraction efficiency.

3.7. Interference studies

The effect of different ions and dyes on the extraction and determination of 0.33 mg L^{-1} of New Fuchsin by the proposed method was studied. A species was considered to be interfering when it caused a variation greater than ±5% in the absorbance of the sample. The results presented in Table I, show the relatively good selectivity of the procedure. The presence of some dyes, such as Morin, Titangelb, Fuchsin acid, and ions such as Cl^-, ClO_4^-, NO_3^-, HPO_4^{2-}, HCO_3^- , K^+, Li^+, Na^+, Ni^{2+}, Cu^{2+} and Mg^{2+} with competitor or complexing potential do not have any significant interference with the mentioned result.

Table I. The effect of foreign ions and dyes on the determination of 0.33 mg L^{-1} of New Fuchsin.

Tolerance ratio(w/w)	Foreign ions/dyes
1	Morin, Titangelb, Fuchsin acid
25	Ni^{2+}
50	Cu^{2+}, K^+, HCO_3^-
500	Cl^-, ClO_4^-, NO_3^-, HPO_4^{2-} Na^+, Co^{2+}, Mg^{2+}
1000	Li^+

3.8. Calibration, precision and detection limits

An analytical curve was obtained using the general procedure for different concentrations of New Fuchsin under the optimized experimental conditions. The calibration graph was linear in the range of 0.013-0.560 mg L^{-1} of New Fuchsin in the initial aqueous solution. The equation of the regression line with and without preconcentration were $A = 1.473\ C + 0.011$ ($r^2 = 0.996$, $n = 7$) and $A = 0.117\ C - 0.028$ ($r^2 = 0.998$, $n = 6$) respectively, where A, C, r^2 and n are the absorbance, the concentration of New Fuchsin in mg L^{-1}, the regression coefficient and the number of data points, respectively. The relative standard deviation (RSD) for 0.33 mg L^{-1} of New Fuchsin (n=5) was 4.07%. The limit of detection of the method based on three times the standard deviation of the blank ($3S_b$) was 5.64 µg L^{-1} (n = 5) of New Fuchsin. The enrichment factor, defined as the ratio of the slopes of the analytical curve with and without preconcentration [34, 35], was 12.58. The preconcentration factor based on the ratio of initial volume in the centrifuge tube to final volume of dissolving solvent was 15. These factors are presented in Table II. Also the average sample throughput was estimated at 20. According to the cited factors, the proposed method has a relatively good ruggedness, robustness and precision.

Table II. Analytical characteristics of the method.

Analytical Performance Characteristics	Data
Linear equation(n=7)	$A = 1.473C + 0.011$
Range of concentration (mg L^{-1})	0.013–0.560
Regression coefficient (r^2)	0.996
Detection limit (µg L^{-1})	5.64
Preconcentration Factor	15.0
Enrichment Factor	12.58
% Relative standard deviation (RSD)	4.07

3.9. Determination of New Fuchsin in real samples

In order to test the reliability of the proposed methodology for the assay of New Fuchsin, it was applied to determine its concentrations in tap water, mineral water and factory water (Dena Sanat) samples under the optimized conditions. Since an official or standard method does not exist for the determination of New Fuchsin, the developed methodology was validated by recovery studies. Good recoveries (93.46–98.01 %) were achieved for all analyzed samples (Table III)

Table III. Determination of New Fuchsin in mineral water, tap water and factory water.

Sample	Slope	Intercept	Regression coefficient	Added (mg L^{-1})	Found (mg L^{-1})	Recovery %
Tap water	1.652	0.033	0.982	0	Nd[a]	---
				0.133	0.126	95.05
				0.266	0.252	95.01
Mineral water	1.532	0.015	0.997	0	Nd	---
				0.133	0.128	96.37
				0.266	0.248	93.46
Factory water	2.188	0.002	0.999	0	Nd	---
				0.133	0.126	94.76
				0.266	0.260	98.01

a Not detected

4. Conclusion

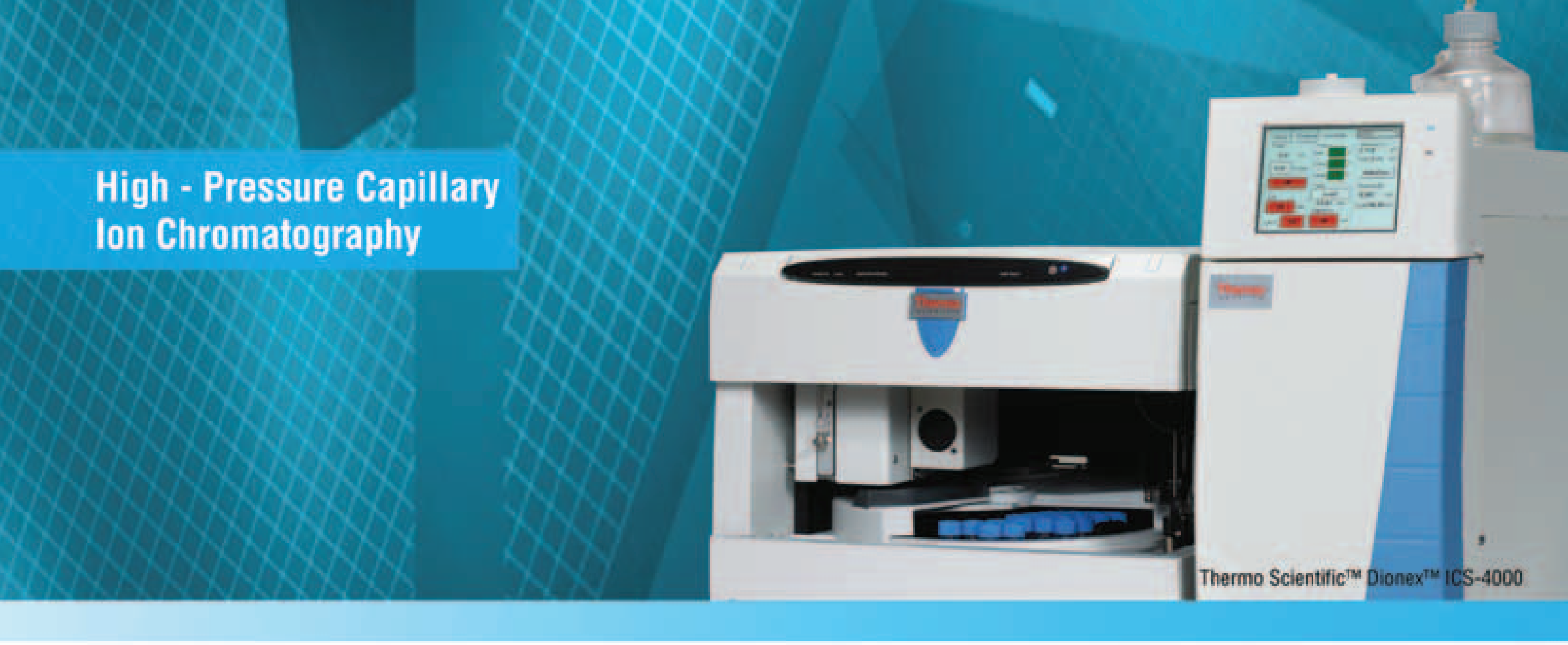
In this paper, a novel, simple, rapid, safe, inexpensive and sensitive cloud point extraction procedure was presented for the extraction, preconcentration, and spectrophotometric determination of New Fuchsin. Method validation yielded good results and included linearity, repeatability, reproducibility, sensitivity, recovery and accuracy. The proposed method can be applied to the determination of New Fuchsin in presence of other dyes from the same family, such as Morin and Fuchsin acid in both water and waste water samples.

Acknowledgements

The financial support of this work by Yasouj University is greatly acknowledged.

References

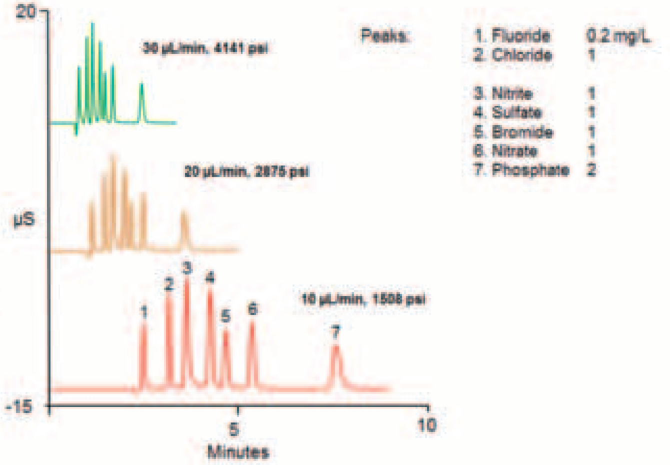
1. Hsiu-Mei, C.; Ting-Chien, C.; San-Dec, P.; Hung-Lungd, C.; J. Hazard. Mater. 2009, 161, 1384.
2. Ghasemi, J.; Mohammadi, D.E.; Microchem. J. 2002, 7, 1.
3. Bastidas, J.M.; Pinilla, P.; Cano, E.; Polo, J.L.; Miguel, S.; Corros. Sci. 2003, 45, 447.
4. Bae, W.; Mehra, R.K.; J. Inorg. Biochem. 1998, 70, 125.
5. http:/www monosan.com
6. Jia-Yi, C.; Shen-Ming, C.; Wen-Hui, W.; Ying, L.; Shauh-Der, Y.; Int. J. Electrochem. Sci 2013, 8, 3963.
7. Liang, R.; Wang, Z.; Xu, J.; Li, W.; Qi, H.; Sep. Purif. Technol. 2009, 66, 248.
8. Afkhami, A.; Noroozi-Asl, R.; J. Hazard. Mater. 2009, 167, 752.
9. Pan, T.; Wang, Z; Zu, J.H.; Wu, Z.; Qi, H.; Process. Biochem. 2010, 45, 77.
10. Shokrollahi, A.; Eslami, S.; Kianfar, A.; Chem. Sci. Trans. 2012, 1, 217.
11. Eiguren-Fernandez, A.; Sosa-Ferrera, Z.; Santana-Rod-riguez, J.J.; Anal. Chim. Acta, 1998, 358 145.
12. Pepper, S.E.; Peterman, D.R.; Tranter, P.J.; White, J.; J. Radioanal. Nucl. Chem. 2009, 282, 912.
13. Liu, W.; Zhao, W.; Chen, J.; Yang, M.; Anal. Chim. Acta, 2007, 605, 41.
14. Alam, M.S.; Kabir-ud-Din, Colloids Surf. B, 2008, 65, 74.
15. Ghaedi, M.; Shokrollahi, A.; Niknam, K.; Niknam, E.; J. Hazard. Mater. 2009, 168, 1027.
16. Pourreza, N.; Rastegarzadeh, S.; Larki, A.; Food Chem. 2011, 126, 1465.
17. Güler, N.; Maden, M.; Bakırdere, S.; Ataman, Y.; Volkan, M.; Food Chem. 2010, 129, 1793.
18. Li, Y.; Hu, B.; Spectrochim. Acta *B.* 2007, 62, 1153.
19. Wang, L.L.; Wang, J.Q.; Zheng, Z.X.; Xiao, P.; J. Hazard. Mater. 2010, 177, 114.
20. Gil, R.A.; Salonia, J.A.; Gásquez, J.A.; Olivieri, A.C.; Olsina, R.; Martinez, L.D.; Microchem. J. 2010, 9, 306.
21. Sun, M.; Wu, Q.; J. Hazard. Mater. 2010, 176, 901.
22. Shokrollahi, A.; Ghaedi, M.; Hossaini, O.; Khanjari, N.; Soylak, M.; J. Hazard. Mater. 2008, 160, 435.
23. Citak, D.; Tuzen, M.; Food Chem. Toxicol. 2010, 48, 1399.
24. Kiran, K.; Suresh K.K.; Prasad, B.; Suvardhan, K.; Lekkala, R.Babu.; Janardhanam, K.; J. Hazard. Mater. 2008, 150, 582.
25. Shah, AQ.; Kazi, T.G.; Baig, J.A.; Afridi, H.I.; Kandhro, G.A.; Arain, M.B.; Kolachi, N.F.; Wadhwa, S.K.; Food Chem. Toxicol. 2010, 48, 65.
26. Shokrollahi, A.; Abbaspour, A.; Azami Ardekani, Z.; Malekhosseini, Z.; Alizadeh, A.; Anal. Methods, 2012, 4, 502.
27. Ruchi, D.S.; Sulbha, A.; Int. J. Pharm. Bio. Sci. 2012, 3, 41.
28. Aghabozorg, H.; Ramezanipour, F.; Soleimannejad, J.; Sharif, MA.; Shokrollahi, A.; Shamsipur, M.; Moghimi, A.; Attar Gharamaleki, J.; Lippolis, V.; Blake, A.J.; Pol. J. Chem. 2008, 82, 487.
29. Moghimi, A.; Sheshmani, S.; Shokrollahi, A.; Shamsipur, M.; Kickelbik, G.; Aghabozorg, H.; Z. Anorg. Allg. Chem. 2005, 631.160.
30. Aghajani, Z.; Aghabozorg, H.; Sadr-Khanlou, E.; Shokrollahi, A.; Derki, S.; Shamsipur, M.; J. Iran. Chem. Soc. 2009, 6, 373.
31. Shokrollahi, A.; Ghaedi, M.; Niband, M.S.; Rajabi, H.R.; J. Hazard. Mater. 2008, 151, 642.
32. Martell, A.E.; Motekaitis, R.J.; Determination and Use of Stability Constants, 2nd ed.; VCH: New York, 1992.
33. Alderighi, L.; Gans, P.; Ienco, A.; Peters, D.; Sabantini, A.; Vacca, A.; Coord. Chem. Rev. 1999, 184, 311.
34. Pinto, C.G.; Pavon, J.L.P.; Cordero, B.M.; Beato, E.R.; Sanchez, S.S.; J. Anal. Appl. Pyrolysis, 1996, 11, 37.
35. Stalikas C.D.; Trends Anal. Chem. 2002, 21, 343.



Melhor desempenho Menor custo

- **Tranquilidade**
- **Menor Custo Operacional**
- **Instrumentos sempre Produzindo Resultados**

- Durabilidade e capacidade das colunas;
- Baixo consumo de eluente (5 litros por ano em CapIC);
- Custo de descarte reduzido;
- Operação sem necessidade de preparo de soluções regenerantes de supressora;
- Operação em modo HPIC em colunas de 4µm – resultados mais rápidos, altíssima resolução;



Peaks:	1. Fluoride	0.2 mg/L
	2. Chloride	1
	3. Nitrite	1
	4. Sulfate	1
	5. Bromide	1
	6. Nitrate	1
	7. Phosphate	2

IC e Preparo de Amostras
3 Anos
Garantia

Toda linha de Cromatografia de Íons e Preparo de Amostras agora com 3 anos de garantia!

Kits de manutenção preventiva com 10% de desconto na aquisição do equipamento

Para maiores informações fale com nossos especialistas.

Thermo Fisher Scientific / Chromatography and Mass Spectrometry Division: Rua Grauça, 389, Vila Sônia, CEP 05626-020 – São Paulo - SP
Tel: 55 11 3731 5140 FAX: 3213 9530
site: www.dionex.com.br | www.thermoscientific.com/dionex

Thermo
SCIENTIFIC

POINT OF VIEW

OPPORTUNITY IN ANALYTICAL CHEMISTRY

I use to say that Analytical Chemistry is the area of chemistry which allows us to understand and describe phenomenon and process through chemical analysis on all areas of interest. In the oil and gas industry, for instance, we have demands on analytical data to support the engineering from the well to the wheel. So, through analytical science we have the opportunity of developing a systemic view of production chain identifying synergies, predicting problems and providing solutions. This reduces the risks and the cost of operation.

Sometimes our capability of understanding an operational event or characterizing a special product obtained in a state-of-art R&D project may be restricted due to sampling conditions, available materials and samples complexity. In such a case the identification of the best analytical approach to overcome this limitation is a challenge and gives us opportunities to develop new methodologies. These methodologies could be made indoors or in partnerships with universities or other research institutes.

Among the drivers for further development I would like to point out two major topics: speciation and time response. Speciation is a challenge concerning the size and the complexity of a sample. The size compromise sample representation and reduce the scope of application for a traditional analytical instrumentation or methodologies. Speciation is necessary to understand and to explain a phenomenon or to describe how the reactions occur in a process helping engineers to make decisions in an initial phase of a project. On the order hand, the response time of an analytical method should be adequate to the process control. This concept of real time operation (RTO) is closely associated to the control of an industrial unit where the goal is to provide instant data to take immediate action when a problem occurs, guaranteeing the operational continuity and the unit integrity, keeping the process under control. Actually, the challenges in analytical chemistry are the same for every industry that is to provide information as fast as possible regardless of the amount of material available in order to make decisions which leads to optimal performance, lower costs, and reduced risks in production.

Therefore, research in analytical methodologies based on concepts like Lab On Chip and nanoscience is expected to extend towards downscaling of low sample and reagent volume consumption, faster analysis and response time meeting the industries targets.

Finally, I would like to summarize my point of view through one word – cooperation. This is the big challenge making people with different knowledge and background to share information and experience in a common effort. A multidisciplinary partnership is the key point to ensure that our scientific community is in the best position to take up the challenges in Analytical Chemistry.

Maria Luiza Bragança Tristão, Ph.D.
Petroleum Chemist, Senior Advisor
Research and Development Center (CENPES) - PETROBRÁS

ANALYTICAL CHEMISTRY ROLE IN FOOD SAFETY ASSESMENT

The process to obtain food can use several food ingredients and different process conditions and technologies. Regulatory compliance demands that all food ingredients and products must be safe for their intended use. During the research, development and production phases, safety assessment is mandatory for tasting trials or human clinical studies. From a chemical food safety perspective, food can be assumed as a complex mixture of chemicals, which can be grouped in two broad categories: intrinsic and extrinsic components. The **Intrinsic Components** are inherent constituents of the food such as compounds that brings the nutritional value of food (macronutrients and micronutrients) and anti-nutrients that are potentially responsible for reducing the nutritional value of food (e.g. protease inhibitors blocking protein digestion). The intrinsic components group also includes the inherent toxicants. This class of compounds is known by their relatively high potential to produce toxicity (e.g. Glycoalkaloids).The **Extrinsic Components** are chemicals of either natural or industrial origin that reaches food through various ways. In this category of compounds are listed food additives that are added to food for technological purposes, contaminants/residues originate from various practices such as agricultural (e.g. pesticide), veterinary (e.g. antibiotic), cleaning (e.g. sanitizers), packaging materials, and contaminants/environmental that can be either of natural (mycotoxins, metals, etc) or industrial (organic pollutants, metals, etc) sources. The extrinsic components also included process-related chemicals formed over processing such as thermal treatment, fermentation, smoking, drying or extraction (e.g. acrylamide).

Any chemical has the potential to produce adverse health effects, i.e. **toxicity**. In this context, toxicity is defined as "*the inherent property of a chemical agent to cause an adverse biological effect*". Since toxicity depends on the chemical properties of the chemicals, it is therefore highly substance-specific. On this core of study the analytical chemistry has a fundamental role: identify and measure. The Analytical Chemistry Science is the sustain pillar to provide reliable information to evaluate risk or toxicity for a particular food ingredient or food product. Several methods can be listed as solutions to provide answers for these questions. Currently you can find running in several Analytical Centers since from simple tests using ELISA specific methods for a broad range of molecules of veterinary drugs and allergenic substances or classical methods to appraised food composition and nutritional labels, until high-ending instrumental techniques using mass spectrometry coupled to gas or liquid chromatography (GC-MS, GC-MS/MS and LC-MS/MS), or plasma source (ICP-MS) for high sensitivity and selective determinations of contaminants such as heavy metals, mycotoxines, hormones, pesticides and several contaminants metabolites. The major aim of analytical science in food safety is provide reliable information to identify potential risks related to safety and compliance of new and existing food products or ingredients with current worldwide regulatory rules. The management of these information always keep the focus on prevention of incidents and crises for human health.

Fernando Vitorino da Silva
Nestlé Quality Assurance Center, São Paulo
Avenida Guido Caloi 1935, Bloco A, 2º Andar, Jardim São Luis
05802-140, São Paulo

TRACE 1300 SERIES GC

FEATURES REVOLUTIONARY USER-EXCHANGEABLE, INSTANT CONNECT INJECTORS AND DETECTORS

NEW VERSATILE GAS CHROMATOGRAPH SERIES

Thermo Scientific, the world leader in serving science, announces the TRACE 1300 Series GC, a compact, versatile gas chromatography system that is designed to increase productivity and lower cost in QA/QC and routine laboratories working on enviromental, chemical and food safety applications. It features unique user-exchangeable instant connect injectors and detectors and an intuitive interface in instruments that deliver reliable results and maximum sensitivity.

The TRACE 1300 Series GC comes in two models, the TRACE 1300 GC and TRACE 1310 GC. The TRACE 1300 GC is ideal for budget-conscious labs carrying out routine analysis; its simplified interface requires limited user interaction, so it can run 24/7 with limited staff oversight. The TRACE 1310 GC, ideal for QA/QC labs or labs focused on method development, features an icon-based touch screen interface for more precise user control.

Both systems benefit from robust design that is durable yet easy to use and maintain. Specific features include:

- User-exchangeable instant connect injectors and detectors that can be mounted in two minutes or less for quick changes if a different injection or detection technique is required.

- Newly designed micro-volume detectors that ensure higher sensitivity and fast peak detection.

- Optional injector back-flush option that eliminates heavy or undesired compounds to protect column and detectors, increase sensitivity and ease maintenance.

- Full control and easy programming with scalable Thermo Scientific Dionex Chromeleon Data System that streamlines path from samples to results offering enhanced operational simplicity.

For additional information please contact
(11) 2162 8080, revista@novanalitica.com.br or visit analiticaweb.com.br

TSQ 8000 TRIPLE QUADRUPOLE GC-MS SYSTEM:
UNCOMPROMISED MS/MS SIMPLICITY & UNSTOPPABLE PRODUCTIVITY

NEW TRIPLE QUADRUPOLE GC-MS SYSTEM

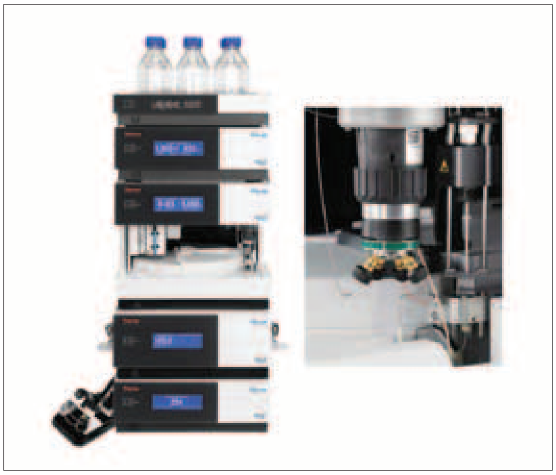
Thermo Fisher Scientific, the world leader in serving science, presents the TSQ 8000 triple quadrupole GC-MS system, a reliable, easy-to-use system that enables faster, more precise, more accurate analyses, saves time and reduces laboratory costs. It is designed for routine applications including the identification of food and environmental contaminants and trace compound quantitation or confirmation in forensic toxicology and sports doping laboratories.

The TSQ 8000 GC-MS/MS system is designed to make it easier than ever to realize the analytical advantages of GC-triple quadrupole methodology. The higher performance provided by the system enables analysts to view their target compounds at lower concentration levels, making new analyses possible and increasing confidence in results. By reducing errors and removing the need for repeat injections of the same sample, the new instrument also reduces costs and saves time in the laboratory. In addition, the greater selectivity of the instrument enables the analysis of more complex matrices, reducing the need for sample preparation and improving turnaround times and throughput.

The TSQ 8000 GC-MS system features state-of-the-art software for rapid and easy set-up and use, even for non-experts. The new system revolutionizes MS/MS method development with AutoSRM, a new program that automates much of the tedious work needed to create and optimize SRM transitions. Whether the starting point is a full-scan or SIM method, or the user is starting from scratch, AutoSRM guides analysts to fully optimized SRM transitions, making triple quadrupole technology more accessible than ever.

The TSQ 8000 system is also compatible with Thermo Scientific TraceFinder software, which can be used by other GC-MS and LC-MS systems, and makes routine quantitation and targeted screening simple, fast and productive.

For more information about the new Thermo Scientific TSQ 8000 system please visit
<thermoscientific.com/tsq8000>
or contact revista@novanalitica.com.br



THERMO SCIENTIFIC™ DIONEX™ ULTIMATE™ 3000 BIORS SYSTEM

GENUINE BIO UHPLC FOR BIOMOLECULE ANALYSIS

Ultra high performance liquid chromatography (UHPLC) provides improved separation speed, throughput, and sensitivity by employing stationary phase particles of around 2 µm or smaller. UHPLC has found widespread use in the analysis of small molecules in pharmaceutical, food, and environmental areas.

As of today the analytical benefits where not so applicable to the separation of larger molecules, such as proteins and peptides. Biomolecules have, apart from their size, other differences from small molecules (e.g. charges, complex structures, etc) that make the application of UHPLC not as straightforward as for small molecules. Apart from smaller particles, special solvents and separation principles beyond reversed phase also need to be supported

The Thermo Scientific™ Dionex™ UltiMate™ 3000 BioRS system represents a platform that is designed with multiple modules to allow configuration for in depth multidimensional analysis or high throughput quality control. The extensive column portfolio extends UHPLC capabilities beyond reversed phase by offering IEX or glycan separation materials in smaller particles and longer columns. Additionally, a ready-to-use buffer set is available to perform pH gradient charge variant analysis. The buffer system allows application of linear gradients to achieve a linear pH profile.

With all these capabilities, Thermo Scientific™ Dionex™ UltiMate™ 3000 BioRS is the system that will work in development, characterization, and QC:

- Optimizing Analysis Time and Resolution for Peptide Mapping
- Method speed up for Protein Charge Variant Analysis using IEX
- Proteins Charge Variant Analysis with pH Gradients
- Glycan Separation Resolution when coupling LC to a Mass Spectrometer

For more information:
Contact: Marc Yves Chalom
Email: marc.shalom@thermofisher.com
Phone: 55 11 3731-5140

EVENTS 2013/2014

- **17º ENQA**
  Encontro Nacional de Química Analítica
  Belo Horizonte, MG – October 6-9, 2013
  http://www.17enqa.com.br/

- **16ª METROLOGIE**
  Congrés International de Métrologie
  Paris, France – October 7-10, 2013
  http://www.metrologie2013.com/

- **53º CONGRESSO BRASILEIRO DE QUÍMICA**
  Rio de Janeiro, RJ – October 14-18, 2013
  http://www.abq.org.br/cbq/

- **ALMA CONFERENCE 2013**
  Orlando, FL, USA – October 22-25, 2013
  http://www.labmanagers.org/

- **EQUALAB 2013**
  Congresso da Qualidade em Metrologia
  São Paulo, SP – August 27-29, 2013
  http://remesp.org.br/enqualab2013/

- **BRAZIL AUTOMATION ISA 2013**
  17º Congresso Internacional e Exposição de Automação, Sistemas e Instrumentação
  São Paulo, SP – November 5-7, 2013
  http://www.brazilautomation.com.br/

- **METROLOGIA 2013**
  7º Congresso Brasileiro de Metrologia
  Ouro Preto, MG – November 24-27, 2013
  http://www.metrologia2013.ufop.br/

- **PACCON 2014**
  Pure and Applied Chemistry International Conference
  Khon Kaen / Thailand – January 8-10, 2014
  http://paccon2014.kku.ac.th

- **PITTCON 2014**
  Chicago, IL USA - March 2-6, 2014
  http://pittcon.org/

- **BIOCOM**
  7º Simpósio Nacional de Biocombustíveis
  Cuiabá, MT – April 23-25, 2014
  http://www.abq.org.br/biocom/

- **XLIII SBBq**
  Reunião Anual da Sociedade Brasileira de Bioquímica e Biologia Molecular
  Foz do Iguaçu, PR – May 17-20, 2014
  http://www.sbbq.org.br/

- **37ª RASBQ – 37ª Reunião Anual da Sociedade Brasileira de Química**
  Natal, RN – May 26-29, 2014
  http://boletim.sbq.org.br/boletim/1077.php

- **SIMPEQUI**
  12º SIMPOSITO BRASILEIRO DE EDUCAÇÃO QUIMICA
  Fortaleza, CE - August 6-8, 2014
  http://www.abq.org.br/

- **ENTEQUI**
  7º ENCONTRO NACIONAL DE TECNOLOGIA QUIMICA
  Vitoria,ES - September 10-12, 2014
  http://www.abq.org.br/

PUBLICATION RULES

BrJAC, the ***Brazilian Journal of Analytical Chemistry***, is published quarterly in English by DKK Comunicacao, in its printed and electronic versions. It is intended for professionals and institutions acting in the Chemistry area.

Papers for publication must be submitted exclusively to ***BrJAC***, in English. Previously published articles will not be accepted.

Categories ***BrJAC*** publishes articles in the following categories:

a) Unpublished papers: new information on analytical research. Such articles should not exceed 25 (twenty five) pages.
b) Reviews: scientific articles on well established subjects, including a critical analysis of the bibliographical references, and conclusions. Such articles should not exceed 40 (forty) pages.
c) Technical and scientific information: case reports, presentation of new techniques, methods and equipment.
d) Miscellaneous articles: Those that do not fit into any of the above categories but are of acknowledged interest for Analytical Chemistry.
e) Letter to the Editor: comments, praises, criticism, suggestions and consultations to Authors are welcome and will be published at the discretion of the Editor-in-Chief.
f) Editorials
g) Book Comments
h) Presentation of novelties, always relevant to the area.

Manuscript preparation: Authors must present the manuscript in the most clear and succinct manner possible.

The Introduction must include important references, concerning both the nature of the problem being investigated and its background. The manuscript must include Introduction, Experiment, Results/Discussion, Conclusion and Reference and should not exceed 25 pages, including tables, figures and diagrams. All pages must be numbered. Authors should indicate, by text or marginal notation in the type script where the figures and tables are to be inserted. All papers must be typed with double spacing using Microsoft Word only, preferably at 12 pt but no smaller than 10 pt. A single file must be generated in the Portable Document Format (pdf) including the entire article, to be sent via online through ***BrJAC´s*** site: www. brjac.com.br.

Organization of the electronic file: all parts of the manuscript must be included in one single file organized as follows: cover page, text, bibliographical references, figures (with subtitles), tables and charts (with subtitles).

1st page: submission letter of the material including name, address, phone number and e-mail address of the principal author, establishing the exclusiveness of publication in ***BrJAC***, in case the article is published.

2nd page: (cover page) Article s title, which must be short, clear and succinct to facilitate its classification. A subtitle may be used, if needed. This page must include the full name of the author(s), their titles and institutions. Other collaborator´s names may be included at the end, in Acknowledgments. In a separate paragraph, indicate the place where the study was performed.

3rd page: abstract with a maximum of 250 (two hundred and fifty) words; it must include the work´s objective, essential data of the methods employed, and the main results and conclusions. Next, list the main terms or words to be used for bibliographic classification (keywords).

4th page: begin the text on the fourth page with the title of the article, without indicating the author(s) or the place where the work was performed.

Bibliographical References: begin a new page. The article must include only the consulted references, numbered according to their appearance in the text. Other authors citations already numbered must indicate exclusively the reference numeral: avoid mentioning the name of the respective author. It is not recommended to mention several authors with identical concept (prefer the author who demonstrated it). It is recommended to avoid citations older than 5 (five) years, except in relevant cases. Include references that are accessible to readers. Personal communications may be accepted exceptionally.

Please use the following model:

- ***Journals:*** name(s) of author(s), full surname, initial(s) for given names; name of journal (abbreviated as defined by the *Chemical Abstracts Service Source Index* - see http://www.cas.org/sent.html); year of publication; volume; number of first page.

1. Barma, T.Y.; Song, B.J.; L..*China Chem. Soc.* 1997, 87, 418.
   - If it is difficult to access the Journal, we recommend that you include its *Chemical Abstract* number, as follows:
2. Chistyvst, L.K.; Andrenev, I.D.; Karol, F.W.; Cyrus, G.H.; *Zabrousk. Tacsh. Wilheim. Lyris.; Huil. Garm. Visijak*. 1987, 28, 816. *(BI 97:89745s).*
   - If the paper does not have a full reference, please mention doi as follows:
3. Striver, J.; Costa, T.C.; Pial, Q.P.; Temiza, V.L.; Vargas, V.N.; *Metalochimica Acta* (2004), doi:20.4598/v. metalacta.2006.15.023.
   - We recommend the use of compound references, whenever possible, instead of individual references, following the style below:
4. Varela, H.; Torresi, R.M.; J. *Eletrochem. Soc.* 2000, 147, 775; Lemos, T.L.G.; Andrade, C.H.S.; Guimaraes, A.M.; Wolter-Filho, W.; Braz-Filho, R.; *J. Braz. Chem. Soc.* 1996, 7, 123; Angelo, A.C.D.; de Souza, A.; Morgon, N.H.; Sambrano, J.R.; *Quim. Nova* 2001, 24, 473.

- ***Books:*** name(s) of author(s): surname and initial(s); title of book (initial letters in capital letters), volume (if a series), edition (if not 1st); city where edited; publisher; year of publication and number(s) of chapter or of page(s) cited.

5. Cotton, F. A.; Wilkinson, G.; *Advanced Inorganic Chemistry*, 5th ed.; Wiley: New York, 1988.

- ***Edited book:*** name(s) of author(s), "in" title of book (initial letters in capital letters); name(s) of editor(s); city where edited; publisher; year of publication and number(s) of chapter or of page(s) cited.

6. Regitz, M. in *Multiple Bonds and Low Coordination in Phosphorus*

Chemistry; Regitz, M.; Sherer, O.J., eds.; George Thieme Verlag: Stuttgart, 1990, Chap. 2.

- ***Patents:*** Please inform the *Chemical Abstracts* number in parentheses:

7. Bash, T.; Dot, K.; Petersen, G.; Orbfield, B.; Cooper, D.; Sabbag, L.; Fce. *Le Kokai Tokkyo Koho 54 95,489* 1975. *(CA 233:P179341v)*
8. McDonald, L.B.; *US pat. 9,236,006* 1987. *(CA 233:P49542y)*

- ***Software:***

9. Sheldrick, G. M.; SHELXL-93; *Program for Crystal Structure Refinement,* Gottingen University, Germany, 1993.

- ***Theses:***

10. Velandia, J. R.; *Doctorate Thesis*, Universidade Federal Rural do Rio de Janeiro, Brazil, 1997.

- ***Material presented in Congresses:***

11. Ferreira, A. B.; Brito, S. L.; *Resumos da 20ª Reunião Anual da Sociedade Brasileira de Química,* Pocos de Caldas, Brazil, 1998.

- ***Internet pages:***

12. http://www.sbq.org.br/jbcs, accessed June 2001.

- ***Unpublished material:***

  - Material accepted for publication

13. Magalhaes, U. H.; *J. Braz. Chem. Soc.,* in press.

  - Material submitted for publication, awaiting approval

14. Magalhaes, U. H.; *J. Braz. Chem. Soc.,* submitted for publication.

  - Unpublished material

15. Magalhaes, U. H.; unpublished paper.

  - Material from personal communication

16. Magalhaes, U. H., personal communication.

Notes:

1. Unpublished results may be mentioned only with express authorization of the persons involved in their achievement;

2. It is recommended that, whenever possible, authors follow the IUPAC standards, particularly the International System of Units.

Illustrations: the illustrations must be numbered according to the order of appearance in the text. For photographs, figures or graphs, use Arabic numbers. Charts and tables use Roman numbers. Subtitles must be properly numbered. In each illustration, identify the sequence number, name(s) of author(s) and the correct position where the illustration (photograph and drawing) should be inserted. Illustrations must have bases measuring 8.5 cm or 17.7 cm. The same result should not be expressed by more than one illustration. Graphs are preferable. For figures, graphs, diagrams, tables, etc. identical to others previously published in the literature, the author must ask for permission for publication to the company or scientific society holding the copyrights, and send such permission to the Editor of ***BrJAC*** together with the final version of the manuscript. If the figures are scanned, they must be high resolution (800 dpi/trace bitmap) with .tif or .jpg extension, provided that they are in the dimensions specified by the Editors. Color photographs or drawings (300 dpi/grayscale) must be submitted with .tif/. jpg extension and maximum total width of 8.5 cm in order not to create problems when applied to the Journal pattern. Other extensions possible include .cdr, .eps, .cdx and .opj. In the particular case of schemes containing chemical structures, they must have always the same dimension in order to be uniformly reduced.

Submission of articles: Please send one single file of the full manuscript in the .pdf format.

Manuscripts to be revised: Manuscripts sent to authors for revision must return to the Editor within at most 45 (forty five) days. After this deadline the manuscripts will be considered withdrawn and the system will terminate the process. To be reopened, a new submission must occur, starting a new process.

Revised manuscripts: Submission of a revised manuscript must be done by the same author, following the instructions for document submission: complete .pdf of the revised version and the answers to Editors, detailing the changes made in the new version and indicating the alterations that were suggested in the opinions and justifying those that were not accepted by the authors. Both files must be sent through the online submission system. As they are received, an e-mail will be sent with the reference number of the paper. The author must be alert to the receipt of this e-mail with the submission code, since non-receipt means that the submission was not completed. The author will have 5 (five) days to complete it; otherwise, the system will not allow the submission and a new process must be started.

Final version: When requested to send the final version, the author will receive specific instructions concerning programs for file submission (text, figures, tables, etc). PDF files will not be requested at this stage.

Measurement standards: Follow the provisions in the General Table of Units of Measurement.

Abbreviations: Abbreviations are not recommended except those recognized by the International Bureau of Weights and Measures or those recorded and established in scientific publications. In case the abbreviations are many and relevant, place their definitions (Glossary) in a separate note. Abbreviations of terms established by Chemistry must follow the international standards traditionally in use, according to standard abbreviations.

Commercial names: The use of commercial names is not recommended.

Copyright: papers submitted for publication in ***BrJAC***, either from universities, industry or any other public or private institution cannot have been either published or being submitted for publication in another journal simultaneously. When the paper belongs to more than one author, it is clear that the other authors agree with the publication in ***BrJAC*** from the moment of its submission.

When submitting their papers for publication in ***BrJAC***, the authors agree that the copyright will become property of the *Brazilian Journal of Analytical Chemistry* – ***BrJAC*** , if and when accepted for publication. The copyright comprehends exclusive rights of reproductions and distribution of the articles, including reprints, photographic reproductions, microfilms or any other reproductions similar in nature, including translations. As to the contents of published articles, the sole responsibility is of the authors, and ***BrJAC*** and its Editors, Editorial Board, employees and collaborators are fully exempt from any responsibility concerning data, opinions or unfounded statements.

Final Considerations: ***BrJAC*** reserves the right to make, whenever necessary, small alterations to the manuscripts in order to adapt them to the Journal rules or make them more clear in style, obviously respecting the original contents. Whatever the nature of the submitted manuscript, it must be original in terms of methodology, information, interpretation or criticism. The qualification of the paper will be attested by two or more consultants indicated by the Editorial Office.

The world is looking at Brazil.

BrJAC focuses on the sustainable growth of companies and country in the future.

The continuous process of innovation, competitiveness and sustainable industrial development is viable by the dissemination of researches information and promoting RD & I.

BrJAC acts in the present with the eyes in the future, supporting the initiatives to keep masters and doctors in business.

According to the Brazilian Ministry of Education, the number of professionals with post-graduation increased by around 100% in Brazil in the last ten years. In 2002, the country had 23,400 researchers with a master degree and 6,800 with PhD degrees. In 2012, 41,300 researchers will obtain a master's degree and 13,300 a PhD degree.

CAPES has just recognized the quality of BrJAC ranking it as B4 in the Qualis classification. For a new Brazilian Analytical Chemistry journal, published in English in order to consolidate the integration of academic, business and research institutes, the news could not be better.

Honor what is ours!
Submit your work for publication in BrJAC.

BrJAC

Brazilian Journal of Analytical Chemistry

www.brjac.com.br