BrJAC

Brazilian Journal of Analytical Chemistry

an International Scientific Journal

Special Edition Celebrating 10 Years of BrJAC

April – June 2021 Volume 8 Number 31

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RIO DE JANEIRO 2021

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Brazilian Journal of Analytical Chemistry

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The *Braz. J. Anal. Chem.* (BrJAC) is a peer-reviewed, open access, scientific journal intended for professionals and institutions acting mainly in all branches of analytical chemistry.

ISSN 2179-3425 printed ISSN 2179-3433 eletronic

Scope

BrJAC is dedicated to the diffusion of significant and original knowledge in all branches of Analytical Chemistry and Bioanalytics. BrJAC is addressed to professionals involved in science, technology and innovation projects in Analytical Chemistry at universities, research centers and in industry.

BrJAC is a quarterly journal that publishes original, unpublished scientific articles, reviews and technical notes that are peer reviewed in the double-blind way. In addition, it publishes interviews, points of view, letters, sponsor reports, and features related to analytical chemistry. Once published online, a DOI number is assigned to the paper.

Manuscripts submitted for publication in BrJAC cannot have been previously published or be currently submitted for publication in another journal.

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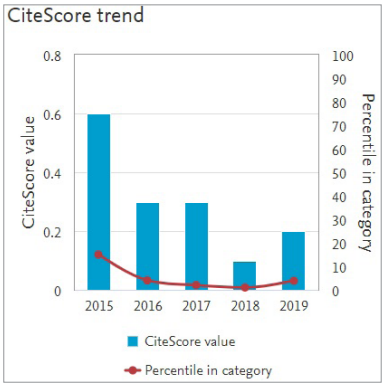
Indexing Sources

Scopus

CiteScore 2019: 0.2; SJR: 0.112; SNIP: 0.086. CiteScore Tracker 2020: 0.3 Access source details



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Expectancy: to be in the SCIE in 2021

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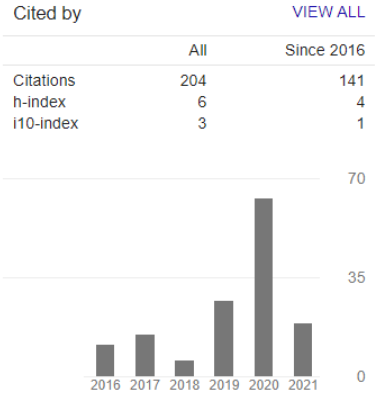

Production Editor
Silvana Odete Pisani

Publisher
Lilian Freitas
MTB: 0076693/ SP
lilian.freitas@visaofokka.com.br

Advertisement
Luciene Campos
luciene.campos@visaofokka.com.br

Art Director: Adriana Garcia

WebMaster: Daniel Letieri

BrJAC’s website: www.brjac.com.br / Contact: brjac@brjac.com.br
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ABEC BRASIL BrJAC is associated to the Brazilian Association of Scientific Editors

VISÃO FOKKA BrJAC is published quarterly by:
Visão Fokka Communication Agency
Av. Washington Luiz, 4300 - Bloco G - 43
13042-105 – Campinas, SP, Brazil
contato@visaofokka.com.br
www.visaofokka.com.br

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EDITORIAL

BrJAC 10 Years

Marco Aurélio Zezzi Arruda

Full Professor

Dept. of Analytical Chemistry, Institute of Chemistry at the University of Campinas (IQ-Unicamp)
Campinas, SP, Brazil

We are partying! This Edition of BrJAC celebrates the 10 years of existence of this scientific journal, which was conceived to act in the academic-industry integration through scientific-technical advances.

A large number of challenges have been or are being overcome, with many achievements obtained along this journey. The initial difficulties that are inherent to the consolidation of a scientific journal, and even to a developing country, were overcome step by step with the participation of people with first-rate scientific ideals who always worked behind the scenes at BrJAC, such as Mr. Carlos Roberto Rodrigues, one of the creators of BrJAC and Director of DKK Comunicação, the first publisher of BrJAC, and now with the Visão Fokka Communication Agency the publisher of BrJAC nowadays, and a great team of experts as Production Editor, Publisher, Advertisement, Art Director, and Webmaster. Last but not least, Prof. Lauro Tatsuo Kubota, the first Editor-in-chief of BrJAC.

The performance of these people and of our scientific community was fundamental, so that the idea sown a decade ago could bear the fruits that we see today. A lot of science, competence, and determination were, and are, the engine that makes BrJAC reach the penetration it has, in dozens of countries. The constant search for the perfecting of the publishing processes, with the implementation of a completely digital platform, the implementation of Webinars, the implementation of the "Young Talent in Analytical Chemistry" award, a first-line Editors' body, among others, and the commitment from everyone, makes BrJAC consolidating itself as a major scientific journal.

To celebrate these 10 years, we have prepared a very special Edition that is full of history and scientific knowledge. We begin with a summary of interviews with high-ranking members of our scientific community, which were conducted throughout our history. Then, the "Point of View" and "Letter" sections bring opinions and more stories from important characters of the Analytical Chemistry life. The Reviews and Articles sections bring exponent names of Brazilian Analytical Chemistry, and other sections of great interest to the entire community finalize this celebrating issue.

The only step between dream and reality is attitude. Therefore, we are working hard to present, in each issue of the BrJAC, the best of analytical chemistry. As Editor-in-Chief of BrJAC, I feel very proud to be part of this reality.

Enjoy the reading!

Cite: Arruda, M. A. Z. **BrJAC 10 Years.** *Braz. J. Anal. Chem.,* 2021, *8* (31), pp 1-2. doi: http://dx.doi.org/10.30744/brjac.2179-3425.editorial.10years

Marco Aurélio Zezzi Arruda, a Fellow of the Royal Society of Chemistry (FRSC), has a degree in Industrial Chemistry from the Methodist University of Piracicaba (1987), a Master's degree in 'Nuclear Energy in Agriculture' from the Center for Nuclear Energy in Agriculture at the University of São Paulo (1990), a Doctoral degree in 'Advanced Analytical Chemistry' from the University of Cordoba (1995) and a postdoctoral degree from the Center for Nuclear Energy in Agriculture (1995-1996). He is currently director of the Institute of Chemistry at the University of Campinas (IQ-Unicamp) and also Full Professor at the Department of Analytical Chemistry in the same institute. He also coordinates the Sample Preparation, Spectrometry and Mechanization Group (GEPAM) and is a member of the advisory board of the Brazilian Institute of Science and Technology (INCT) for Bioanalytics.

Brazilian Journal of Analytical Chemistry
2021, Volume 8, Issue 31, pp 3-13
doi: 10.30744/brjac.2179-3425.interview.10years

INTERVIEW

Compilation of 30 Interviews with Senior Researchers published in the 10 years of BrJAC

In this special edition celebrating 10 years of BrJAC, a compilation of the interviews given during this period by renowned researchers is presented. This set of interviews provides the reader with a broad and diversified view of the evolution of analytical chemistry over the decades, both in Brazil and abroad. This compilation is also very enjoyable, as it brings back personal and curious memories of the interviewees. Enjoy reading!

Carol Collins

The first impression of Professor Collins is that of a person in love with Analytical Chemistry. And the last impression, too. Her academic and professional background – as wide as her passion – developed during 50 years of work in the United States, Belgium, Taiwan, the Philippines, and Indonesia. She settled in Brazil in 1974, the year she came to State University of Campinas to work in Radiochemistry. She became a Brazilian citizen and a full professor of Analytical Chemistry at Unicamp Chemical Institute, a position she held until her retirement. “Retired, but not inactive,” she hastens to say. Currently, she dedicates most of her time correcting and translating to English over 200 articles every year written by her Chemistry Institute colleagues, for publication in journals indexed abroad. During this interview she recalls a little of the history of Analytical Chemistry and talks about the future trends of this science.

Access this interview published in the BrJAC Vol 1 #0, 2010.

Elias Zagatto

A resume does not say everything about a person. This is the conclusion drawn by those who personally know Elias Zagatto, Professor at the Center for Nuclear Energy in Agriculture, University of São Paulo (CENA/USP) and Member of the Brazilian Academy of Sciences. After analyzing his resume it is normal that one would expect him to be a flashy guy, almost arrogant, supported by his brilliant academic career and many awards around the world. In practice, Zagatto is a simple guy who likes a good conversation and does not miss the opportunity to say that he doesn’t know everything and still has much to learn from life and people. He received BrJAC for an interview about the changes towards a cleaner chemistry, the interaction between industry and university and the highlight of Brazil on the international scene, especially with regard to the pioneering application of flow injection analysis on a large scale basis.

Access this interview published in the BrJAC Vol 1 #1, 2010.

Cite: Compilation of interviews published in the 10 years of BrJAC. *Braz. J. Anal. Chem.,* 2021, *8* (31), pp 3-13. doi: http://dx.doi.org/10.30744/brjac.2179-3425.interview.10years

Glaucius Oliva

When it comes to scientific research, Brazil is still considered a “young country, with many challenges ahead”. Despite this, the outlook is positive in the opinion of the Professor Glaucius Oliva, President of the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq), the most important federal Brazilian agency for research support. Prof. Oliva thinks that the country has progressed significantly, promoting a continuous growth in the generation of new knowledge and training qualified personnel for R, D&I. In an exclusive interview to BrJAC, Prof. Glaucius also spoke about the key challenges faced by the Brazilian Science, the underserved areas of science and what is needed to meet them, the criteria adopted by the CNPq for the evaluation of researchers, the contributions provided by the Laboratory of Protein Crystallography and Structural Biology, Institute of Physics of São Carlos University of São Paulo, among other issues.

Access this interview published in the BrJAC Vol 1 #2, 2010.

Ramon Barnes

The BrJAC invited Professor Ramon Barnes to tell us about our last conquers and what we, Brazilians, still should work on to foster analytical chemistry and spectrochemistry nationally. Prof. Barnes is an old friend of Brazil: he has been coming to our country for more than two decades to meet with Brazilian researchers, and thus, he speaks knowingly. The conversation ranged from a critical view of the analytical chemistry research in Brazil to the future steps we should take to broaden and strengthen the relationship with industry. His counsel is precious.

Barnes received a Ph.D. in analytical chemistry from the University of Illinois (1966), and he was a postdoctoral research fellow at Iowa State University (1968-1969). From 1969 to 2000 he taught analytical chemistry and maintained an international research program at the University of Massachusetts, where he is now Professor Emeritus. He has published more than 300 papers and edited four books.

Access this interview published in the BrJAC Vol 1 #3, 2011.

Marco Tadeu Grassi

Water and air pollution, destination and toxicity of wastes are today political problems that affect the whole world population in different degrees and with different complexities. This is one of the areas of study of Professor Marco Tadeu Grassi, from Federal University of Paraná, in southern Brazil. Professor Grassi is a Brazilian chemist dedicated to the field of Environmental Chemistry. Grassi has created and found infrastructure and financial support for laboratories in the field of Environmental Chemistry in Brazil. He has been researching in the field since the 90s, and has many ongoing research projects dealing with water, oil, instrumentation for chemical analysis, aquatic sediments, and pollutants in public water supply and in rivers and watersheds, plastic containers for water, bioavailability of heavy metals and many other themes. He talked to BrJAC about Environmental Chemistry in Brazil.

Access this interview published in the BrJAC Vol 1 #4, 2011.

Paschoal Senise

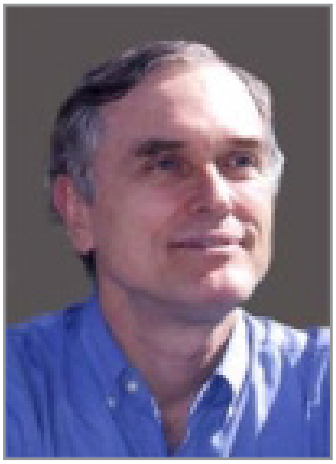
One of the most important teachers, researchers and analytical chemists of his generation, Professor Paschoal Senise built a long and solid academic career at the Institute of Chemistry at the University of São Paulo (IQ–USP). His carrer started back in 1935, when he and a small group of students formed the first class of Chemistry of the University. He was often consulted on academic matters, because of his deep knowledge of the system, its administrative structure and also because of his relevant role in Analytical Chemistry studies in Brazil. As an extremely dedicated teacher and researcher, Professor Senise has brought innovations and teaching techniques to USP and, in two different periods in the 1970s, he was director of the Institute as well. However, even when he was in this function, Professor Senise never stopped teaching.
This was one of the last interviews given by Professor Paschoal Senise, who received the Journal at the Institute of Chemistry, USP, on March 30, 2011. He died on July 21, 2011 at the age of 93.

Access this interview published in the BrJAC Vol 1 #5, 2011.

Antonio Celso Spínola Costa

In a recent publication prepared by the co-workers and colleagues of Professor Antonio Celso Spínola Costa, from Federal University of Bahia, Brazil, as a tribute to him, all testimonials (and they were more than 20) are unanimous in referring to him as an extremely dedicated teacher and mentor. He is the "guru" for most chemists from Bahia, and for many in the whole country. The title of the publication is indeed "Example of a professor and scientist for his and for future generations", and it shows that Spínola was always concerned about the education in Chemistry in the country, in the school and high school years to begin with. Such recognition, added to the many prizes and medals he won in his career, did not come by chance. It comes from hard work, intelligence, from leadership and from a deep love for teaching.

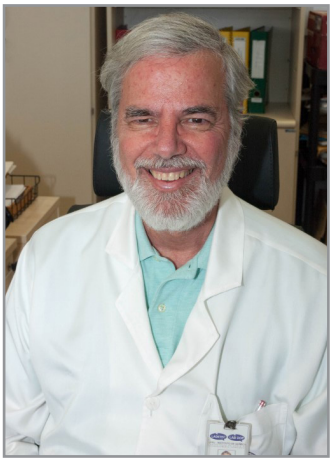
Access this interview published in the BrJAC Vol 1 #6, 2011.



Ivano Gutz

Prof. Gutz was only seventeen when he first started teaching. So interested he was in science, technology and in unravel the mechanisms of production, that even younger than all technicians and managers in the company, Ivano Gutz was invited by a large textile industry in Brazil to teach process technology to the new apprentices. At that time, a fruitful marriage between science and teaching began and, as we can see in this interview, only death will make them apart: after 35 years of a very intense professional life, with a nomination for the Brazilian Academy of Science, Prof. Gutz takes care of his health in order to keep teaching, researching and producing knowledge. This was one of the most difficult interviews to edit in this scientific journal, because the broad, and at the same time deep view of the work field that Prof. Gutz shows us was very hard to cut down to 10 pages: it is really a brilliant essay on the state-of-the-art of Brazilian Analytical Chemistry, both from the science and the professional perspectives.

Access this interview published in the BrJAC Vol 2 #7, 2012.



Francisco Radler

Prof. Radler coordinates nothing less than eight different laboratories at the *Universidade Federal do Rio de Janeiro*, ranging from technological support to chromatography, geochemistry, calibration and residue analysis. There, he has been doing research even before graduating, in undergraduate research programs. The broad scientific content in his research line is evident: the fields of knowledge range from the oil industry to the doping control in sports, from air quality to toxicology, from forensics to pharmacology. From this observation, one could assume that his contribution is shallow: on the contrary, Prof. Radler shows us, in this very interesting interview, his consistent view of the need of quality in analytical chemistry in Brazil, discussing the certification of laboratories, the experience of studying abroad and the teaching of chemistry in elementary school with a critical view.

Access this interview published in the BrJAC Vol 2 #8, 2012.

Roy Bruns

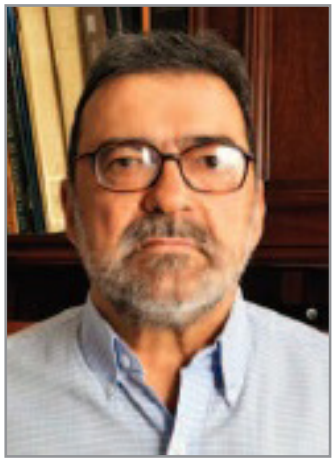
Prof. Bruns is an American chemist who came to Brazil in the 1970s to work as a researcher and professor in Universidade Estadual de Campinas (Unicamp), and became the pioneer in chemometrics in our country. In this exciting field of analytical chemistry, Prof. Bruns started at a time when computers were enormous structures occupying entire rooms, and when laboratory instruments did not have an interface with computers. However, in a typical Brazilian way, he was able to overcome difficulties with little resources, and started a highly productive career. Prof. Bruns talked with BrJAC about his studies, the history of chemometrics in Brazil, the development of the field since then and the newest mathematical tools, created in the last years. He also explained the role of analytical chemists in the development of research models in the future and shows that there is a large field to explore in analytical chemistry research.

Access this interview published in the BrJAC Vol 3 #9, 2012.

Ivano Gutz

In May 1983, a Brazilian Normative Act 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, 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. In this second interview for BrJAC, Prof. Gutz analyzes what this period represented for the analytical chemistry scientific and commercial contexts. This is a detailed narrative from someone who personally faced 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.

Access this interview published in the BrJAC Vol 3 #10, 2012.



Orlando Fatibello

Prof. Fatibello talked to BrJAC about the history of electroanalytical chemistry in Brazil. It was a privilege to hear about this from him, because Prof. Fatibello is one of the pioneers in the area in Brazil. As the founder of the first analytical chemistry lab at the Federal University of São Carlos, he describes his field of work as a growing, promising research area.

With new ideas for investigation arising in happy hours with friends or during a shower, Prof. Fatibello is a tireless researcher who made a large contribution to the Brazilian rank of publications in analytical chemistry by publishing interesting papers on its development. Following this interview, the reader can get a very nice picture of the historical development of electroanalytical chemistry in Brazil, largely concentrated in São Paulo State but now spreading all over the country.

Access this interview published in the BrJAC Vol 3 #11, 2013.

Wilson Jardim

Prof. Jardim, from the University of Campinas, SP, Brazil, is one of the pioneer scientists that did not overlook the social and political role of chemists that are involved with environmental chemistry. He talked to BrJAC for one hour about the history of this field of investigation in Brazil, of which he is a very important representative. Prof. Jardim expressed his concerns about water, energy, sanitation, “green dogmas” and many other issues that are interesting for the experienced chemists and also those in the beginning of the career.

For the beginners, Prof. Jardim guarantees: “With a mosquito repellent, a sun blocker and a good idea, any responsible and ethic scientist can explore the outdoor laboratories available in Brazil: Rain Forest, Pantanal, Cerrado, Caatinga, and many other ecosystems in our country. Chemists can leave the traditional labs and build their own lab in the open field. There is a lot to study”.

Access this interview published in the BrJAC Vol 3 #12, 2014.

Joaquim Nóbrega

Prof. Joaquim Nóbrega gave a nice interview to the BrJAC. Among many subjects that were discussed, Prof. Joaquim talked about the Group for Applied Instrumental Analysis (GAIA) started in 1994 when Dr. Ana Rita Araujo Nogueira (Embrapa Pecuária Sudeste) and him started a Scientific and Technological Development Support Program (PADCT) with support from the “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq). Prof. Joaquim explained that GAIA’s main motivation is to support students in performing research dedicated to solving demands for analytical applications and also improving the capability of modern instruments performing elemental analysis based on atomic absorption and emission processes, and mass spectrometry. Prof. Joaquim also talked about the GAIA Institute of Spectrometry, whose main goal is the development of human resources in the field of spectrochemical analysis and sample preparation.

Access this interview published in the BrJAC Vol 4 #13, 2016.

Marcos Eberlin

With a vivid personality, Prof. Eberlin motivates everyone with his positive energy and joy of living. The passion for chemistry came as a child, when a teacher showed him how fascinating chemistry can be. Since then, he has traced his way into the world of chemistry, specializing in mass spectrometry. Prof. Eberlin believes that analytical chemistry is the art of revealing in details the chemical composition of a sample. He continues: “In regard to mass spectrometry, analytical chemistry is the art of going to the molecular and atomic level and inspecting a sample with the ‘hands’ of a mass spectrometer. Analytical Chemistry is therefore a very exciting journey into revealing the molecular secrets of our Universe, from nature to all man-made products! For the freshman I would say: discover new ways to ‘see’ the ballet of molecules and atoms that compose the chemistry of our life and universe! Innovate! Try the craziest ideas! Make things as simple as possible in a way that brings analytical chemistry to all!”.

Access this interview published in the BrJAC Vol 4 #14, 2017.

Celio Pasquini

Asked about the importance of scientific initiation programs in analytical chemistry, Prof. Pasquini replied: “Scientific initiation programs are the basis for the renewal of research and researchers in any area of science, including analytical chemistry. In Brazil there are already established actions that contribute to the scientific initiation to fulfill its role in the identification of new talents and to give continuity to the development and improvement of the quality of the national research. In my opinion, the earlier a student becomes aware of and engages in research activities, the better. It is better because they can contribute to the future of science by having their vocation awakened very early. However, if this doesn’t happen, at least they will have the knowledge and insight to defend research and science, even if they don’t follow a career in research. In the case of analytical chemistry, its intrinsic multidisciplinary character, and the scope of its direct action in problems related to the daily life of society, elects it as one of the most versatile areas to awaken vocations”.

Access this interview published in the BrJAC Vol 4 #15, 2017.

Isabel Cristina Jardim

At a certain point in her career Prof. Isabel observed that the overuse of pesticides was becoming an imminent danger due to environmental contamination and thus food insecurity; there was an urgent need for efficient and rapid methods to detect, identify and quantify pesticides. Thus, she started a new line of research on the development and validation of chromatographic methods for the determination of pesticides in different types of environmental matrices. During the interview, Prof. Isabel stated: “Teaching provides me a complete realization, because my eagerness to transmit my knowledge is great and the satisfaction of seeing smiles on the faces of the students as they understand the subject being taught is immeasurable. I always put the mission of teaching in the foreground, so that good professionals could be formed and sent into the market. Research is challenging and when you achieve the goals outlined you enjoy happiness without limits”.

Access this interview published in the BrJAC Vol 4 #16, 2017.

Fernando Lanças

Prof. Lanças, founded the Laboratory of Chromatography (CROMA) at the University of São Paulo in São Carlos, which acts in the development of new equipment and methodologies for the preparation of samples, separation and detection, especially Mass Spectrometry coupled with High Resolution Chromatographic Techniques. In addition, he is the founder of the Latin American Congress of Chromatography (COLACRO) and also the Brazilian Symposium on Chromatography (SIMCRO). In this interview Prof. Lanças stated: "A career in the field of chromatography is gratifying because it involves aspects of teaching, research and extension. As it is a technique eminently applied it can solve fundamental problems of Brazilian society. For those who want to ingress in this field, I suggest always seek to understand the principles behind the technique, as well as the fundamentals of the instrumentation involved, to become a chromatographer and not just an equipment operator".

Access this interview published in the BrJAC Vol 4 #17, 2017.

Norberto Peporine Lopes

The research interests of Prof. Peporine are centered on natural product chemistry and mass spectrometry in both basic chemical aspects and their biological and ecological importance. He has dedicated himself to contributing to the understanding of the phase one metabolism of natural xenobiotics. His laboratory, therefore, has special interest in all aspects of the discovery of new natural products and has a strong track record for innovation. Five spins off were born from his group, with most of them being created by former postdoctoral researchers.

When asked to mention important achievements in the world of analytical research he replied: "I think that one of the greatest social impacts of current analytical chemistry is the doctorate of Dr. Lívia Eberlin with Prof. Cooks. Bringing the mass equipment into the surgical room in the hospitals will strongly impact the mass spectrometry field".

Access this interview published in the BrJAC Vol 5 #18, 2018.

Mário César Ugulino de Araújo

Prof. Ugulino has been the coordinator of the Lab of Automation and Instrumentation in Analytical Chemistry and Chemometrics (LAQA) at the Federal University of Paraíba. The LAQA is a consolidated lab in the northeast region of Brazil, specializing in the areas of analytical instrumentation, automation of analytical processes, and chemometrics. When talking about the LAQA, Prof. Ugulino said: "Being the LAQA coordinator is a huge passion. It was extremely difficult to set up this lab due to financing issues in Paraíba. I stay at the LAQA for at least 10 hours every day, except when I am traveling, in meetings, in classes, or resting at weekends and on holidays. I am extremely passionate about my career as a professor and researcher in analytical chemistry. My advice for professionals from any area is: do everything in your professional life with passion and dedication. If you do not have passion for your profession, immediately seek another profession that will provide this. Doing things in life without passion can be very sad".

Access this interview published in the BrJAC Vol 5 #19, 2018.

Nelson Stradiotto

Prof. Stradiotto, whose career has been marked by an effective contribution in the training of human resources for science, has also excelled in the development of research in Electrochemistry and Electroanalysis. The subject of his research is related to sensors, electrochemical detectors coupled to chromatographic techniques, and electroanalytical methods in the bioenergy area, with emphasis on biofuels, bioproducts, biomass, and bio-refineries. With his critical and balanced thinking, Prof. Stradiotto has assisted university institutions in finding solutions for technical and administrative issues, in the development of scientific knowledge, and in the training of human resources. As a result of the respect of his peers, he was nominated to coordinate the "Bioenergy Research Institute", a project developed at UNESP jointly with UNICAMP and USP, as well as representing UNESP at the Institute of Studies Brazil Europe (IBE).

Access this interview published in the BrJAC Vol 5 #20, 2018.

Alice Aparecida Chasin

Prof. Alice Chasin has experience in toxicological analysis and toxicology education. When asked about the most important moments of her 30 years of dedication to the scientific research, Prof. Chasin said: "One of the most important was the moment of my decision to pursue academic life when I was working for 10 years at the Legal Medical Institute. The job I was doing fascinated me. Being able to speak for those people who have died (what happens in the post mortem analyzes) has always thrilled me and very early I was aware that without science, it is impossible to carry out this task with such responsibility. I understood that in academic life I could reconcile these two aspects of my work, and it happened. Another important moment was the invitation for me to teach toxicology classes at Universities. I am very pleased to have been able to make this link between academia and practice. However, without a doubt, my greatest achievement was to have participated in the training of professionals who are now professors and experts who carry out their work with ethics and competence. This is my pride!".

Access this interview published in the BrJAC Vol 5 #21, 2018.

José Luís C. Lima

Prof. Lima is an Emeritus Professor at the Faculty of Pharmacy, University of Porto, PT. With an extensive and prestigious academic career, Prof. Lima collaborated and continues to collaborate with many Brazilian research groups. About the career that a recent graduate can expect in the field of analytical chemistry, Prof. Lima said: "The speed of technological evolution recommends moderation in the predictions that can be made regarding any activity. I would stress, however, that given the nature and areas of intervention of analytical chemistry, it will always need specialists who respond to society's growing concerns in areas such as food safety and environmental control, as well as public health. Such a scenario assures newcomers good perspectives and a lot of work, whether they face their future activity in teaching, research or control, or whether they work in public or private entities".

Access this interview published in the BrJAC Vol 6 #22, 2019.

Luiz Alberto Colnago

Since 1986, Dr. Colnago has been a prominent researcher at the Brazilian Agricultural Research Corporation (Embrapa). He is a specialist in *in vivo* or *in vitro* Nuclear Magnetic Resonance (NMR) applied to biological systems and in the development of NMR instrumentation for analysis of chemicals, food, fuels and other products. Early in his career, Dr. Colnago develop a low field NMR spectrometer for non-destructive determination of the oil content in corn seeds. In recent years, Dr. Colnago and his team have proposed the use of low field NMR using permanent magnets to investigate adulterations in fresh and processed foods, biofuels and biomaterials, and to monitor electrochemical reactions *in situ*, among other applications. The researcher's dream is that one day these NMR devices can be in supermarkets so that consumers can assess the quality of the products they are buying.

Access this interview published in the BrJAC Vol 6 #23, 2019.

Fabio Augusto

Prof. Fabio Augusto, a pioneer researcher in Brazil in the development of modern analytical separation techniques works in two major areas: development of systems and methodologies in GC×GC for applications in petroleomics, food analysis and microbiological metabolomics, and microextraction techniques for chromatographic analysis. For young scientists who wants to pursue a career in Chromatography, Prof. Fabio Augusto says: "One should always keep in mind that analytical chemistry is essentially an applied science. Anyone wishing to make a significant contribution to the development of chromatography must be aware of the demands of industry, academia, and society. To know where the relevant problems lie, we should always listen and interact with professionals in fields such as medicine, agronomy, food science, and environmental science, even though, in many cases, this is a naturally difficult dialogue".

Access this interview published in the BrJAC Vol 6 #24, 2019.

Matthieu Tubino

In 1970, Prof. Tubino and three colleagues were invited to work at the then-beginning Institute of Chemistry of the University of Campinas. Shortly after the invitation, the four newly formed chemists went to the city of Campinas, SP, to visit the campus of that university. Of this trip, Prof. Tubino remembers: "I can say that this campus was a vast cane field, no longer cultivated, but some sugarcane clumps were still there. There was only the building of the university rectory and two more sheds. In one of the sheds, the Institute of Chemistry was located, along with other institutes and colleges. The building of the Institute of Chemistry was still under construction, and its occupation began in 1971. Given the conditions at that time, two of my colleagues gave up, but one colleague and I agreed to stay. Those were hard years, both in terms of working conditions, as everything had to be done, and in political terms because the political regime in Brazil was dictatorial and no one felt safe, even those who had no involvement with politics".

Access this interview published in the BrJAC Vol 6 #25, 2019.

Ronei Poppi

When asked about what has changed in the student profile since the beginning of his career, Prof. Ronei said: “Classes need to be more dynamic and with greater student participation. We need to teach students how to think chemically (or analytically) and not simply show how to push buttons on a device, because the robots will push the buttons in the near future. I can mention an interesting fact in a laboratory class, where it was necessary to know the molar mass of a given compound to prepare a solution. A student took out his smartphone and simply asked the molar mass, which was promptly provided. This exemplifies the fact that much information is readily available and may no longer need to be memorized. The worst is that even today there are professors who prohibit the use of smart phones in class”.
This interview was given in March 2020 and regrettably Prof. Ronei Poppi passed away on April 25, 2020.

Access this interview published in the BrJAC Vol 7 #26, 2020.

Jose Manuel Riveros Nigra

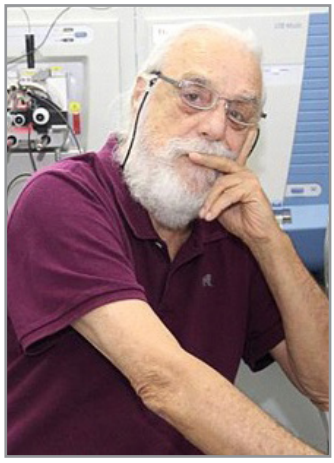
Prof. Riveros is an Emeritus Professor at the Institute of Chemistry of the University of São Paulo (IQ-USP). Internationally known for his important contributions to the field of gas phase ion-molecule reactions using a combination of mass spectrometric techniques and electronic structure calculations, Prof. Riveros continues to contribute to the IQ-USP as a Senior Professor. Recalling the beginning of his studies, Prof. Rivero said: “In 1962, I was admitted as a graduate student at Harvard University with a full scholarship. There, I undertook my Ph.D. thesis on molecular structure studies by microwave spectroscopy under the supervision of Prof. Bright Wilson. My experience at Harvard, from 1962 to 1966, was very stimulating because of the opportunity to interact with some of the great names in Chemistry and Physics. I also had the great pleasure of sharing an apartment in Cambridge, MA, USA, with two other graduate students and great friends, one of whom, Tom Steitz, would earn the Nobel Prize in Chemistry in 2009”.

Access this interview published in the BrJAC Vol 7 #27, 2020.

Érico Flores

Asked about the recent advances and challenges of Analytical Chemistry in Brazil, Prof. Érico replied: “Currently, Analytical Chemistry is one of the most vigorous fields of chemistry in our country and has researchers of great international recognition. There have been substantial advances in all areas of Analytical Chemistry, largely due to advances in microelectronics and new materials with properties that allow the construction of detectors, reactors, instruments, etc., with many advantages over the instruments produced a few years ago. Despite all these advances, one of the biggest challenges for scientific research in Brazil is to transform the knowledge generated into applications that impact the daily lives of different sectors of society, from the economy to social and environmental well-being. For this, we will have to advance even more in the popularization of science, with...”.

Access this interview published in the BrJAC Vol 7 #28, 2020.



Gilberto Domont

Prof. Domont heads the Proteomics Unit of the Institute of Chemistry at the Federal University of Rio de Janeiro. Talking about what motivated him to become a proteomist, Prof. Domont remembered: “I was trained and did classical protein chemistry until 1989/1990 when I spent the winter months working on modern protein sequencing techniques at the Yale School of Medicine. Fred Richards and William Konigsberg, as well as John Fenn were also there. By memory, I recall Fred and Bill chatting in the large school corridor on Fenn’s mass spectrometry electron spray experiments. The discussion was about the consequences of Fenn’s electrospray technique to scientific research. No one had any idea where it would lead or what could happen with the science that was done at the time, but everyone was sure that it would revolutionize research in the health sciences. I found the electrospray technique to be my calling. Years after, dinning a hot soup in the relaxing hotel restaurant after the promenade of the II ESPRIT and I EuPA Congress, Valencia, Spain, 2007, I told John how I was hooked by his ESI at Yale.

Access this interview published in the BrJAC Vol 7 #29, 2020.

Brazilian Journal of Analytical Chemistry
2021, Volume 8, Issue 31, pp 14-15
doi: 10.30744/brjac.2179-3425.pov-pvoliveira-10years

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POINT OF VIEW

The Role of Analytical Chemistry in Agrofood

Pedro Vitoriano Oliveira
Departamento de Química Fundamental
Instituto de Química – Universidade de São Paulo
São Paulo, SP, Brazil

The State of Food Security and Nutrition in the World program for sustainable development presents a transformative vision, recognizing that our planet is changing, bringing with it new challenges that must be overcome if we want to live in a world without hunger, food insecurity, and malnutrition, in any of its forms [1]. At the same time, there is a direct relationship between the quality of food and health. The production of high-quality food in high quantities is an emerging concern. However, the challenges are enormous, as demonstrated by the following: i) there is a demand for food production, occurring amid a declining rural labor force; ii) there is an increasing amount of raw material production (*e.g.*, biomass generated) for a market that has been only mildly explored; iii) there is a lack of simpler and cheaper analytical alternatives to apply in undeveloped countries, whose developments is highly dependent of agriculture; and iv) there is the necessity to adopt more efficient and sustainable production methods that are adapted to climate change [2]. On almost all these fronts, the field of analytical chemistry has a lot to contribute.

Small-scale and affordable agricultural production, close to the consumed markets, can be a way to expand food production at more modest costs. For this, the development of sustainable, low-cost, and easy to use analytical devices that can meet technological demands and monitor the quality of irrigation water, soil nutrients or hydroponic solutions, and substance levels from pesticides could help to overcome some of these challenges [3]. On the other hand, the environmental impacts caused by the intense use of nitrogen and phosphate fertilizers can already be seen, especially in countries where agriculture is produced intensively and extensively. The consequences can be seen in the short term, with the irreversible impairment of cultivated soils [4]. One way to remedy these impacts is to use technology to produce food in a more sustainably way. The internet of things is a trend that aims to establish a technical and integrated evolution, with the objective of making processes and products more efficient. For this, integrated machines and sensors can use decision-making routines to work towards a common product or solution. The expansion of this technical revolution in the value chain of complex areas, such as agriculture, food production, and health, requires the implementation and connection of sophisticated methods [5]. In this way, the integrated control of parameters, such as humidity, pH and soil composition, dosage of nutrients or pesticides, leaf composition, and physical–chemical characteristics of the harvested agricultural product, can be monitored in situ using optical (*e.g.*, LIBS) or electrochemical sensors (*e.g.*, electrodes and microelectrodes), which can be monitored remotely to minimize costs, reduce environmental impacts, and increase production efficiency.

From the point of view of the most fundamental studies, there are still many unanswered questions in the food production sector that cannot do without analytical chemistry support, such as in the evaluation of the synergistic effects between the elements during translocation to plants (*e.g.*, the role of rare earth elements [6] and selenium [7] in protecting against abiotic stress, especially stress associated with metals), in the control of residues and organic contaminants [8], and in the use of proteomic and metabolomic strategies for identification of target and non-target species in plants and food [9] to compare, for example, transgenic

Cite: Oliveira, P. V. The Role of Analytical Chemistry in Agrofood. *Braz. J. Anal. Chem.,* 2021, *8* (31), pp 14-15. doi: http://dx.doi.org/10.30744/brjac.2179-3425.pov-pvoliveira-10years

and non-transgenic soybean strains [10]. In addition, analytical chemistry can support studies related to the production of food fortified with inorganic nutrients, evaluating the translocation efficiency, generating information about the concentration of elements and species that can help to understand physiological effects on plants and monitor safe and quality of the food produced. The deficiency of micronutrients (*e.g.*, Fe, Se, and Zn) in the diet of the populations is increasingly prevalent. The concern to decrease this deficiency is related to the current concept of biomedical agriculture [11], requiring interdisciplinary actions between different experts to ensure that the food production cycle, aligned with this concept, is respected [12]. In the field of fortification and biomedical agriculture, chemical speciation is particularly one of the specialties of analytical chemistry that could be very useful.

Finally, in addition to the development of easy, cheap, robust and environmentally friendly methods, in this field, analytical chemistry has to work in a more integrated, inter and multidisciplinary way in order to help solve the great challenges of the agrofood sector, producing high-quality and safe food, while respecting life and the planet.

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Pedro Vitoriano Oliveira is currently Full Professor at the Institute of Chemistry at the University of São Paulo (IQ-USP), São Paulo, SP, Brazil. Works mainly on Optical Methods of Analysis (AAS, ICP OES and ICP-MS) and sample preparation for total elements determination and speciation in biological, food, environmental and technological samples. Coordinates the Spectrometry Analysis and Research Group (GAPE) at IQ-USP. CV Curriculum Vitae

Brazilian Journal of Analytical Chemistry
2021, Volume 8, Issue 31, pp 16-21
doi: 10.30744/brjac.2179-3425.letter-cblatt

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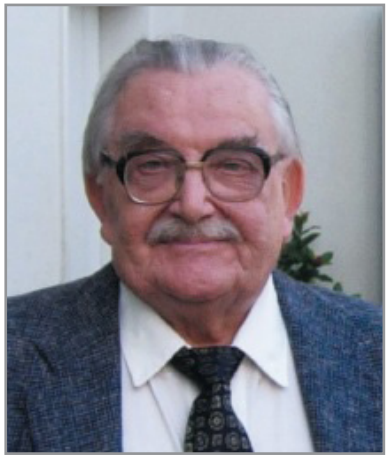
LETTER

Professor Remolo Ciola, a Master and a Genius

Celso Blatt ✉
GC and GC/MS Application Scientist
Agilent Technologies Brasil
Barueri, SP, Brazil



Remolo Ciola

In 1984, when I was in my last year as an undergraduate in Industrial Chemistry at the Federal University of Santa Maria (UFSM), Santa Maria, RS, Brazil, I heard about Professor Remolo Ciola. A Professor from UFSM introduced me to the book "Fundamentos da Catálise" written by Prof. Ciola (Figure 1). I liked the subject catalysis and contacted Prof. Ciola to see whether I could discuss the possibility of doing a Master's degree at the University of São Paulo (USP) in São Paulo, SP, where he was a Professor.

Arriving in São Paulo for this initial conversation, I found out that Prof. Ciola had a company called "Instrumentos Científicos CG Ltda", on Avenida Domingos de Moraes, in the south area of the city of São Paulo, where the meeting would take place. When I arrived at the company, Prof. Ciola welcomed me, but said that despite having guided several people in catalysis, he was no longer working with this technique at USP. He made me a proposal that surprised me: "How about working with gas chromatography and analyzing sugar?". Until that moment I had never heard of gas chromatography and no chromatography was used at UFSM at that time. Prof. Ciola clarified that to analyze sugars I would use chemical reactions to attack the OH groups of the sugars and exchange them for an acetyl or trimethyl silicon and make these sugars volatile. To convince me, he said that he would have to test catalysts for these derivatization reactions.

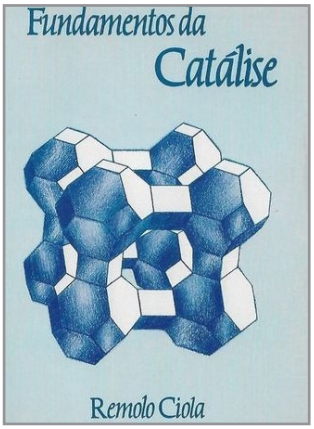


Figure 1. Cover of the book entitled "Fundamentos da Catálise" by Remolo Ciola.

At UFSM, the first Master's class would open in 1985, so I took a qualification test for the Master's degree in Santa Maria and at USP. As the São Paulo Research Foundation (FAPESP) scholarship came out first, I decided to come to São Paulo.

Prof. Dr. Remolo Ciola was already well known and respected in 1985 when I arrived at USP. He was born in Italy in 1923 and came to Brazil at a young age. He graduated in chemistry at USP in 1948 and taught at the Technological Institute in Aeronautics (ITA) in São José dos Campos, SP, and at the Engineering School of Mauá in São Caetano do Sul, SP, before teaching at USP. Prof. Ciola defended his Master's degree in the United States in 1958 and his Doctorate at USP in 1961. For many years he was director of the research center at the "Refinaria União" (Petrobrás oil refinery) in Mauá, SP. Despite having already started experiments with chromatography in 1954, Prof. Ciola built the first gas chromatograph with Thermal Conductivity Detector (TCD) in 1958 to meet the needs of the refinery. He was a pioneer in Latin America, building this first gas chromatograph with parts

Cite: Blatt, C. Professor Remolo Ciola, a Master and a Genius. *Braz. J. Anal. Chem.,* 2021, *8* (31), pp 16-21. doi: http://dx.doi.org/10.30744/brjac.2179-3425.letter-cblatt

and supplies developed in his laboratory. At that time, the refinery acquired a lot of knowledge of catalysis and polymers.

The company “Instrumentos Científicos CG Ltda” was created in 1961 in the garage of Prof. Ciola’s house, together with his nephew Ivo Gregori. In the early years of the company, Prof. Ciola was the chemist and inventor and his nephew played the role of electronics and mechanic. It took me years to discover that the name CG was not Gas Chromatography, but the initials of the surnames Ciola and Gregori. The company’s objective was to produce gas chromatographs to supply industries, universities and laboratories that performed chemical analysis. The CG Company grew a lot due to the market reserve in Brazil that could not import equipment if there was a similar national product, and also with the Brazilian Pro-alcohol program where many plants bought gas chromatographs for alcohol analysis.

Dr. Ciola, as many called him, is the author of more than 170 scientific and technological papers, of several patents on catalytic projects and analytical instruments and the author of books on catalysis and gas and liquid chromatography. The chromatography books of his own are shown in Figure 2.

Figure 2. Covers of the three books on chromatography by Remolo Ciola. From left to right: “Introdução à cromatografia em fase gasosa”, “Fundamentos da Cromatografia a Gás”, “Fundamentos da Cromatografia a Líquido de Alto Desempenho”.

I started postgraduate classes in 1985 at USP, with the practical part being performed in the CG Company itself, which was moving to a new address on Avenida Vereador Jose Diniz in the neighborhood of Brooklin, São Paulo. Postgraduate classes with Prof. Ciola were very good and always well attended. A certain course that I took, Prof. Ciola applied a chemistry test on the first day of class on basic knowledge such as chemical structure, pH and chemical functions; half of the class did not get a score of 5 in the test and were prevented from taking the course. He did not admit those to a postgraduate course who did not have a basic knowledge of chemistry. The postgraduate courses given by Prof. Ciola at USP were Catalysis, Polymers, Gas chromatography and Liquid chromatography. Prof. Ciola has always impressed me with his knowledge of basic chemistry, organic chemistry, synthesis, polymers and catalysts. In addition, he was very good at designing and creating technological solutions to solve problems or create new things. He did the sketches, and then passed them on to a professional designer at CG Company where the workshop produced the piece.

During my Master’s I used the gas chromatograph in Figure 3 to perform the analysis of derivatized sugars. It was a gas chromatograph with a split injector and flame ionization detector (FID) and an oven temperature that was constant and set on the button in the red square in Figure 3. This chromatograph did not have a display to indicate the real temperature in the oven, but had red LEDs above the mentioned button. If the LED on was the LED to the left of the central LED, it indicated that the temperature had not yet reached the set temperature. The illumination of the central LED theoretically indicated that the oven had reached the expected temperature. I needed to make a temperature ramp for my analysis, which required

me to use a stopwatch. I injected the sample and every minute I turned the knob up by 10 degrees. I worked like this for a year until Prof. Ciola provided a marvel of the time, which was the box on the side of the Figure 3, a linear temperature programmer. With this box we no longer needed to use the timer for temperature ramps, and it has since become automatic. I even saw other users in the CG Company using the recorder, but Prof. Ciola was generous and gave me an integrator/recorder with which to handle the data.

Figure 3. Original gas chromatograph Model 37 from CG Instrumentos Científicos Ltda.

It caught my attention that the employees at CG Co. did everything from manufacturing all the pieces to preparing the packed and capillary columns. I even helped to pack very long columns where a person was on an upper floor transferring the stationary phase with a funnel into the column and I was on the lower floor hitting the column with a piece of wood, for a good packaging of the column. I also followed how the capillary columns were prepared. CG Co. bought the fused silica tube and made the preparation and deposition of the stationary phase film. Working at CG Co. was very pleasant, and it felt like a family. The photo below (Figure 4) taken at Prof. Ciola's house shows his wife Celia and son Remolo on the left, and me and other graduate students Silvana, Celina and Rivana on the left.

Figure 4. Photo taken at Prof. Ciola's house. From left to right: Célia and Remolo Ciola, seated; Remolo Ciola Filho, Celso Blatt, Silvana Pisani, Celina Takayama, Rivana Marino, standing.

Dona Celinha, as Prof. Ciola called his wife, was an engineer trained in Rio de Janeiro, Brazil. She told me how difficult it was to be a female engineer when studying at the Institute of Nuclear Engineering in Rio de Janeiro. Son Reminho also worked at CG Co. and taught me how to make spreadsheets in Lotus 123, the precursor to Excel.

Prof. Ciola was always very innovative, read a lot and subscribed to several scientific journals. At that time there was no Google, and Prof. Ciola asked me to do a bibliographic review of the last decade. I spent about 3 months in the USP library consulting the famous Chemical Abstracts; when I found a reference, I had to look for the journal with the article, take a Xerox copy and take it to read at home. In the Master's, I tested several reactions to derivatize the sugars and found that with hexamethyldisilazane (HMDS) and imidazole as a catalyst, the reaction with OH groups happened quickly and efficiently. To write the thesis, I used a computer, but in 1987 there was no Word, no spell checker, and no Paint, or any of the other wonders we have today. The computer used was an Apple, the text editor Wordstar had no accent; the printing was done on a dot matrix printer. The figures had to be glued to the text and we used a concealer to avoid showing glue in the figures. In defense of my Master's thesis, Prof. Ciola pointed out that it was one of the first USP theses typed on a computer, but a Professor at the examining board complained that it had no accents in the text. Thanks to the arguments of Prof. Ciola my Master's was approved.

In 1986, when Professor Ciola and I went to the 1th Latin American Chromatography Congress (Colacro) in Rio de Janeiro, we realized that chromatography using supercritical fluids was one of the most talked about subjects at the congress. In 1987, when I defended my Master's degree, Prof. Ciola made me the offer to work at CG Co. as an employee and also invited me to do a doctorate in Supercritical Fluid Chromatography (SFC) and Supercritical Fluid Extraction (SFE). For someone who came to São Paulo with plans to do a Master's degree and return to the South of Brazil in two years, it was a turnaround that today means I have spent more than 35 years in São Paulo. Prof. Ciola's doctoral proposal was to study chromatography with supercritical fluids and also perform extraction using supercritical fluids. At the time, there was no commercial equipment, so we had to develop the hardware, which was what encouraged Prof. Ciola. To use carbon dioxide as a fluid, we needed liquid CO_2; at the time, it was uncommon. It was easy to solve this, however, as we took a cylinder of CO_2 fire extinguisher and put it upside down and took the liquid. To pump the CO_2, we used a Haskel multiplier pump that Prof. Ciola had bought to pack the HPLC columns. For restricting flow after the column, Prof. Ciola bought tubes of fused silica with 5 and 10 microns. In addition to chromatography, the extraction process using supercritical CO_2 was studied; the genius Prof. Ciola took action again by designing a heated restrictor after extraction and inside the injector of a GC/MS. We then had an online system SFE-GC/MS. This online coupling using the heated restrictor shown in Figure 5 yielded my first article in an international journal in 1991 and participation in the International Symposium on Capillary Chromatography in Riva Del Garda, Italy. The drawing shows how Prof. Ciola had a brilliant mind and was a good draftsman. The drawing below was done by a professional draftsman, but the sketch and idea were by Prof. Ciola.

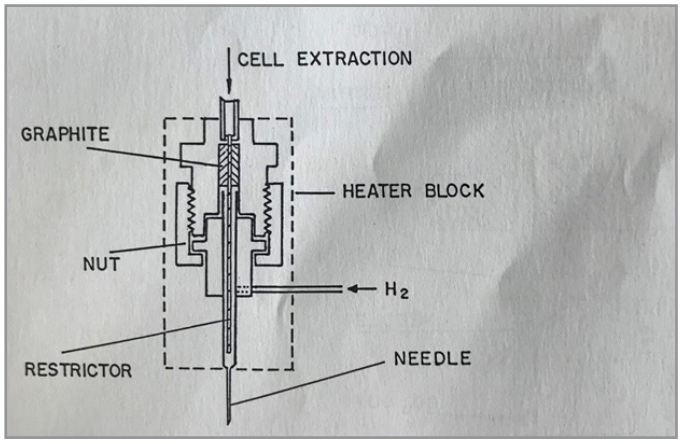


Figure 5. Schematic diagram of the interface between extraction cell and PTV insert in online SFE-PTV-GC.

During my doctorate, talking to Rita who made the capillary columns at the CG Co., I discovered once again how Prof. Ciola was a genius. After cutting the 30 meters of a capillary column and wrapping it in the basket, the first part of the preparation process was to clean the tube and leave the surface wettable and then lock the phase in the silica. As they had difficulties with this first stage, Prof. Ciola changed the process to treat the OH groups of silica with HMDS and imidazole, which worked. Prof. Ciola's genius was that he used my Master's degree to solve a problem in the production of capillary columns.

The CG Co., in addition to manufacturing its own gas chromatographs, also represented Shimadzu for a while and that's when I learned to use GC/MS. I even installed some GC/MS and gave training. Times started to get difficult in CG Co. because they lost Shimadzu's representation and the market reserve in Brazil ended. In this way, eight CG employees, including myself, made a deal and left CG Co. to set up a new company in February 1992, named Analítica, known today as Nova Analítica. Prof. Ciola remained at CG Co. until 1998, and the company's name was changed to "CG Científica". I stayed at Analítica for six months, when, in September 1992, the company Hewlett Packard invited me to work in support of the SFE and SFC instruments that they were producing and selling. In 1999, Hewlett Packard separated the areas of computing and measurement. The measurement area became Agilent Technologies, where I work today.

In 1999, Professor Ciola started his own company called Cromacon and continued to produce national gas chromatographs and make packed and capillary columns. Figure 6 is a photo of Prof. Ciola at the Cromacon Ciola booth at an instrument exhibition.

Figure 6. Remolo Ciola at the Cromacon Ciola booth at an instrument exhibition.

The photo below (Figure 7) was taken on the occasion of Prof. Ciola's birthday in 2009. He used to say in classes that they washed their hands with benzene at the "Refinaria União". At the time nobody knew that benzene is a carcinogen substance. Prof. Ciola passed away in 2010, but he lived his 87 years very well.

Figure 7. Remolo Ciola and his wife Celia Ciola.

Thank you Prof. Dr. Ciola for your teachings, for all your dedication as well as for your contribution to the Brazilian chromatography.

For more information about Prof. Ciola and his biography, I recommend the In Memoriam entitled "Remolo Ciola, uma mente inventiva, um verdadeiro pioneiro" by Luiz Bravo and Silvana Pisani, published in the journal Scientia Chromatographica, 2010, Volume 2, Nº 3, pages 94-96, available through the link: http://iicweb.org/scientiachromatographica/

Celso R. Blatt is graduated in Industrial Chemistry (1984) from the Federal University of Santa Maria, Santa Maria, RS, Brazil, and took his Master's (1987) and Doctorate (1992) in Analytical Chemistry at the University of São Paulo, SP, Brazil. From 1987 to 1992, he was a Researcher in Analytical Instrumentation at Instrumentos Científicos CG Ltda, SP. In 1992, he started working as a Support Engineer at Hewlett Packard Co. then Agilent Technologies Brasil where he still works as an Applications Scientist.

Brazilian Journal of Analytical Chemistry
2021, Volume 8, Issue 31, pp 22-50
doi: 10.30744/brjac.2179-3425.RV-62-2020

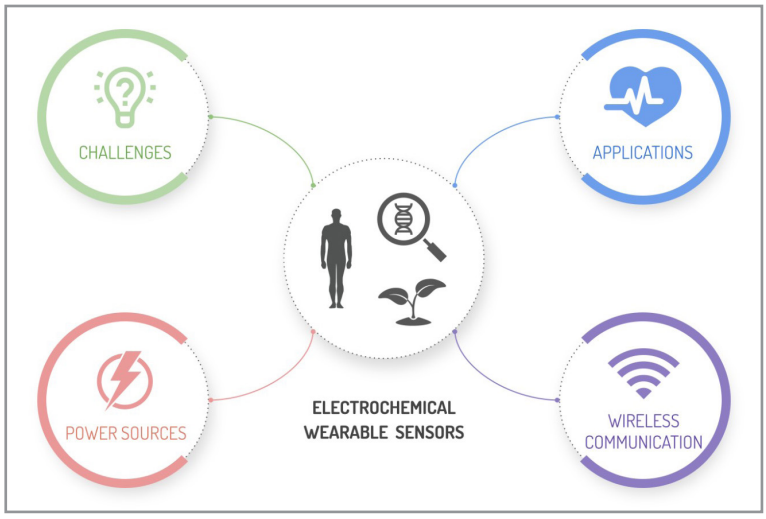
BrJAC

REVIEW

Wireless Wearable Electrochemical Sensors

Thais Maria Rabelo Alves, Patricia Batista Deroco, Dagwin Wachholz Junior, Lourenço Henrique Bittar Vidotto and Lauro Tatsuo Kubota

Instituto de Química, Universidade Estadual de Campinas (Unicamp), Rua Josué de Castro, Cidade Universitária, 13083861, Campinas, SP, Brazil



The demand for wearable sensors has been grown rapidly over the past few years, mainly those related to monitor health, fitness and their surroundings. Consequently, wearable chemical sensing has become a crucial appliance area for wireless sensors and has proved to be a very challenging and multidisciplinary area. The great advantage of coupling wireless communication to different types of wearable sensors is the enhancement of the sensor's scope for remote and resource-limited settings with the possibility of obtaining real-time data acquisition and application in different areas like homeland defense, home-based healthcare, and food logistics. Being the electrochemical sensors considered attractive and promising to use in the wireless chemical sensor field, due to its features such as simple structure, the possibility of miniaturization, comfort, simplicity of operation, high sensitivity, fast response, relatively low energy consumption and low manufacturing cost. Furthermore, wearable electrochemical sensors enable obtaining insights into individuals health status through the noninvasive monitoring of clinically relevant biomarkers in different biofluids without complex sampling, manipulation and treatment steps. In this review, we present the main advances in technologies used in the development of fully integrated wireless wearable electrochemical devices, such as communication protocols, data collection and privacy concerns and power sources. We also discuss in a critical way the main challenges, trends, strategies and new technologies that will drive this research line in the future. Lastly, we highlight the progress in the last few years in healthcare, sports, security and defense, and forensic applications.

Keywords: wearable sensors, electroanalysis, flexible electronics, Internet-of-Things, continuous monitoring.

Cite: Alves, T. M. R.; Deroco, P. B.; Wachholz Junior, D.; Vidotto, L. H. B.; Kubota, L. T. Wireless Wearable Electrochemical Sensors: A Review. *Braz. J. Anal. Chem.*, 2021, *8* (31), pp 22-50. doi: http://dx.doi.org/10.30744/brjac.2179-3425.RV-62-2020

Received 23 October 2020, Revised 10 March 2021, Accepted 13 March 2021, Available online 26 March 2021.

INTRODUCTION

Interest in the development of wearable sensors has been steadily increased over the last years, following advancements in material science, flexible electronics, microfabrication, miniaturization techniques, and the rapid growth of the Internet-of-Things (IoT), as it becomes easier to integrate features such as real-time data acquisition and wireless communication into such devices. The IoT is a new prototype that is being developed in the current wireless telecommunications landscape, and its concept is based on a wide network of connected things, such as sensors, actuators, identifiers, and products, which can interact with others autonomously and also cooperate to achieve a common goal [1,2]. The IoT is not just an idea, but it is already a technological application from which our life can benefit [3].

In the field of analytical chemistry, these concept of connected wireless communication with chemical (bio)sensors allow analytes to be monitored in situ from a distance, even in hazardous places. Using the right wireless protocol, multiple devices can be linked together in networks, providing real-time data over large areas [4]. Therefore, the choice of a wireless technology depends on the connectivity requirements for its application. The most common technologies recently in use for wireless communication include Bluetooth, ZigBee, radio-frequency identification (RFID), and near-field communication (NFC), each with its own set of features and advantages [5]. Smartphones are also worth mentioning in this context, as they have been increasingly used in chemical sensing, due to its pervasiveness and computational capability. A smartphone can be used to perform data acquisition and analyses in real-time, typically using built-in radio protocols. Furthermore, integrated cameras enable it to also serve as the detector in optical sensor systems [6].

Chemical sensors can be classified according to their transduction element, which can be electrochemical, optical and piezoelectric. In the scope of wearable devices, electrochemical sensors have a great domain in their development due to characteristics such as the possibility of miniaturization, flexibility, comfort, simplicity of operation, compatibility with different materials and most importantly, can operate with inexpensive electronic devices powered by battery instrumentation [7,8].

Currently, there is a great expectation for the development of fully integrated devices, with a bio(sensing) signal processor and powered by battery. These fully integrated devices operate as a “microcomputer” connecting all detection processes, from the collection and processing of information to deliver the results [9]. Wireless communication is essential to ensure complete mobility of wearable electrochemical sensors. Therefore, the combination of these components brings a new class of hybrid devices, the wireless wearable electrochemical sensors [2].

Wireless electrochemical sensors offer real-time monitoring of analytical data not only in hospital settings but also during regular day-to-day activities [10-12]. This represents an opportunity to decrease healthcare costs, improving preventive and personalized medicine approaches [10]. While most these new wearable sensors devices have focused on fitness and healthcare applications, recent efforts appear in the development of on-body sensors for diverse security and defense, environmental and forensic applications. Consequently, several studies have described wearable electrochemical sensors that can monitor, for example, toxic gases [13,14], pesticides [15], illicit and licit drugs [16-18], explosives and heavy metal [19], electrolytes [20], and metabolites [21] on-body via biofluids, such as saliva [22], tears [23], and sweat [24]. This progress has been the subject of several recent review articles [2,25-29].

This growing demand for real time analysis and the continuous monitoring of relevant (bio)molecules has been spurred the development in the field of wearable technology aiming a noninvasive data collection at a low cost. As a result, the wearable market had a historic growth in 2019, which was more than double compared to 2014 [30]. As encouraging as these developments are, the impact of existing wearable electrochemical sensors fully integrated with wireless data transmission technology still remains at an early stage. There are still challenges and limitations to be overcome in order for these devices to be marketed. This review focuses on these challenges and technological gaps in the field of wearable electrochemical sensors. The main topics discussed include the development of available wireless communication protocols, data collection and privacy concerns, power sources for wearable devices, challenges involving

electrode materials, sampling, sensitivity, stability and applications in healthcare, sports, environmental security and forensic analyses.

WEARABLE ELECTROCHEMICAL SENSORS CHALLENGES

Principles of electrochemical sensor

The great advantages of coupling wireless communication to different types of wearable sensors are the mobility of these class of devices, the possibility of obtaining real-time data acquisition and application in different areas like homeland defense, home-based healthcare, and food logistics [31,32].

Electrochemical sensors are the most used in the wireless chemical sensor field, due to features such as simple structure, possibility of miniaturization, comfort and simplicity of operation, combined with high sensitivity, fast response, low energy consumption and low manufacturing cost [7]. Basically, a wireless electrochemical sensor transduces the electrochemical interaction of an analyte at an electrode into a voltage or current signal, which can be transmitted through a wired or wireless communication module. The electrochemical signal can be amperometric, voltammetric, potentiometric and impedimetric [33].

The predominant transduction methods for the development of wireless electrochemical sensors are potentiometric, which measure the potential difference between an indicator electrode (ion-selective electrode) and a reference electrode; voltammetric, which measure current flow varying potential and amperometric, which measure current at a constant potential.

The potentiometric methods are the most used, due to less complexity in its electrochemical transduction components [2]. The development of wearable potentiometric sensors has been reported for the measurement of various electrolytes present in sweat, including sodium [34], potassium [12], chloride [35], ammonia [36], calcium and hydrogen ions [37]. Although potentiometric sensors operate easily wirelessly and without a battery, their sensing capability is limited to electrolyte levels and therefore are not suitable to detect other physiologically relevant species, such as metabolites, drugs, and proteins [38].

Wearable amperometric devices typically use enzymes to achieve selectivity. The incorporation of enzymes for specific target analytes/metabolites may require the use of high voltages to trigger the enzymatic reaction, which can generate interference signals. To overcome this problem, an electron transfer mediator can be incorporated to decrease the redox potential of the target reaction [12]. This technique has been described for the sensing different species such as glucose [39], lactate [20] and ethanol [40] in sweat. In all mentioned examples, the used mediator was Prussian Blue with the specific enzyme for each species, ensuring good selectivity to the device.

Despite the techniques of amperometry and potentiometry being the most used techniques in the development of wireless wearable electrochemical sensors, other techniques have been reported, such as square wave anodic stripping voltammetry (SWASV) [41], cyclic voltammetry (CV) [42] and differential pulse voltammetry (DPV) [12]. An advantage of voltammetric techniques in relation to amperometry is the possibility of coupling pre-concentration techniques to trace levels, thus obtaining better detection limits.

Although electrochemical sensors are promising analytical tools in the development of wearable devices with wireless communication, there are still several requirements to be addressed to achieve an ideal architecture, such as: the type of biological fluid sampling –(which can be sweat, saliva, tear, interstitial fluid and breath), the substrate for the construction of the platform –(needs to have flexibility, high mechanical resistance and biocompatibility), sensing materials, applicability accessories, electrode fouling and/or drift and power. Issues such as the need for calibration and device stability are essential for its optimal performance. It is also crucial that the sensor be free of external interferences from different sources [43]. Besides that, it is necessary for the construction of wireless wearable sensors to incorporate a flexible and active electronic system, such as transistors and integrated circuits for data processing and transmission for wireless communication systems [26,32,44].

Several studies have shown that electrochemical sensors can be structured on different platforms such as glasses [20], bracelets [45], rings [13], gloves [15] embedded in fibers or cloths [46], temporary tattoos [40] on the skin and contact lenses [47] and despite the great advances in their knowledge and use, this

field has experienced unbalanced advances in research and development [20,31]. The acceptance of this wearable sensor technology needs the creation of products that can be really integrated into user's lifestyle.

Sampling with wearable sensor

One of the main requirements for the development of wearable electrochemical sensors for continuous monitoring of physiological parameters is how the biological fluid will be sampled, in order to be concomitant with the sensing. From this configuration it is possible to classify the platforms in two ways: minimally-invasive and noninvasive [48,49].

For minimally invasive devices there are some controversies about their definition, despite containing the sensing and sampling sets coupled. The most common definition is that they are types of devices that penetrate an external layer of the body such as the skin or internal epithelial tissues, like the nasal mucosa or the intestine in less than 1 mm and do not require a trained personal doctor for its application. Interstitial fluid (ISF) is the most accessible body fluid in a minimally invasive manner and contains chemicals in similar concentrations as blood, like glucose and ethanol, with less content of proteins, offering therefore a wide range for clinical application. This fluid bathes the epidermal tissues (<1 mm below the surface of the skin) and can be analyzed, extracted, and accessed in situ trans dermally through near-infrared spectroscopy, ultrasound or microneedles, thus offering a new set for sampling [48,50].

Preliminary studies have shown a needle-type biosensor with integrated RF capability to monitor cholesterol [51]. However, a more adequate approach is the use of micro-needles, allowing the miniaturization of the device. Some studies show the use of microneedles in the electrochemical device for detection of glucose and lactate [52]. Recently, Mohan et al. developed a skin-worn electrochemical sensor array used for continuous real-time monitoring of subcutaneous alcohol at the ISF. The device was consisted of an assembly of pyramidal microneedles integrated with Pt and Ag wires, each with a microcavity that was modified with the enzyme alcohol oxidase (AOx). The microcavity present in the microneedle array lends to create a stable enzyme layer without affecting its skin penetration capability. With this type of sensor, it is possible to couple a wireless system for then develop an alcohol minimally-invasive real-time remote monitoring device in individuals while eliminating blood sampling complications [53]. The measurements obtained by this method can be corrected due to the time lag between variations in blood and interstitial levels via validated compartmental models, and despite being a promising approach, there are several improvements to be made in terms of sensing platform such as durability and sensitivity. Another issue to be evaluated in this type of system is that despite being minimally invasive it can cause bruising and skin irritation, tending to be inconvenient and uncomfortable [51].

Regarding noninvasive sensors, they offers the opportunity to not require any drilling or incision procedure, and in addition, it can detect analytes and metabolites in different biological fluids such as sweat, saliva, tears and epidermal interstitial fluid [54,55].

Tear is a very complex extracellular fluid that contains proteins, electrolytes and metabolites. Currently, its use in the construction of noninvasive electrochemical devices is directed to the detection of glucose [54]. As an example, Keum et al. developed an intelligent contact lens system, being a noninvasive wearable device with electrochemical detection for glucose monitoring in tears. The device was built on a biocompatible polymer fabricated by the chemical cross-linking of silicone hydrogel precursor solution containing a PET film, flexible electrical circuits and a microcontroller chip for real-time biosensing, wireless power management, and data communication [47]. Despite the technological advancement, a major challenge in wearable eye detection is obtaining an adequate energy source.

Saliva is an another complex biofluid that contains many components present in the blood such as enzymes, hormones, antibodies and so on. Thus, it is possible to monitor the emotional, hormonal, nutritional status of the human body [56,57]. The detection in this type of biofluid has been demonstrated for the detection of urea [58], glucose [39], uric acid [22], lactate [59] and lithium [60]. Despite being a very promising type of device, several challenges still need to be overcome, such as the low concentration of biomarkers in saliva compared to the blood and surface biofouling interference from salivary macromolecules [27].

In order to obtain the interstitial fluid in a noninvasive way, the combination of the reverse iontophoresis technique and biosensing in a single device has been demonstrated. Reverse iontophoresis is based on the extraction of interstitial fluid trans dermally. This method consists of applying a small controlled electric current to the skin (< 0.5 mA cm^{-2}), with the aim of increasing the transport of low molecular weight substances through the skin, such as ions and neutral polar compounds. Due to the application of electric current, the transport rate of the compounds is greater than their passive permeabilities, and the main transport mechanisms are electromigration and electroosmosis, where the contribution fraction of each mechanism is dependent on the physicochemical properties of such as the need for calibration, not causing discomfort or irritation to the skin due to the current or potential applied to the fluid extraction. In addition, there is a limitation on the amount of fluid extracted and a long extraction time is not suitable for biosensing.

The development of wearable electrochemical sensors has been largely directed towards the detection of species present in sweat. Several components of sweat have been used as target analytes to validate this design [61]. The development of wearable electrochemical sensors has been largely directed towards the detection of species present in sweat. Among these components are sodium [34], potassium [20], calcium and pH [37], lactate [62] and ethanol [40], among others [61]. The advantages of choosing sweat as a biofluid in noninvasive wearable systems include the possibility to be sampled at very low fluid generation rates and external contamination, as well for continuous access in addition to the possibility of being stimulated on demand with local iontophoresis. In contrast, its use still raises several challenges such as being irregular without iontophoretic stimulation, old sweat can mix and contaminate new sweat, changes in skin pH and low sample production rates [63].

A significant effort in research has been reported in the literature for noninvasive devices so that the platforms provide both the sensing and the possibility of data transmission. A major view over the possibilities for different wearable electrochemical devices can be seen in the section "*Application fields for wireless wearable electrochemical devices"*.

Materials and methods employed to construct wearable electrochemical sensors

For the construction of a wearable sensor, it is widely known that the material of the platforms needs to be biocompatible, functional/active and with low manufacturing cost. In addition, for substrates that are in contact with skin it is necessary that they are flexible, with high density, robust, and present high mechanical resistance [64].

Several works have been reported using extensible materials as substrates, such as elastomers and elastomer composites with metallic fillers, which provide mechanical robustness, high device density, and scalability. The use of substrates based on polymers is given due to unique characteristics such as greater mechanical resilience and possibility of an adequate conformational adjustment between the contact surface and the sensor, in addition, it offers lower noise values in the response of the analytical signal. An example is the silicon elastomer polydimethylsiloxane (PDMS), which has been widely used in wearable sensors, and has the characteristics of biocompatibility, high tensile strength, hydrophobicity and is not flammable [38].

Recently, Lee et al. [65] presented an extensible electrochemical sensor for glucose monitoring based on PDMS with polyurethane nanofibers, incorporated with cotton fabric. The electrodes were formed directly on the PDMS substrate, in which the working electrode and the counter electrode were composed of nanoporous gold and the reference electrode by an Ag/AgCl layer. In addition, the sensor was incorporated into a microfluidic system to perform sweat collection for subsequent measurement of glucose levels. This on-body sensing shows great potential for continuous monitoring of metabolites, not only in sweat but also in other body fluids and also prove to be a great prototype for the incorporation of wireless systems. PDMS was selected as the best choice of flexibility stretchable substrates, and despite being a promising material, the use of PDMS has disadvantages like poor thermal stability due to the limitation at preparation temperature of the electrode and long-term instability due to its high permeability [38,66].

Others polymers have been used as options for building wearable devices such as polyimide (PI), parylene, poly(methyl methacrylate) (PMMA), polyurethane (PU), polyurethane acrylate (PUA), polyethylene terephthalate (PET), polyethylene naphthalate (PEN), poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (PEDOT:PSS) polystyrene-block-poly-(ethylene-ran-butylene)-block-polystyrene (SEBS). Among those mentioned, PI and PET are widely used as a flexible bioelectronics material in biosensor integration technology owing to their good performance and user comfort. PET exhibit excellent deformability and optical transparencies and PI plays important role due to its high mechanical strength and applicability as support layers for skin sensors [38,61,67,68].

Other materials have been widely used for the construction of wearable systems, such as fibers, paper and textiles [66]. Being flexible and widely used in our daily life, textiles such as wool, cotton, and nylon have been extensively exploited as the substrate for integrating various electrochemical biosensors, even as paper that can be readily and rapidly modified with biomolecules and nanomaterials for electrochemical-sensing applications [33,69].

The materials used as well as the manufacturing techniques for the wearable devices depend on the desired application, such as the biological fluid and analyte/metabolite of interest and the type of sensor. There are different manufacturing methods described in the literature, including soft-lithography, photolithography, electron-beam evaporation, inkjet printing and screen-printing [70,71]. Among the aforementioned methods, the screen-printing has been widely used in the manufacture of electrochemical wearable devices due to features such as the versatility of modern thick-film, fabrication techniques enable large-scale manufacture in different geometries, low cost, robustness, and suitable electrochemical performance. In contrast, the lithographic methods are capable of producing high-performance electro-analytical devices, however, they have the high cost as a major disadvantage [70].

Conductive electrodes are the main components in wearable electrochemical sensors, so it is extremely necessary that the electrodes have mechanical flexibility and stability. The ideal electrode material must have characteristics such as robustness, high conductivity, low resistance, low processing temperature, flexibility and extensibility [66]. In addition to all the challenges imposed on the electrode material, there is still the incompatibility of properties and foundational mechanics that could appear during stretching [67], thus disabling the sensor due folding of the device [61,72].

The most used materials for the construction of flexible electrodes are carbon nanomaterials and metallic nanoparticles. Nanomaterials such as metallic nanoparticles (NPs), nanowires (NWs) and carbon nanotubes (CNTs) have been used, due to their thermal, mechanical and electrical properties, in addition to offering different surface areas. NPs and NWs are excellent conductive materials providing high mechanical flexibility and electrical conductivity [38,66,73]. Carbon materials such as carbon black (CB), CNTs and graphene have excellent properties. The limitation of CB is the difficulty of dispersion in elastic or solvent matrices due to its aggregation. CNT features excellent intrinsic conductivity, high optical transparency and thermal stability; and several of graphene-based electrodes as sensors have been studied due to material characteristics such an excellent electron mobility, ultrahigh strength, and low contact resistance with organic electronic devices [74].

Among the nanostructured metals, gold nanoparticles (AuNPs) are being the most common in flexible electrodes [38], moreover to the use of AgNWs or AgNPs on flexible platforms due to its optical transparency, high electrical conductivity and high mechanical flexibility [75]. Silver nanoparticles are well known as an effective antimicrobial agent and already used widely in consumer products, such as clothes, shoes and toys. Some studies had shown that AgNPs are able to enter the cells, whereas for the corresponding metal ionic species either no or a relatively low penetration could be seen. As well no toxicity was observed for AgNPs due to low release of silver ions within short time periods, so, being a safe substrate for electrode printing in wearable sensors [76]. Although some concerns need to be addressed for other metal nanostructures, considering its potential toxicity for human health and environment and the lack of studies demonstrating its safety.

In addition, materials such as printing inks have helped to promote advances in printing technology on different types of substrates [66]. The printing inks used to build flexible electrodes are basically composed of fillers, binders and additives. Fillers are the active component of the paint that provides the characteristic features needed for each application and can be metallic, ceramic, organic or a combination nanomaterial, such as nanosheets, nanowires and nanoparticles. Among these materials, carbon-based inks have received great prominence due to the numerous properties of carbonaceous nanomaterials such as high surface area and conductive properties [77].

The design of solid-state reference electrodes without a liquid junction, the called pseudo-reference is a crucial point in the construction of wearable sensors. The purpose of the reference electrode is to provide a stable potential relative to the solution, which the potential of the working electrode is referred to this potential [78]. The easiest and most common employed type of reference electrode is the Silver/Silver Chloride (Ag/AgCl) pseudo-reference electrode. This electrode has several favorable traits, such as fast reaction kinetics and a lower toxicity than calomel-based reference electrodes [79,80].

The main difference between the Ag/AgCl pseudo-reference electrode and the normal Ag/AgCl reference electrodes is that the Cl^- concentration is not maintained constant by a liquid compartment enclosing the solid electrode, making the electrode susceptible to oscillations of its potential when the Cl^- concentration in test solution varies. Thus, it is very important that the chloride ions concentration in the test solution, be kept constant to provide a stable and reproducible potential. This concentration of chloride ions must be at moderate concentration to ensure that the small amount of chloride ions present in the analyzed samples can be negligible and does not alter the accuracy of the pseudo-reference electrode [80].

Although several technological innovations have been made in relation to flexible substrates and materials, there are still challenges in the construction of this type of device such as incompatibilities in properties and also in basic mechanics causing the deactivation of the sensor [61].

Calibration, long-term stability and sensitivity of the wearable electrochemical sensor

Despite the significant progress in the development of electrochemical bioelectronics for wearable devices, some key challenges need to be addressed, considering that the continuous exposure to a living biological system increases complications of the sensor [81]. The physical and mental traits of a human body constantly change and behave differently depending on time, age, environment, and circumstances. For example, the sweat rate changes under stress, coercion, intense physical activity, and so on. Other factors such as the fluid pH, volume, and flow rate could also potentially affect the accuracy of in situ monitoring and suitable detection mechanisms need to be introduced to achieve better accuracy for wearable devices. Due to the difference of the analyzed biofluids, it is also necessary that sensors can accurately measure compositions at small volumes and have minimal calibration with fast response time, long-term stability, extended lifetime, and durability [54]. Furthermore, minimal drift, high sensitivity and selectivity, anti-fouling, internal calibration strategies, repeatability as well as mechanical reliability and system robustness need to be addressed when used continuously in wearable devices [70]. Also, the amount of sample and sampling strategies are problems that need to be circumvented too, as well as mechanical material degradation or failure due to stress [7].

An important aspect to consider is the quantification approach used for wireless monitoring with wearable devices. It is important to have in mind that electrochemical methods are not absolute, and their results need to be compared with a calibration curve or performed by the standard addition method. However, the last one is not viable for in vivo analysis because it implies contaminating the individual/sample. Therefore, the usual strategy relies on sample collection and conduction of comparative ex situ analyses to correlate the results obtained by the on-body sensor and that of the collected sample. This correlation in some cases can be performed considering a population mean, but ideally it should be customized/personalized to take into account the differences between individuals (metabolism, excretion, sample production, temperature, and so on) [26]. A calibration-free approach is an important example of how calibration issues can be overcome, also for other application areas [54]. As different detection methods usually have varying

susceptibility against the same interference, rational response can be estimated more reliably from the ratio of both single responses. Unfortunately, calibration-free approaches are few and might work, depending on the environment, but most have still not been tested for very long-term functionality. Self-calibration, incorporation of calibration reagents or reference items maybe the best solution for an accurate calibration but also become less realistic the more sensor entities shall be deployed, considering cost, replacement or maintenance efforts, and stand time of reagents [26]. The use of orthogonal approaches (colorimetric/potentiometric/fluorescence/electrochemical/volumetric modes in the same sensor) [82] could also be an important alternative for calibration issues, as well the use of amperometry, chronoamperometry and exhaustive coulometry as self-calibration strategies [26].

Eliminate fouling/passivation of the electrode surface is one of the most challenging aspects of electrochemical wearable devices [70]. The nonspecific adsorption (fouling) is one of the first primary steps to diminish long-term stability in wearable devices. Some studies have shown that serum albumin and other endogenous protein or biomolecules fragments can adsorb onto the electrode surface diminishing the diffusion of analytes of interest to the sensor surfaces and making the detection impossible. For that reason, numerous materials have been developed to form a protective coating to reduce nonspecific adsorption on sensors, as well as the use of some electrochemical techniques to prevent this phenomenon. Some materials used for anti-fouling include protective and selective membranes, with durable solid membranes, such as monolayer protein [83], Nafion [84], polyethylene oxide (PEO) [85], poly(ethyleneglycol) (PEG) [86], oligo(ethylene glycol) (OEG) [87] and polycarbonate [88]. Zwitterionic polymers containing both cationic and anionic groups on the same monomer residue have been also proved to be biocompatible and multifunctional to prevent fouling in biosensors [89]. A study showed that the zwitterionic polymer coatings reduce nonspecific protein adsorption not only from single protein solutions such as BSA, fibrinogen, antibody and lysozyme, but also from complex media, such as undiluted blood plasma [90]. Some approaches to shield the sensing element and sensor from interferents with filters [91], and cleaning/renewing of the sensing element/membrane (with irradiation, heat or UV light) [92,93] are also realistic contributions toward the long-term reliable operation. The use of specific electrochemical techniques has also shown that fouling could be prevented in electrode surface when fast scan cyclic voltammetry (FSCV) or chronoamperometry were used. Due to the fast scan rate, the FSCV limits the diffusion distance of the charged molecules to the electrode and minimizes electrode fouling in vivo [50]. Amperometric signal modulation is another strategy, shown years ago by Rocklin et al. which enabled a cleaning of the electrodes [94]. So, this approach can be an interesting alternative or be imagined as an additional measure to reduce fouling effects.

Operational challenges of wearable electronics represent another paradigm, where special conditions and pretreatments of the sensors are desired to enhance the sensitivity, selectivity, accuracy, limit of detection, and so on [81]. Some of these treatments such as chemical treatments to restore the initial stable values cannot be applied while sensors are installed on wearable devices. The simultaneous monitoring of multiple analytes is also challenging as cross-talk between different sensors influences their selective detection. Increasing the density of sensors, data processing units, and wireless communication channels would require more power and is, therefore, challenging when trying to maintain the same level of operation for long durations. Besides that, some challenges for real application of wearable sensors have to be addressed for continuous growth of this area, especially when nonenzymatic approaches are used because strategies to increase selectivity and sensitivity have to be proposed for real applications [70]. The use of biological recognition agents and (bio)chemical modification of the electrode surface to improve the selectivity and sensitivity are almost mandatory to allow direct on-body sensing, as well as the coupling of additional strategies and devices to collect, preconcentrate, and incubate the biofluids during on-body measurements. The majority of developed wearable sensors are functionalized with specific enzymes to enhance the selectivity and sensitivity toward a specific analyte and they commonly rely on affinity-based recognition which is hard to regenerate in vivo with current technologies [81]. Some common metabolites, such has glucose or lactate have their detection based on this approach. However, electroactive molecules

could be directly detected considering the loss or donation of electrons on the electrode surface when a redox potential is applied to the sensing electrode. Usually, linear sweep voltammetry (LSV) and cyclic voltammetry (CV) are employed as the first strategy to evaluate the electrochemical behavior of these electroactive molecules on a specific working electrode due to the simple and rapid operation. However, in electrochemical detection, these methods result in low sensitivity due to a large background current arisen from charging at the electrode surface [50]. Pulse techniques, such as DPV or square wave voltammetry (SWV) could be utilized as attractive approaches to decrease the limit of detection enhancing the sensitivity. In such techniques, the currents are recorded before the beginning and at the end of the potential pulse, and the current difference is monitored concerning to the voltage, minimizing background current. Owing to the minimized effect of the charging current, high sensitivity down to nmol levels can be reached, making it possible to use flexible and wearable devices to monitor ultralow levels of electroactive molecules in body fluids or other matrixes [95]. Some other approaches are related to enhancing the sensitivity of electrochemical wearable devices, considering the nature of the modified working electrode. Instead of directly using printed metallic or carbon electrodes, nanomaterials functionalized or modified electrodes may be utilized to increase the electroactive area and the sensitivity of the direct electrochemical detection through different electrochemical techniques. However, the biocompatibility of those materials and substrates is another major concern for wearable electronics, especially for sensors mounted on the skin/epidermis. The physical, mechanical, and chemical properties of these materials and substrates need to be matching to avoid thermal, electrical, and multi-layer integration mismatches. Furthermore, although nanomaterials play an important role in flexible electrochemical bioelectronics toward improving the sensing performance, their potential toxicity and biocompatibility need to be carefully evaluated [50].

Although some of these related approaches have distinct advantages on simplicity, sensitivity and low cost, major challenges on selectivity and biofouling need to be overcome in order to achieve continuous, stable, and selective measurement in vivo. To address these challenges, new material innovations and quantification strategies are strongly desired. Owing to its high portability, and low cost, the development of new strategies for electrochemical sensors with high sensitivity and selectivity hold great promise toward point-of-care and wearable applications.

WIRELESS TECHNOLOGIES FOR WEARABLE SENSOR

Communication Protocols

Wearable sensors are expected to be able to communicate wirelessly with other devices, such as a smartphone. Various types of wireless technology and networks allow these devices to send data to each other without cables. There are a number of different wireless technologies that can be implemented in hardware products for the IoT. Such technologies include Bluetooth, ZigBee, RFID, NFC, which are the most common for the development of wireless wearable sensors. Each of these wireless protocols has its own specific advantages and limitations. Selecting the right one when developing a wearable device is an important step, as it affects the system's performance and features, such as size, communication range, data transfer rate, and power consumption [2].

The wireless protocols can be classified into active and passive based on the role of power supply required to operate the devices. The active ones require power to operate, for instance Bluetooth and ZigBee, whereas the passive ones do not rely on power (RFID and NFC). Active wireless systems can both transmit and receive information over a distance, meaning they need a power source, typically a battery. So, sensors based on Bluetooth and ZigBee, for example, have a transceiver which communicates with a reader device or other sensors in a network with a range of 10 up to 100 meters. Based on the application's requirements of range and data rate, the energy consumption can be adjusted, but the system needs an energy supply to power both the data transmission and sensor circuitry [2]. One recent example of use of a low-power energy-efficient Bluetooth microcontroller (Texas Instruments CC2640) is an eyeglasses-based sensor merged with a fluidic sampling for tears alcohol analysis [96]. The platform was composed of an integrated microfluidic system, attached to the eyeglasses, to guide the tear sample to the electrode, which was based on the enzyme alcohol oxidase.

In contrast, passive wireless systems, like RFID and NFC, communicate with a reader device only when it requests information, by means of modulation of the electromagnetic field generated by the reader. A disadvantage is that the reader must be in close proximity, just a few centimeters, < 5 cm (NFC) up to several meters < 15 m (RFID), making the range very short when compared to active systems. Also, since there is no power source, data cannot be logged autonomously. This makes the system rather inconvenient for wearable applications, once it requires human intervention to bring the reader close to the sensor for data collection, limiting the prospect of having autonomous wearable sensors [2]. Despite the disadvantages, RFID/NFC remains a viable option due to its low power requirements. An example of using NFC protocol was demonstrated in the development of a sweat Na^+ sensor, build on a flexible printed circuit board (PCB), becoming a fully integrated wearable skin patch. Potentiometric data from the ion selective sensor was transmitted to a smartphone using NFC. The sensor was passively powered by the smartphone, so it had no battery. Its operational lifetime is only dependent on the sensor chemistry [97]. Due to increasing smartphone ownership and easy availability for developing tools and hardware, the two most explored wireless technologies in wearable sensors are Bluetooth [22,98,99] and NFC [100-103]. In cases where continuous connectivity is not a requirement, the data can be logged locally on the sensor platform and later requested by a device such as a smartphone. One important aspect of designing a wireless sensor system is thinking about what data to show the user. Sending all the information generated by the sensors would be both unhelpful and wasteful, since wearable devices often have power restraints. Therefore, providing only relevant information that can be easily understood by the user is a desirable goal from an energy consumption standpoint and also user-friendliness.

Power Sources for Wearable Sensors

Wearable devices often performing tasks such as data analysis, communication, and detection simultaneously, it is of no surprise that their power consumption is of great concern. To help minimizing the problem, techniques such as modulating the device's sampling frequency can be employed with great effect. The use of low-power energy-efficient electronics is another approach. Nonetheless, the development of better and alternative power sources has become essential to address this issue. Thus, research is being carried out on solutions such as wearable batteries [104,105], biofuel cells [106,107], solar cells [108], supercapacitors [109-111], and piezoelectric/triboelectric energy sources [112-114], each with its own benefits and drawbacks. Energy extracted from body motion, sweat or friction could also be used as an alternative to the traditional batteries.

The most common way to provide power to wearable devices is through a battery. Lithium-ion batteries have long life cycle, low self-discharge rate, and high energy efficiency. Their disadvantage is their weight and size, because they are usually heavier and bigger than the device that they are powering. Also, their body is rigid, rendering them unsuitable for skin-interfaced devices. This is addressed in recent developments in flexible and stretchable batteries [115-117], although their performance is not yet on par with their conventional counterparts. Another important advancement is the development of an ultrafast rechargeable battery [118].

Recently, supercapacitors have been considered as an alternative, given their safety and capacity for fast charge and discharge [119]. They can be used together with energy harvesting techniques to store and provide power as needed [120]. However, there are still complications to be solved, such as improving energy density, which is affected by multiple factors, including electrode materials, binder, and electrolyte [121]. In addition to the improving efficiency, some researchers are focusing on developing transparent epidermal supercapacitors, which would improve optical interfaces to the skin. One example uses an Ag/Au/polypyrrole core-shell mesh based on a nanowire on a silicone substrate as the supercapacitor electrode. The system shows good transparency across the visible range (73% at 550 nm for 2 layers of the mesh) and good charging/discharging cycling properties (580 μF cm^{-2} at current density of 5.8 μA cm^{-2}) [122].

Other novel alternatives are wearable solar cells and biofuel cells. Biochemical energy from biofluids, such as blood, tears, and sweat, which are always available and are rich in metabolites, can be used in

biofuel cells to generate electricity. Since the flow of biofuel is controlled by human physiology, continuous generation of constant power is difficult. One example is a screen-printed highly stretchable (500%) biofuel cell. The device uses a special stretchable ink based on carbon nanotubes as the conductive component. The cell displays a maximum power density of ~50 μW cm^{-2} at a voltage of 0.25 V in the presence of 20 mM glucose [122].

Harnessing energy from light is another tough challenge. Even though the field is advancing, there are still major challenges ahead, since solar energy is a very low-density power source. Thus, suitable levels of power require a panel with a large surface area. Also, the highest conversion efficiency is achieved when sunlight reaches the panel perpendicularly. Taking into account the shape of the human body, its constant motion and the sun's motion, only a negligible fraction of sunlight will fall normal to the panel, converting a low fraction of sun light into effective energy for the device. Given these challenges, researchers are exploring ways of improving conversion efficiency [123].

Data Analysis and Privacy

The emergence of the so-called big data for health-related fields like wireless (bio)chemical sensors raises questions about privacy, security, and data ownership [124]. Extensive amounts of highly personal data will be generated by physical and chemical sensors, and also by many new smart devices, networks, and software applications. Data privacy is, therefore, of crucial importance in the healthcare domain and its security should be enforced at every step of the system, including at the sensor level at which the data is collected [125].

As more people rely on wearable sensors, they become exposed to potential security breaches. Providing the wearer with the most relevant analytical information in a simple way is important for widespread adoption of wearable technology. This involves data mining to analyze and filter relevant information before presenting it to the user. However, current data mining algorithms will not be able to cope with the amount of data that wearable sensors are expected to output [126]. Efforts to address these problems are being performed with new algorithms and expanding data mining protocols to work with heterogeneous information [127]. If wearable sensors are able to output information at high data rates, they must have powerful data storage and processing capability. This is challenging given the constraints associated with a compact wearable device. Cloud computing could be a way of mitigating this problem [128], removing the need for dedicated electronics for storing and analyzing raw sensor data. However, remotely handling the data must go hand-in-hand with security measures.

APPLICATION OF WIRELESS WEARABLE ELECTROCHEMICAL DEVICES

Health and Fitness monitoring

Over the past few decades, the tremendous advance in electronic, medicine, chemistry, biocompatible materials and nanomaterials has led to the development of technologies that provide rapid and patient-centred diagnostics, especially for those with limited access to health services, while conventional disease diagnostic tests commonly used in laboratories and hospitals are time-consuming and costly, and require highly trained personnel [50,81]. More recently, a considerable progress in "epidermal electronics" area, including miniaturized wearable, implantable and flexible devices has been reached on medical and sport field enabling diagnosis and prognosis through small sensors and biomedical devices, greatly improving the quality and efficacy of health care, and the monitoring of physiological parameters for fitness and sport proposes. These flexible bioelectronics allows natural interaction between electronics and the soft human body and enables real-time and continuous health monitoring (e-Health) and transmission of biological data to the Internet for further processing and transformation into clinical knowledge [129]. This devices provide a continuous physiological status of individuals and personalized health profile to keep track of an individual's well-being [130,131] and are becoming important for long-term health monitoring due to the increasing elderly population throughout the world [81]. Although it all started as a cost-effective alternative to deliver diagnostic strategies in the developing world, especially in rural, remote, and underserved

communities, it has rapidly proliferated as a revolutionary tool that may enable virtual appointments between patients and doctors [132,133].

The current market for wearable electronics is still limited to tracking physical and vital signs from patients or users. The monitoring of human physiological activities such as body motion, respiration, heart rate, electrocardiograms, skin temperature, oxygen concentration and blood pressure are the most common and found in every smartwatch available on the market [81,129]. To provide a complete picture of the health state of an individual, it would be also very attractive to measure other physiological biomarkers, such as glucose, lactate, intra- and extracellular ions, and specific diseases biomarkers. Some recent studies are being conducted for the development of wearable hybrid devices that could simultaneously measure both chemical and electrophysiological parameters in a single epidermal patch [50,134]. Although these devices are mostly based on optical detection principles, an alternative can be the use of electrochemical assays through electron transfer during redox reactions for health markers monitoring [54,70]. In the following paragraphs, the main wireless wearable electrochemical devices used in health monitoring will be reviewed, given special attention to the different monitored analytes, dividing it in four main groups: glucose- and ion-selective-sensors, and sensors for organic compounds- and specific diseases. Some examples of wearable electrochemical devices for health and fitness monitoring are summarized in Table I.

Wearable glucose sensors

Glucose sensors are one of the most common biosensors related in literature. Due to the major problem of diabetes diseases caused by disorder or depletion of insulin leading to various major complications, the patient's glucose levels should be constantly monitored and controlled [135]. Therefore, attachable devices that monitor glucose to improve diabetes management and blood glucose control are very attractive, as they help the continuous real-time monitoring of patients for quick self-assessment [9,81]. Currently, interstitial fluid is the most used in continuous monitoring of blood glucose levels for its glucose correlation with blood glucose concentrations. Others physiological fluids such as urine, sweat, saliva, ocular fluid, and breath contain glucose biomarkers that could also be used for glucose monitoring [81,136].

Numerous studies are related for glucose wearable sensors and the smart watches are one of the most popular types. GlucoWatch® biographer (Cygnus Inc., Redwood City, CA, USA) was the first device to have a commercially approval for noninvasive glucose monitor by the Food and Drug Administration (FDA) [137]. It works acquiring electrochemically information about glucose concentration extracted by reverse iontophoresis from skin interstitial fluid. The direct measure of glucose concentration on the human skin is a promising approach for the future devices. Lee et al. has created a wearable patch-type sensor made from graphene and gold to enable direct sweat analysis with a wireless connection to a smartphone using an Android application via Bluetooth [138]. These flexible devices used gold mesh and gold-doped chemical vapor deposition (CVD) graphene electrodes to ensure high conductivity, optical transparency and mechanical reliability for stable electrical signal transmission, with an internal interference elimination system: when the relative humidity is detected at the critical sweating point, the temperature and pH is measured and corrected at the same time. The developed wireless device shows also an interesting way for direct drug delivery system, once when a high concentration of glucose was measured, the internal heater dissolved the phase-change material and released metformin in a feedback percutaneous drug delivery reaction through biocompatible microneedles direct into the human body. Another study, conducted by Lee's Group developed a thin and stretchable microfluidic device that was made with nanoporous gold electrodes in a poly(dimethylsiloxane) (PDMS) substrate, enabling collection and accurate delivery of sweat from skin to the electrode surface, with excellent replacement capability. The integrated glucose sensor patch demonstrated excellent ability to continuously monitor the sweat glucose level with high accuracy and good skin adaption. For the on-body detection, copper wire leads were attached to the electrodes of the sensor patch with silver paste to connect them to a portable electrochemical analyser (EmStat Blue 3+, Palm Instrument B.V.). This portable electrochemical analyser was then controlled wirelessly using a PStouch Android application (Figure 1A) [65]. Other examples for glucose wireless electrochemical sensors are summarized in Table I.

Wearable ion-selective sensors

These sensors have one of the simplest forms of sensing modality as the response is determined by ion exchange/transfer processes at the selective membrane–liquid interface. With major developments of sensing materials chemistry, sensitive and selective sensors can be quickly achieved for ionic detection in biofluids. The selective membrane typically consists of ion-selective ionophores dissolved in complex polymeric matrix with high mechanical stability and decides the sensitivity and selectivity of the ion-selective sensors. Each of these layers is critical to produce high-performance ion-selective electrodes for long term usage in wearable devices. These wearable potentiometric sensors integrated into a conformal platform to achieve coherent structure and easy wearability depending on applications have been widely investigated for detection of a variety of analytes in different matrixes [50].

Currently, the most common wearable potentiometric sensors are ion-selective sensors for detection of small ions such as Na^+, K^+, and H^+. Recently, a noninvasive wearable electrochemical device for continuous monitoring of ionized calcium and pH in body fluids using a disposable and flexible array of Ca^{2+} and pH sensors that interfaces with a flexible printed circuit board was developed by Javey's Group. The sensor was made with selective membranes for both ions supported over a PET substrate. The accuracy of Ca^{2+} concentration and pH measured by the wearable sensors was validated through inductively coupled plasma-mass spectrometry technique and a commercial pH meter, showing that the wearable sensors had high repeatability and selectivity to the target ions. Real-time on-body assessment of sweat was also performed, and the results indicated that calcium concentration increases with decreasing of pH [37].

Sodium ion is a potential biomarker for hydration status. In a study, the authors showed that sweat Na^+ concentration increases abruptly after approximately 2 h of outdoor running when there is no replenishment for lost water. Among various ions found in human body fluids, detection of Na ions has been the most prominent in wearable research due to its presence at high concentration in biofluids and physiological importance for maintaining fluid and electrolyte balance [34]. A potentiometric sodium ion sensor was developed by Alizadeh et al. depositing a chloride membrane into the internal layer of electrochemical deposited poly (3,4-ethylenedioxythiophene) (PEDOT). The solid-state Na^+ ISE was based on the calix[4] arene tetraester Na^+ ionophore as well as the reference electrodes (RE) were fabricated using a 175 µm PET substrate onto which conducting carbon and dielectric insulating layers were screen-printed using a DEK 248 printer. The developed sweat patch and associated electronics module were monitored remotely via Bluetooth for the detection of sodium concentration during a session of exercises [139].

Other flexible and attachable body fluid monitoring smart system was developed by Shen Group. In this work, they reported a wearable self-powered-like sweat monitoring system with integrated micro-supercapacitors (MSCs). The monitoring system is composed of ion selective membrane based [Na^+] and [K^+] sensors, and $NiCo_2O_4$-based MSCs as the power source of the sensor arrays, displayed an excellent electrochemical performance with high sensitivities of 0.031 nF mM^{-1} for [Na^+] and 0.056 nF mM^{-1} for [K^+]. By further integrating with wireless transmission technology (WiFi), the system could easily and accurately realize the real-time monitoring of perspiration [140].

Chloride is an important biological parameter for cystic fibrosis diagnosis [141]. Considering that the sweat Cl^- has been the gold standard for this diagnosis, researchers developed a skin-worn wearable platform for local sweat extraction and Cl^- monitoring using flexible ion selective sensors. A device developed by Javey´s Group allows a simultaneous detection of sweat rate based on the change in impedance between two parallel metal electrodes and monitoring of ion (H^+, Na^+, K^+, Cl^-) concentration with an ion-selective electrode. The signal from the impedance is sampled by the on-chip analogical-to-digital (ADC) converter, and a discrete Fourier transform (DFT) algorithm is used for impedance calculation. A 16-bit ADC (LTC1864) is used to measure the potential difference between working and reference electrodes. Both parts are controlled by a microcontroller which sends data to Bluetooth module via UART protocol. Finally, the Bluetooth module wirelessly transmits the data to mobile phone interface for display. The incorporation of sweat rate monitoring further assists in the understand of inter-related sweat parameters due to secretion rate (Figure 1B) [142].

Wearable sensors for organic compounds

Besides glucose, other organic compounds present in body fluids are closely related to human health and play important roles in the regulation of metabolism and are vital for maintaining the health of the entire human body. So, wearable biochemical sensors for detecting such organic compounds are really in need for continuous monitoring in order to assess human health and wellbeing since adverse effects occur in the absence or deficiency of these biomarkers. Many of these sensors are based on corresponding enzymatic electrodes, which are similarly fabricated as enzymatic glucose sensors, but since some analytes have good electrochemical activity a direct monitoring of such biomarkers could be conducted without an enzyme mediator.

Uric acid (UA) is an important final product of purine metabolism in human body. If the excretory system of uric acid is not working normally, an excess of UA is detected in human fluids, which leads to change in the pH of the fluid and affects the normal function of human cells. Some diseases such as gout occur if UA content in the body is neglected for a long term. In 2015 a mouthguard enzyme-based biosensor for uric acid was proposed by Kim et al. The enzyme (uricase)-modified screen-printed electrode system was integrated with anatomically miniaturized instrumentation wireless electronics composed by Bluetooth 4.0 link for data transmission. The mouthguard amperometric biosensor system offers high sensitivity, selectivity, and stability towards uric acid detection in human saliva, covering the concentration ranges for both healthy people and hyperuricemia patients [22].

Neuro biomarkers like stress indicators were also explored by some authors [143]. Parlak et al. also introduced an integration of an electrochemical transistor and a tailor-made synthetic and biomimetic polymeric membrane for selective molecular recognition of human stress hormone cortisol. The sensor and a laser-patterned microcapillary channel array were integrated in a wearable sweat diagnostics platform, providing accurate sweat acquisition and precise sample delivery to the sensor interface. The device was controlled using an Arduino Uno R3 microcontroller unit, which was used to power the individual components as well as to acquire and report detection data [144].

Lactic acid (LA) is a chemical substance produced in the glycolytic system and is known to be closely related to physical activity in anaerobic exercise such as muscle training. Blood lactate is already widely used in evaluation of training intensity in professions sports but sweat is becoming an interesting alternative for biological lactate monitoring in athletes and patients. A wearable electronic sensor capable of simultaneously measuring lactate, hydrogen ions, and sodium ions in human sweat through temperature sensing for internal calibration, equipped with microfluidic sampling and wireless reading electronics was proposed by Anastasova et al. The amperometric-based lactate sensor consists of doped enzymes deposited on top of a semipermeable copolymer membrane and outer polyurethane layers. For data acquisition, custom wireless sensing electronics based on the nRF51822 (Nordic semiconductors) IC with Bluetooth Smart wireless transmission were employed. A sensor interface was designed with a LT1638 (Linear Technologies) operational amplifier for measurements of the lactate sensor. An Android application was developed to capture the data from the wearable sensors and displays on other devices, like smartphones or tablets. A correlation between lactate and exercise frequency in sweat was observed by the authors once lactate concentration increased with a higher level of exercise effort [145].

Future efforts towards continuous lactate monitoring will focus on miniaturization and integration of the electrochemical circuits and electronics for data acquisition, processing, and wireless transmission, as well as critical assessment of all potential toxicity and biocompatibility concerns.

Wearable sensors for specific diseases

Despite the monitoring of some biological analytes in body fluids for healthcare, the impressive advances in the development of wearable sensors are allowing the researchers to perform clinical diagnoses in real-time based in specific biomarkers for a disease, without sophisticated instrumentation requirements. The specific biomarker analysis is one of the cornerstones of medical evaluation and the use of wearable devices has demonstrated the potential to become a fundamental medical tool when rapid, sensitive, and user-friendly disease diagnosis in non-laboratory environments is required.

Considering the advances reported in the last few years in point-of-care (POC) devices integrated in wearable systems, a noticeable increase in the number of publications on biosensors for specific diseases detection has been observed. These devices can provide a multiplex detection of different biomarkers in a small volume of sample or the screening of numerous samples using a single chip, allowing the possibility of a personalized healthcare monitoring for each patient, making the diagnosis of some diseases simpler, cheaper and faster. Some PoC devices combined with microfluidic systems are already related to detect biomarkers at ultra-low concentrations for early diagnosis in asymptomatic patients of diseases such as cardiovascular disease, neurological disease, cancer, urinary tract disease, dengue fever [146], malaria, and recently also for SARS-CoV-2 [129].

The efforts in development of wearable devices for specific diseases detection can be seen in some previous works. For example, a portable electrochemical microfluidic paper-based immunosensor device (E-μPID) was developed by Zhao and co-worker for detection of immunodeficiency virus and hepatitis C virus antibodies in serum, exhibiting high precision, sensitivity, and selectivity. The transmission of detection results to remote sites or health databases, via Internet or mobile networks, was related for the first time and can be used for telemedicine or patient's health-data storage and analysis. The platform was consisted of an electrochemical microfluidic paper-based immunosensor array (E-μPIA) and a handheld multi-channel potentiostat, with a capacity of performing enzyme-linked immunosorbent assays simultaneously on eight samples within 20 min. The circuit architecture of the potentiostat includes a microcontroller unit, a signal multiplexing/demultiplexing unit, a signal processing circuit (for converting an electrochemical current into a voltage), a 9 V battery and a Bluetooth wireless communication unit [147].

For Parkinson's disease (PD), several approaches have been developed for wearable monitoring systems during the last years, with detection of bradykinesia and tremor. Physical and cognitive parameters like tremors, balance dysfunction, motor rhythms and confusion are the most one monitored by wearable devices for PD diagnosis. These sensors do not use electrochemical or microfluidic principles, but the main concepts can be exploited in this manner. De facto, a microneedle sensor was developed by Wang's Group for continuous monitoring of Levodopa (L-Dopa) in interstitial fluids. L-Dopa is the most effective medication for treating Parkinson's disease, which is metabolized to dopamine. However, because dose optimization is currently based on patients' report of symptoms, which are difficult for patients to describe, the management of PD is challenging. So, a real-time and continuous monitoring device is important for an individual healthcare protocol for each PD patient. The reported sensing platform was made with different carbon paste electrode transducers assembled into a two-working electrode microneedle array for parallel independent electrochemical probing. While a direct electrochemical detection of L-Dopa was possible with SWV, a second microneedle electrode used chronoamperometric measurements for tyrosinase (TYR)-based biocatalytic detection (Figure 1C). The designed biosensor used an Ag wire reference electrode, and no counter electrode was used. The new sensor device displays an attractive analytical performance with high sensitivity, a low detection limit, high selectivity, and good stability in artificial interstitial fluid. The attractive analytical performance and potential wearable applications of the microneedle sensor array was demonstrated in a skin-mimicking phantom gel as well as upon penetration through mice skin [148].

Figure 1. Example of wireless wearable electrochemical devices for different applications. **A** – Stretchable non-enzymatic glucose wearable sensor: (a) Optical images of fabricated sensor (with no microfluidic channel); (b) Optical images of the stretchable microfluidics-integrated biosensor patch attached on the forehead (left) or arm (right) of a subject for real-time measurement. [Reprinted with permission from [65] Copyright© 2019, American Chemical Society]. **B** – Schematics of the wearable sweat sensing patch for H^+, Na^+, K^+, Cl^- monitoring and sweat rate determination. The sweat sensing device is directly placed to the skin and can be comfortably worn on a user's wrist. The analyzed data is wirelessly transmitted to a cellphone via Bluetooth platform. [Reprinted

with permission from [142] Copyright© 2018, American Chemical Society]. **C** – Microneedle sensor for L-Dopa detection for Parkinson's disease monitoring. (a) microneedle sensor with the corresponding reagent layers, including the CP, tyrosinase, and Nafion layer; (b) Schematic representation of a mannequin hand wearing the microneedle sensor; (c) the microneedle sensor for L-Dopa monitoring in ISF; (c) Portable wireless electro analyzer enabled with wireless data transmission to a smart device. [Reprinted with permission from [148] Copyright© 2019, American Chemical Society]. **D** – Schematics of robotic fingers: (b) Image illustrating the robotic hand used to develop the glove-based sense fingers. (c) Prototype of the screen-printed robotic sense fingers with long connections to the wrist where the electronic interface is located. (d) Images and corresponding electrochemical data of (i) robotic sour-finger dipped in orange juice and the SWV signature of ascorbic acid, (ii) robotic sweet-finger in cherry juice and amperometry data of glucose, (iii) spicy-finger on green-pepper and SWV feedback response to the presence of capsaicin. [Reprinted with permission from [153] Copyright© 2018, American Chemical Society]. **E** – Tattoo-based transdermal alcohol sensor. (a) Schematic diagram of an iontophoretic-sensing tattoo device, containing the iontophoretic electrodes and the three sensing electrodes. (b) Photograph of an alcohol iontophoretic-sensing tattoo device with integrated flexible electronics applied to a human subject. (c) Schematic diagram of a wireless operation of the iontophoretic-sensing tattoo device for transdermal alcohol sensing. In the diagrams of the tattoo-base device, blue and red highlights show the active zones during iontophoresis and amperometric detection, respectively. (d) Schematic diagram of constituents in the iontophoretic system (left) and of the reagent layer and processes involved in the amperometric sensing of ethanol on the working electrode (right). [Reprinted with permission from [40] Copyright© 2016, American Chemical Society].

Bacteria are already well known for causing numerous diseases and disorders in the human body, such as urinary tract infection, diarrhoea, and sepsis. A real-time monitoring device is an opportunity for a direct tracking for possible bacteria contamination and infection. So, Mannoor and co-authors have developed a wireless graphene nanosensor, using biomaterials to remotely detect and monitor bacteria present in breathing or saliva. The fully integrated biosensing platform was fabricated with graphene printing onto water-soluble silk substrate, followed by functionalization of antimicrobial peptides (AMPs) as biorecognition moieties. The selective binding of the bacteria with the AMPs alters the electrical resistance and allows rapid bacterial quantification and monitoring. Upon recognition of specific bacterial targets by the immobilized peptides, the electrical conductivity of the graphene film was modulated and wirelessly monitored using an inductively coupled radio frequency (RF) reader device. The developed sensor was used in an integrated system in tooth enamel for bacteria detection in saliva and represented a versatile approach for detection of other biochemical targets using a fully wearable device [149].

Even considering that dehydration does not have specific biomarkers for its diagnosis, the monitoring of some biofluids by wearable devices has been shown to be efficient in the monitoring of people affected by this disease. The use of these wearable devices is even more important for monitoring the body condition of high-level athletes and sports practitioners, serving as an essential platform for real-time detection of water loss due to excessive physical activity. Bioelectrical impedance is the most popular and conventionally used technique for measuring dehydration and human body water content.

It consists of two electrodes mounted on the human body with a low amplitude alternating current passed through them. Frequent modifications are done to the bioelectric impedance model based on the placement of electrodes on the human body or based on putting conductive strips in wearable media, along with data readout through wireless communications, with a ZigBee-based mobile wireless sensor network [150]. Further improvements in incorporating sensors on conformable materials, especially on textiles, are also expected, enabling the wearability of such devices more efficiently for real-time analysis during exercises [151]. Wireless epidermis sensors mounted directly on human skin represent another interesting approach for measuring hydration and strain levels in the human skin for sport and fitness proposes [152].

Wireless wearable electrochemical sensors for other applications

As demonstrated above, many efforts have been directed towards the development of wireless wearable electrochemical devices to directly sense different biomarkers (metabolites, electrolytes). While the majority of these wearable sensor systems has focused on fitness and healthcare applications, there are growing demands for developing such sensors in various other research and industrial fields, such as security, environmental and food quality, and towards monitoring of drug abuse [154].

In particular, there is a limited number of studies describing wireless wearable electrochemical devices addressed to these other fields of applications. Wang's research Group is the pioneer in the development of fully integrated electrochemical wearable platforms with wireless data transmission for screening of food and additives flavors, monitoring hazardous chemicals for diverse security and environmental applications and drug controlling.

In the food field, Wang's group described a sensing robotic fingers device for rapid screening of food flavors and additives [153]. The device consisted of three stretchable finger electrochemical cells printed on the robotic glove, and it was integrated with a circuit board to transmit the data wirelessly, via Bluetooth to a laptop or tablet. The printed middle, index, and ring robotic fingers allowed accurate discrimination among sweetness, sourness, and spiciness, using electrochemical detection of glucose, ascorbic acid, and capsaicin in a wide range of liquid and solid food samples as can be seen in (Figure 1D). The researchers hope that these advanced wearable taste-sensing systems at the robot fingertips will pave the way to automated chemical sensing machinery, facilitating robotic decision for practical food assistance applications, with broad implications to a wide range of robotic sensing applications.

For protection and personal safety of high-risk professions, which can be exposed to toxic and hazardous species, wearable sensors fully integrated with wireless data transmission represents a very promising technology. The advantage of this technology is the integration of sensors in the clothes that are worn on a daily basis, providing the ability to continuously monitor the users and their environment to warn of an imminent risk, helping in fast and effective decision-making process [155]. Few wearable devices for detecting chemical threats have been described in the literature. Most of them are based on the detection of organophosphate (OP) nerve agents. Such compounds represent a serious concern worldwide, due to their high toxicity. Some types of OP compounds are routinely used as pesticides in agricultural and domestic environments; however, the main concern revolves around OPs such as the G series agents, sarin and soman, as they are extremely toxic and fast-acting agents of war due to its irreversible inhibition of acetylcholinesterase [156].

Wang's research group has developed promising wearable devices based on platforms such as glove [15], tattoo [157,158] and textile [158] for detecting OP compounds. Such devices consist of electrochemical sensors coupled to a miniaturized electronic interface based on a potentiostat for signal processing and wireless data transmission, via Bluetooth. In all of these systems the detection of OP compounds was based on an organophosphate hydrolase enzyme biosensor, where the OP hydrolysis product (p-nitrophenol) is subsequently oxidized at the working electrode. In these studies, important points were considered. For instance, the authors used elastomeric stress-enduring inks for printing the electrodes, which ensure resilience against mechanical stress expected from the wearer's activity without compromising the biosensing performance. Moreover, the electrochemical detection was carried out using a soft and flexible electronic board, that maintains its functionality under mechanical stress and retains conformal contact with the skin.

The use of wireless wearable electrochemical devices has been also studied for the continuous real-time monitoring of licit and illicit drugs, because it plays an important role in personal and automotive safety, as well as for forensic applications [159]. Consequently, new noninvasive/few-invasive wearable chemical detection platforms, operating on readily sampled biofluids, such as sweat, saliva or tears, have attracted considerable interest as an alternative for monitoring alcohol or drugs in real time, which can be easily used by police, health workers and individual consumers.

Thus, several wireless wearables electrochemical devices have been developed for this purpose. An interesting example is the wearable tattoo-based biosensor for noninvasive alcohol monitoring in induced sweat developed by Kim and collaborators [40]. The device is formed by an integrated system for sweat-inducing direct iontophoresis with amperometric enzyme biosensing, along with a thin flexible printed electronic circuit for controlling the entire operation and a wireless real-time data collection, Figure 1E. The on-body iontophoresis operation was optimized on the human skin and the epidermal sensor was tested in nine volunteers before and after consumption of alcoholic beverages. In all experiments, the tattoo biosensors were placed on the subject's arms, and ethanol sweat measurement was performed using the iontophoresis-amperometry operation. The proposed skin-compliant biosensor displayed a highly selective and sensitive response to ethanol. The authors consider that future studies are required to calibrate the devices and ensuring data security and privacy safeguards. In addition, they point out that the attractive design and performance of the new wearable iontophoresis detection platform also offers considerable promise for noninvasive monitoring of additional target analytes.

In general, studies have pointed out the consumption of alcoholic beverages as one of the main factors responsible for the high incidence of accidents with victims. Furthermore, alcohol consumption is often combined with other psychoactive drugs, such as cannabis, and their combined synergistic effects may greatly increase the risk of fatal accidents [160]. Thus, researchers of Wang's group developed a fascinating wearable electrochemical sensing device for the simultaneous detection of salivary alcohol and Δ^9-tetrahydrocannabinol (THC), which is the main psychoactive ingredient in cannabis [16]. The sensing platform contains a dual working electrode, which offers direct SWV detection of THC and amperometric biosensing of alcohol with no apparent cross talk and high sensitivity. Both electrochemical techniques have been executed using the potentiostatic circuit embedded within the ring body. Such ring-based integration of miniaturized wireless electronic interface facilitates selective "on the spot" detection of cannabis and alcohol. The authors point out that the proposed wearable sensing can be readily expanded for the monitoring of other drugs of abuse.

Other interesting wireless wearable electrochemical devices in these fields and its characteristics are summarized in Table I.

Although there are many challenges to be overcome for the real application and commercialization of these devices, they are fundamental to encourage the development of new improved wearable devices.

Table I. Wireless wearable electrochemical devices for fitness, healthcare and other applications

Analyte	Electrode	Linear Range	Matrix	Technique	Platform	Characteristics	Ref.
Glucose	GOx membrane (MEMS)/Pt	5–1000 μM	Saliva	Amperometry	Mouthguard	Long-term real-time monitoring (more than 5 h)	[161]
Glucose	GOx/Sol-gel membrane	0–2 mM	Tears	Differential module	Contact Lens	Wirelessly powered by radiated RF waves	[162]
Na^+	Ion selective membrane	–	Saliva	Potentiometry	Mouthguard	Bluetooth low-energy wireless telemetry	[163]
K^+	Ion-selective membrane/ screen-printed	0.1–100 mM	Tears	Amperometry and Potentiometry	Eyeglass	Nernstian slope of 58.0 ± 4.3 mV Bluetooth wireless data transmission	[20]
Latic acid	PPD-LOx/ carbon-PB-printed electrode	0.1–0.5 mM	Saliva	CA	Mouthguard	High sensitivity, selectivity and stability using whole human saliva samples	[59]
Latic acid	LOx/ glutaraldehyde/ BSA/PU/Pt	0–1 mM	Tears	Amperometry	Contact lens	Quick response time Sensitivity of ~ 53 A mM^{-1}	[164]
Dehydration	AgNW/PDMS	–	–	Impedance	Skin	Ultralow power microprocessor with Bluetooth wireless connection	[165]
Glucose, AA, caffeine (CAF) and capsaicin (CAP)	Carbon-printed electrode and GOx/PB/carbon-printed electrode	1–6 mM AA and glucose; 10–50 ppm CAP; 0.1–0.9 mM CAF	Food	SWV and Amperometry	Nitrile glove	Robotic sensors that mimic the human sensing capabilities to discriminate sweetness, sourness, and spiciness	[153]
Organophosphorus nerve agent (methylparaoxon and methylparation)	OPH/Nafion/ carbon-printed electrode	–	Surfaces	SWV	Nitrile glove	"Lab-on-a-glove" that withstand extreme mechanical deformations	[15]
G-type nerve agents simulant (diisopropyl fluorophosphate)	OPH/Nafion/PANi/ carbon-printed electrode	10–120 mM	Vapor and liquid phases	Potentiometry	Tattoo	Temporary tattoo paper and miniaturized potentiometric printed circuit board transducer integrated	[157]
Organophosphorus nerve agent (methylparaoxon)	OPH/Nafion/ carbon-printed electrode	90–300 mg L^{-1}	Vapor phases	SWV	Tattoo and textile	Maintains its functionality under mechanical stress and retains conformal contact with the skin	[158]
Explosive and nerve-agent threats (DNT, H_2O_2 and methylparaoxon)	Carbon and OPH/Nafion/ carbon-PB-printed electrode	10−100 ppm; 2−10 mM; 0.25−1.25 mM	Vapor and liquid phases	SWV and Amperometry	Ring	Multiplexed chemical sensor platform with battery-powered stamp-size potentiostat	[13]

Table I. Wireless wearable electrochemical devices for fitness, healthcare and other applications (Continuation)

Analyte	Electrode	Linear Range	Matrix	Technique	Platform	Characteristics	Ref.
Ethanol	AOx/BSA/chitosan/ carbon-PB-printed electrode	0−36 mM	Sweat	Amperometry	Tattoo	Wireless epidermal iontophoretic-biosensing system	[40]
Ethanol	AOx/PPy/Au	1-50 mM	Interstitial fluid	Amperometry and potentiometry	16-gauge syringe	Continuous and long-term monitoring	[166]
Ethanol, glucose and vitamins (C, B_2, B_6)	Carbon-PB-, GOx/ BSA/carbon-PB- and AOx/BSA/ carbon-PB-printed electrode	–	Tears	SWV and amperometry	Eyeglasses	Noninvasive biosensing fluidic system allow real-time tear collection and direct biomarkers measurements	[96]
Ethanol and Δ^9-tetrahydrocannabinol (THC)	AOx/BSA/chitosan/ glutaraldehyde/ carbon-PB- and MWCNT/carbon-printed electrode	0.2–1.0 mM ethanol; 1–6 μM THC	Saliva	SWV and amperometry	Ring	Powerful wireless electronic board into a ring platform; Dual-sensor electrode cap, for the simultaneous detection	[16]
Drug (Caffeine)	CNTs/Nafion/ carbon printed electrode	10–40 μM	Sweat	DPV	Wrist band	Complete system with signal transduction, conditioning, processing, and Bluetooth transmission functionalities to relay electrochemical signals to a user interface	[45]
Abuse drug (fentanyl)	MWCNT-PEI-IL/ carbon printed electrode	10–100 μM	Liquid and powder forms	SWV	Glove	Selective detection in the presence of common cutting agents such as acetaminophen, caffeine, glucose, and theophylline	[167]

GOx = glucose oxidase enzyme, MEMS = microelectromechanical systems; PPD = poly(ophenylenediamine), LOx = lactate oxidase enzyme; PB = Prussian blue; CA = Chronoamperometry; BSA = bovine serum albumin, PU = polyurethane; AgNW = nanocomposite of Ag wires, PDMS = poly(dimethylsiloxane); AA = ascorbic acid; SWV = square wave voltammetry; OPH = organophosphorus hydrolase enzyme; PANi = polyaniline; AOx = Alcohol oxidase enzyme; PPy = polypyrrole; DPV = differential pulse voltammetry; CNTs = carbon nanotubes; MWCNT = Multiwalled carbon nanotubes; PEI = polyethylenimine; IL = ionic liquid.

CONCLUSION AND OUTLOOK

The area of wearable sensors is being developed in an extraordinary way. Advances in fields such as nanotechnology and the Internet-of-Things have boosted the ability of such devices to extract quantitative and innovative information in real-time, especially in remote areas and with limited resources, revolutionizing user health, well-being and safety practices.

Electroanalytical methods have received great attention in the last decade for the manufacture of these wearable platforms. This is because such methods allow the manufacture of fully integrated devices in a miniaturized, flexible and robust way, allowing the directly analysis on the human body, processing and transmitting data wirelessly to a portable device or a data center. Moreover, the functionalization of electrochemical sensors, supported on this wearable platform, with nanomaterials or bioreceptors can improve the analytical performance of the entire system, improving its sensitivity, selectivity and versatility.

Most of the developments in wearable sensors has been focused on health/fitness applications, however, these devices are extremely important for various scientific and industrial fields. Recent efforts have demonstrated its potential for in field monitoring that are still little explored, such as security and defense, agri-food and forensic applications.

Although there are unquestionable advantages in using wireless wearable electrochemical devices, most of the works described in this review have as their main objective the manufacture of the devices, demonstrating only the potential of the proposed devices. Little attention has been paid to critical and fundamental steps towards the final purpose of the device from the point of view of real applications and commercialization.

Importantly, the impact of this technology is still at an early stage. As with most new developments, there are many challenges and opportunities for this relatively new field, both in fundamental and applied aspects. These challenges involve the improvement of sensitivity, selectivity, long-term stability and reliability of devices and multiplexing capabilities. More researches are needed to manufacture large-scale wireless wearable electrochemical sensors with reliable performance for relevant analytes in real samples and viable scenarios, in order to make it the best option to solve real demand. The improvement of the multidisciplinary synergy of chemistry, biology, electrical engineering and software engineering will play a central role in the creation of innovative portable and wireless wearable microdevices with excellent performance.

Acknowledgements

The authors gratefully acknowledge the financial support from the Brazilian funding agencies São Paulo Research Foundation (FAPESP: grant numbers 2013/22127-2 and 2019/00166), National Council for Scientific and Technological Development (CNPq Grant no. 136386/2019-9), CAPES and National Institute of Science & Technology in Bioanalytics (INCTBio).

This work is dedicated to our friend Gustavo Arantes Lorga, who left us so early! The four loves of his life were: God, family, friends and science. Unfortunately, science became orphaned by a great and dedicated researcher, disposed to make relevant contributions to the scientific community. Gustavo was involved with great affection for each of the members of his research group, showing special appreciation for Prof. Lauro Tatsuo Kubota, whom he greatly admired. We are grateful for all the time by your side, Gustavo!

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Brazilian Journal of Analytical Chemistry
2021, Volume 8, Issue 31, pp 51-73
doi: 10.30744/brjac.2179-3425.RV-64-2020

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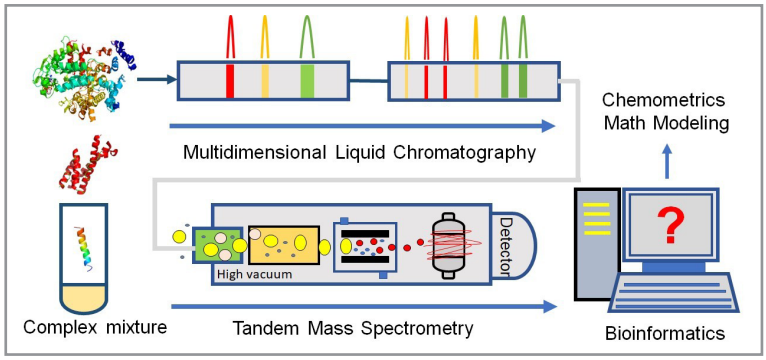
REVIEW

The Importance and Challenges for Analytical Chemistry in Proteomics Analysis

Weliton Pedro Batiston[1,2], Emanuel Carrilho[1,2*]

[1]*Instituto de Química de São Carlos, Universidade de São Paulo, Av. Trabalhador São-carlense, 400, 13566-590 São Carlos, SP, Brazil*
[2]*Instituto Nacional de Ciência e Tecnologia de Bioanalítica – INCTBio, 13083-970, Campinas, SP, Brazil*



Although Linus Pauling had an exceptional scientific contribution to the study of chemical bonds, reported in his book *The Nature of Chemical Bond*, the lousy image he got for the X-ray diffraction drove him to an unstable structure with an unreal DNA triple helix publication. Oppositely, for the consecration of James Watson & Francis Crick, they had the opportunity to enter science history using the right image of X-ray to propose the famous DNA double helix structure correctly. This chapter of science is an excellent example of how analytical chemistry performance affects horizons and scientific advances. Today the complexity of the systems is more significant and understanding how all proteins truly work into cells and organisms is the current challenge from proteomics. Comprehending how analysis is carried out and how instruments work could promote new insights to improve the analytical performance in proteomics. Here we described an overview based on our expertise on the analytical chemistry toolkit for proteomics analysis: *shotgun*, *bottom-up*, *middle-down*, *top-down*, and *native* proteomics, and their inherent instrumentation technologies. In addition, a detailed discussion of the analytical figures of merit in proteomics analysis is provided. We also address the limitations in multidimensional liquid chromatography and tandem mass spectrometry platforms. Furthermore, we present some perspectives in bioinformatics, mathematical modeling simulations, and chemometrics tools, as well.

Keywords: instrumentation, liquid chromatography, mass spectrometry, bioanalysis, biopharmaceuticals.

INTRODUCTION

The importance of studies on the proteins: implications in medicine and economics

The advances in proteomics have many implications for the economic and scientific fields because *i)* the modern pharmaceutical industry is developing biopharmaceutical, most of them based on proteins; *ii)* the current bet to the next advances in medicine is molecular diagnosis and the therapy linking proteome anomalies to a more in-depth characterization of diseases; *iii)* the final products from

Cite: Batiston, W. P.; Carrilho, E. The Importance and Challenges for Analytical Chemistry in Proteomics Analysis. *Braz. J. Anal. Chem.*, 2021, *8* (31) pp 51-73. doi: http://dx.doi.org/10.30744/brjac.2179-3425.RV-64-2020

Received 2 November 2020, Revised 25 March 2021, Accepted 6 April 2021.

the machinery of living beings are proteins. Therefore, the future will be even more exciting to molecular information from proteomics than currently from genomics [1,2].

In any of these examples, the chemical elucidation of proteomes and protein is critical to advancing the subject. However, when macromolecules are introduced in to an analytical equipment for characterization, the complexity of its chemical structure imposes various drawbacks that affect several analytical figures of merit. Giant molecules have many possibilities to change conformation due to the free rotation of any *sigma* bond. They show a complex nature driving to several intra- and intermolecular interactions possibilities and many structural conformations. For that, the next generation of analytical scientists needs to overcome the challenges of analyzing complex macromolecular samples that mean understanding how to drive the proteomics analysis and the instrumentation modes to its maximum. Although the theme is old and pertinent, the discussion in the proteomics community needs more insights [1,3,4].

Proteins provide essentials functions in all biological steps. They are catalyst agents in biochemistry reactions, carry and store other molecules, provide signal transduction, they are essential constituents in the structure of various biologics organisms, etc. Thus, reliably unraveling the biological process of proteins in the cell is the path to the solution and understanding of various diseases, suggesting advances in chemistry, pharmacy, biology, biochemistry, medicine, and economic development [1,2].

In practice, scientists in molecular medicine compare healthy samples with samples containing the questioned disease. Using analytical and bioinformatics techniques, they monitor the appearance of analogous proteins in the cell or monitor the variation in their quantity, or even map changes in the three-dimensional structure that can represent the origin of the disease [3]. Some proteins have the function of controlling the normal condition of cells. Characterization of them and understanding the amount suitable to an individual's necessities opens the opportunity for creation of supplements or drugs that promote the normal functionality of cells, preventing the proliferation of disease into an organism. They are the basis of biologicals and biosimilar medicines, most of them made up of monoclonal antibodies. These biopharmaceuticals have revolutionized the treatment of many previously untreatable diseases. Making it one of the top products of the modern pharmaceutical industry [4].

In the 90s, BASF Pharma, in partnership with Cambridge Antibody Technology, developed the incorporated drug of the protein Adalimumab whose market name is Humira®. It is intended for the anti-inflammatory treatment of several chronic diseases, such as *i)* arthritis, *ii)* ankylosing spondylitis, *iii)* Crohn's disease, *iv)* colitis, and *v)* psoriasis. The Institute for Applied Economic Research (IPEA) mentioned that this product generates US$ 10 billion a year of revenue to the German chemical company [5]. Several Brazilian pharmaceutical companies have started the production of biosimilars in the last decade. In Brazil, despite the economic crisis, this sector presented annual growth of 10%. The current increase in the aging of the population in emerging countries drives the need for constant innovation, but the expiration of patents for biosimilar medicines drives the plans of the pharmaceuticals companies for expanding the market.

State of the art: multidimensional proteomics for cell biology

A proteome concerns the set of all proteins expressed in a cell, tissue, or organism, from the functional expression of a specific genome. Thus, the term proteomics encompasses strategies for the identification and quantification of all proteins from a specific proteome. The proteome is a dynamic system in which each protein has interconnections in the cell that can have many different properties (chemical, physical, and biological dimensions). When analyzed altogether, it describes the phenotype of the cell/organism. Although it is possible to obtain relevant information from proteomes, the diversities, and the dynamic nature of living systems infer several drawbacks to measure these properties, meaning that is a great challenge to analytical chemistry and bioinformatics fields to overcome them. Also, there are many different proteomic profiles for the same biological system that depends on proteins expressed at the moment of sample collection. This fact represents a true challenge to get a representative sample and to control the statistical variable in terms of random errors [6].

Thus, bioanalytical chemistry and bioinformatics seek strategies for a systemic understanding of the proteome, which includes different experiments to elucidate the different properties of proteins, such as *i)* total abundance, *ii)* expression of isoforms, *iii)* turnover number, *iv)* post-translational modifications, and *vi)* molecular interactions. The connection of the results on the different properties of proteins and proper biochemical interpretation is currently called multidimensional proteomics. That allows a global view of the proteome, suggesting several scientific advances, especially in drug development and understanding of currently incurable diseases. In Table I, Professors Mark Larance and Angus I. Lamond of the University of Dundee, described the main experiments used to measure the different properties (dimensions) of the proteome [6].

Techniques applied to proteomics

Proteomics experiments can be classified in two ways: *i)* analysis of peptides from final products of proteolytic digestion from specific enzymes, named bottom-up, shotgun, and middle-down proteomics, or *ii)* analysis of intact proteins, named top-down and native mass spectrometry. Analysis of peptides allows greater identification of species, and it is currently the most suitable technique for comprehensive characterization of a proteome and comparison between sample treatments. Analysis of intact proteins allows better structural characterization of macromolecules and, generally, they come from a less complex mixture [7–12]**.** The readers must have in mind that if there are various ways of analyzing a proteome, a single technique cannot solve alone completely all questions to understand them. Each technique is complementary to one another.

Table I. Some of the main dimensions and complexities of a proteome. There are, however, other dimensions not described in this table. [Reprinted (adapted) with permission from [6] Larance, M.; Lamond, A. I. Nat. Rev. Mol. Cell Biol., 2015, 16 (5), pp 269-280 (https://doi.org/10.1038/nrm3970). Copyright© (2021), Springer Nature.]

Dimension	Examples of techniques used*
Abundance (absolute and relative)	Label-free quantitation
	SILAC
	^{15}N-labelling
	NeuCode SILAC
	Dimethyl-labelling
	TMT
	iTRAQ
Cell cycle regulation	Centrifugal elutriation
	Chemical inhibitors of cell cycle regulators
	FACS (for DNA content or phase-specific markers)
Tissue distribution	Dissection
	FACS (for cell-type-specific markers)
Interactions	Affinity-enrichment (endogenous immune-precipitation or tagged fusion protein pull-down)
	Protein correlation profiling
	Proximity-labelling
Post-translational modifications	Affinity enrichment: TiO_2
	Affinity enrichment: IMAC
	Modification-specific antibodies
	Liquid chromatography: IEX
	Liquid chromatography: HILIC
	Liquid chromatography: ERLIC

Table I. Some of the main dimensions and complexities of a proteome. There are, however, other dimensions not described in this table. [Reprinted (adapted) with permission from [6] Larance, M.; Lamond, A. I. Nat. Rev. Mol. Cell Biol., 2015, 16 (5), pp 269-280 (https://doi.org/10.1038/nrm3970). Copyright© (2021), Springer Nature.] (Cont.)

Dimension	Examples of techniques used*
Localization	Centrifugation
	Protein correlation profiling
	Proximity-labelling
	Detergent solubility
Turnover	Metabolic pulse-labeling
	Cycloheximide treatment
Isoform expression	High sequence coverage to identify isoform-specific peptides Targeted mass spectrometry analysis may be used to detect isoform-specific peptides
Solubility	Thermal denaturation followed by differential centrifugation
Activity	Analog-sensitive kinases
	Activity-dependent binding domains
Tertiary Structure	Protease sensitivity
	Crosslinking

*ERLIC, electrostatic repulsion hydrophilic interaction chromatography; FACS, fluorescence- associated cell sorting; HILIC, hydrophilic interaction chromatography; IEX, ion-exchange chromatography; IMAC, immobilized metal affinity chromatography; iTRAQ, isobaric tags for relative and absolute quantification; LC-MS/MS, liquid chromatography followed by tandem mass spectrometry; SILAC, stable isotope labeling by amino acids in cell culture; TiO_2, titanium dioxide; TMT, tandem mass tag.

Bottom-up proteomics refers to the analysis of proteolytic digests made under different chromatography fractions of the whole proteome of interest. Shotgun proteomics is the proteolytic digestion performed under the entire proteome (without previous fractionation of the sample). Both bottom-up and shotgun seek complete cleavage of peptides bonds, therefore, products with low molecular weight. Analysis of small molecules such as peptides simplifies sample handling, separation, and identification performance in the analytical instruments. However, after analyzing the results (a deluge of peptides), it is imperative to use bioinformatics that provides the sequencing of the peptides and proposes the proteins identity. This subject refers to protein coverage; the ideal condition would be the sequencing of all peptides from all proteins, meaning 100% coverage of each protein from proteome. However, this fact has proved to be very challenging—nearly impossible—because of the limitations from shotgun proteomics, where most peptides are lost or not detected in the analytical method [13,14]**.** Thus, the software uses other metrics to indicate the accuracy of relative protein identification. Besides the coverage of the proteins, other issues are the *score*, the *number of peptides*, the *number of unique peptides*, and the *number of proteins* [15–17].

Top-down proteomics is the study of proteoforms, *i.e.,* the analysis of intact proteins, usually molecular masses of 30 kDa. Top-down proteomics tolerates structural changes arising from the conditions of the method or sample preparation. In native protein mass spectrometry, another class of intact protein analysis, non-covalent interactions (molecular interactions) between proteins, are kept. Thus, the technique uses analytical method conditions close to the biological middle of origin. Therefore, it is useful to study functional and structural dynamism from interactions that occur into proteins complexes, *i.e.,* in the quaternary state of proteins (protein-protein and protein-ligand complexes). Intact protein analysis shows many challenges in sampling, separation, and detection processes than peptides analysis [10,11].

Middle-down proteomics is the analysis of peptides of larger molecular weight than shotgun and bottom-up (> 5 kDa) or limited peptide cleavage. This approach allows studies on the relative characterization of proteoforms with a good number of biomolecular species for identification. The technique is the middle ground between bottom-up/shotgun and top-down/native protein mass spectrometry. Figure 1 summarizes the different techniques applied to proteomics analysis [7].

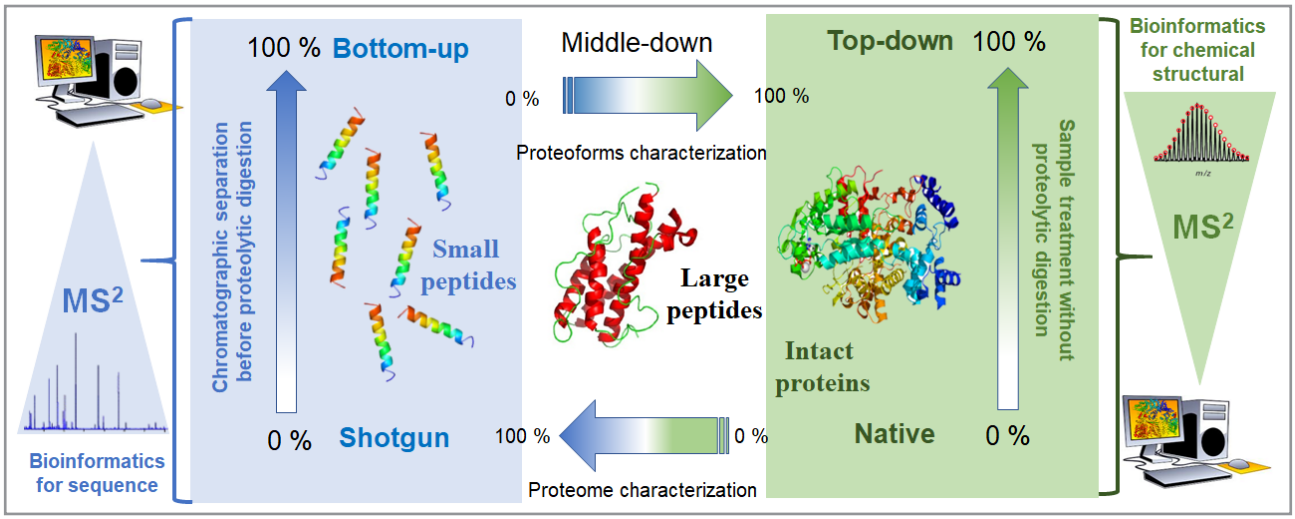


Figure 1. Different types of proteomics analysis and strategies. Left (blue) and center (white): Peptide analysis (shotgun, bottom-up, and middle-down proteomics). Right (green): Protein analysis (native and top-down proteomics). In bottom-up proteomics it is necessary the chromatography fractionation before proteolytic digestion, in shotgun proteomics is not required, but after proteolytic digestion liquid chromatography is always used. In top-down proteomics is necessary a mild sample treatment, in native is not substantially required. Mass spectrometry and bioinformatics for peptides analysis is essentially to sequence peptides into proteins. Mass spectrometry and bioinformatics for proteins analysis is essential for characterization of proteoforms. Peptides analysis allows better characterization of the proteome, whereas protein analysis allows better characterization of proteoforms.

State of the art in analytical instrumentation for large scale identification for proteomics

Proteomic analysis is complex, time-consuming, and expensive. To solve the proteome's biomolecular constituents before detection and identification is essential to use high separation power techniques, preferably automated and reproducible. Currently, multidimensional liquid chromatography (LC×LC) has the most appropriate attributes for that. Also, a molecular detection technique for accurate characterization is essential. Thus, mass spectrometry is the crucial tool because of its versatility. It allows detection in MS^1 and characterization after fragmentation in MS^2 quickly and, most importantly, at the molecular ion level. Besides, this technique provides an incredible separation power, separating ions by a difference as low as 0.0001 *m/z* in high resolution instruments. Such versatility of MS adds to the separation power of the LC×LC. Therefore, in terms of high orthogonality of separation, the state of the art in proteomic studies is multidimensional liquid chromatography coupled to high-resolution tandem mass spectrometry [18–22].

Multidimensional or two-dimensional liquid chromatography (LC-LC and LC×LC)

Multidimensional chromatography is designed for techniques that combine two or more chromatographic columns, so for each column, an analogy of a separation dimension is made. Its applicability becomes necessary for samples with high complexity, usually > 400 compounds. Figure 2 summarizes when two-dimensional chromatography (2D-LC) becomes necessary. It is historically verified that the isocratic 1D mode allows the separation of up to 10 compounds in an average of 10 minutes of analysis indicated by blue area in Figure 2. In contrast, for samples with approximately 100 compounds, it is essential to use a gradient, as indicated by yellow area. For samples above 1000 compounds, it is necessary to use 2D LC, green area.

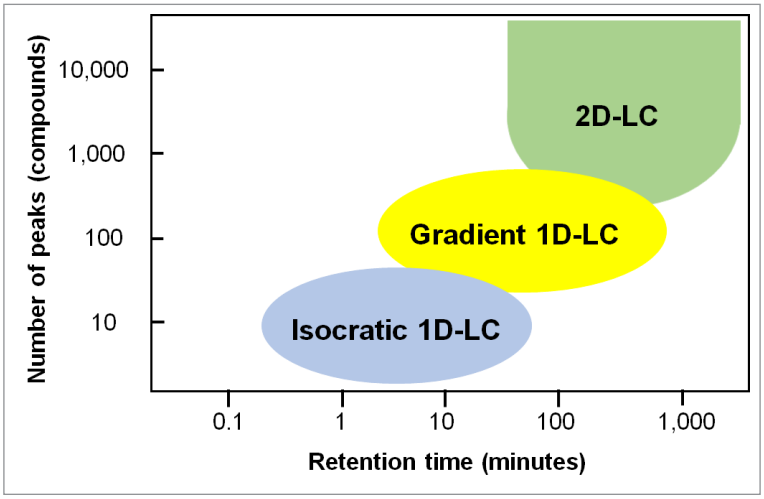


Figure 2. Representation of the evolution of different liquid chromatography strategies on the complexity of a sample**.** The different colors indicate the historical domain of the different modes of separation. [Reprinted (adapted) with permission from [22] Stoll, D. R.; LI, X.; Wang, X.; Carr, P. W.; Porter, S. E. G.; Rutan, S. C. J. Chromatogr. A, 2007, 1168 (1-2), pp 3-43 (https://doi.org/10.1016/j.chroma.2007.08.054). Copyright© (2021), Elsevier.]

Peak capacity definition means the maximum number of components that can theoretically have separation in the chromatographic column for a specific time interval of the gradient. The main advantage of 2D-LC is the high peak capacity (*n*), as indicated by Figure 3, where two peaks in 1D are separated into four peaks in 2D. This phenomenon occurs due to the differences in selectivity in the solute partition process between the stationary phases of the different dimensions [22–24].

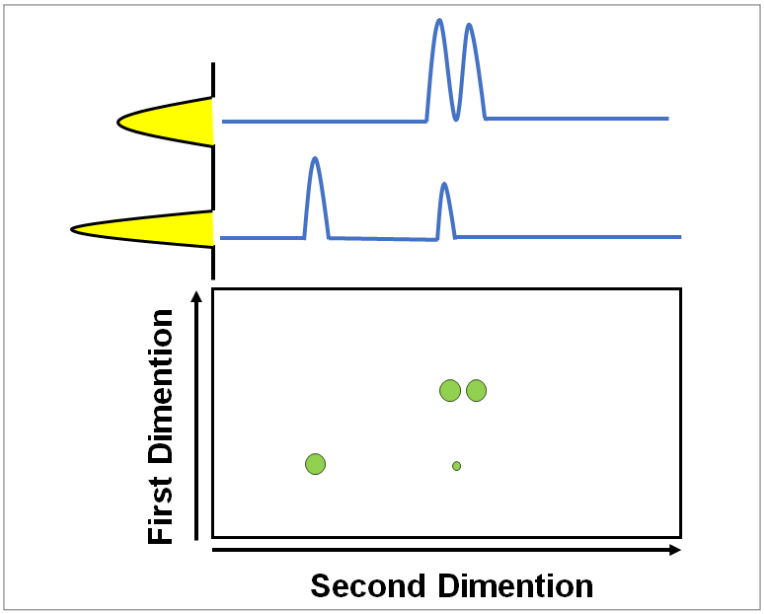


Figure 3. Scheme of separation from two chromatographic columns (bidimensional or multidimensional) illustrating peak capacity. [Reprinted (adapted) with permission from [22] Stoll, D. R.; LI, X.; Wang, X.; Carr, P. W.; Porter, S. E. G.; Rutan, S. C. J. Chromatogr. A, 2007, 1168 (1-2), pp 3-43 (https://doi.org/10.1016/j.chroma.2007.08.054). Copyright© (2021), Elsevier.]

J.W. Dolan et al. discuss different ways of measuring peak capacity, where the purpose is to accurately measure all the factors inherent to the random errors of chromatographic analysis. However, Equation 1 and Equation 2 are the most used due to their simplicity, where, *n* is peak capacity, t_R and t_1 are the

retention time of the last and the first chromatographic peak, respectively, W_b is the average of the base width of all peaks, and t_g is the gradient time [24,25].

$$n = \frac{(t_R - t_1)}{W_b} \qquad \text{Equation 1}$$

$$n = 1 + \frac{t_g}{W_b} \qquad \text{Equation 2}$$

The expectation is to obtain high values of *n*, so the LC strategies to achieve that are: *i)* decrease the rate of the organic phase gradient, *ii)* use columns of greater length, with a proportional increase in the gradient time, and a consequent increase in pressure of the system, and *iii)* employ columns packed with smaller sorbent particles. Achieving better performance in multidimensional chromatography means that the separations in the different columns have orthogonality. It is expected that the physical-chemical mechanisms in the analyte partition process for each chromatographic dimension are independent; in other words, they provide different selectivities [24–26].

Figure 4 defines the theoretical meaning of orthogonality. We can quickly notice the origin of the word orthogonality, which comes from trigonometric concepts. That is, each chromatogram is projected at an angle of 90°. Thus, when the analytes are randomly distributed in the chromatographic area, they have little correlation and high orthogonality. Therefore, excellent peak capacity. If the analytes do not have randomness and high correlation, there is low orthogonality and inefficiency in peak capacity [24].

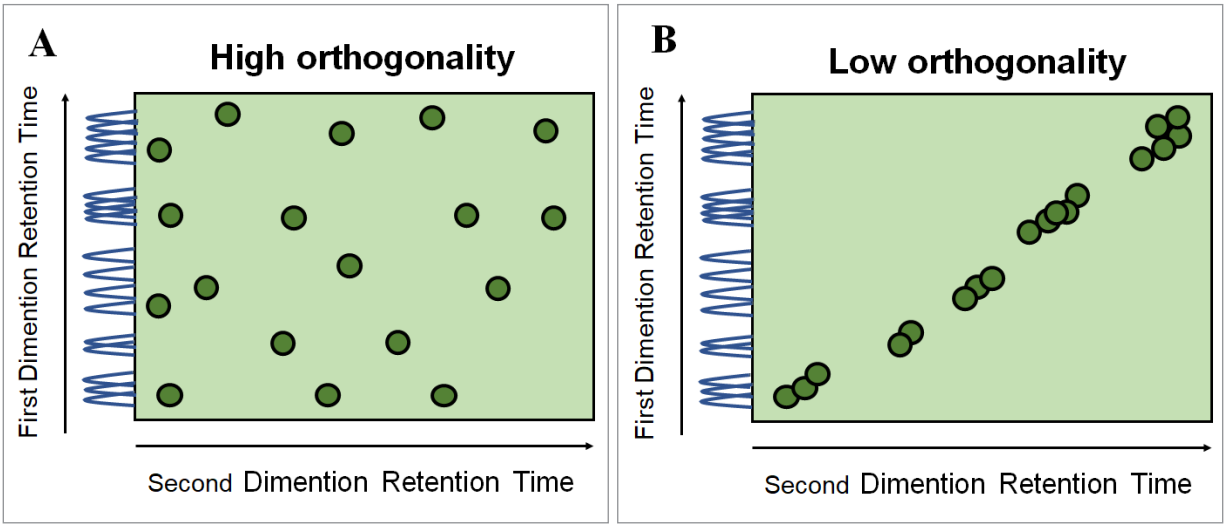


Figure 4. Theoretical representation of orthogonality in multidimensional liquid chromatography. A) low correlation and high orthogonality. B) high correlation and low orthogonality. [Reprinted (adapted) with permission from [24] François, I.; Sandra, K.; Sandra, P. Anal. Chim. Acta, 2009, 641 (1-2), pp 14-31 (https://doi.org/10.1016/j.aca.2009.03.041). Copyright© (2021), Elsevier.]

In 1995, J.C. Giddings proposed that the theoretical peak capacity of multidimensional chromatography is the multiple products of the peak capacities of each separation dimension. Thus, when it is combining the orthogonal LC modes of the peak capacity of n_1 = 60 and n_2 = 100, the result is an n_c of 6000, according to Equation 3 [27].

$$n_c = n_1 \times n_2 \times n_3 \times \ldots n_n \qquad \text{Equation 3}$$

Where n_c is the total peak capacity, and n_1, n_2, and n_n are the peak capacities of each chromatographic dimension. Therefore, the peak capacity depends on the orthogonality of the separation dimensions. If the selectivity of the separation modes is not entirely orthogonal (different), the maximum attainable peak capacity is less than expected.

Setup in multidimensional liquid chromatography

The technique can have two types of configurations: off-line or online. The off-line setup is based on collecting fractions from 1D and the injection of fractions into 2D. The online mode performs the direct transfer of the eluent fractions from the first dimension to the second dimension, without interruption in the flow of the mobile phase, usually using switching valves, that can be named column-switching (Figure 5A and B) [23,28].

The off-line setup offers greater instrumental simplicity and flexibility when the mobile phases between the dimensions are different since the fractions can be: diluted, concentrated, or dissolved in appropriate solvents. The disadvantages are a higher risk of loss and contamination of the sample due to manipulation and difficulty in automating the system, resulting in longer analysis times [19,28].

In online mode, sample handling and analysis time are reduced, which helps avoiding dead volumes, sample losses, and analyst errors. However, this technique has a strict requirement in the choice of solvents for the mobile phase because they must be compatible in both dimensions; otherwise, there is a need for a trap column to clean the fractions and change the eluent before the analytes are transferred to the second column. Consequently, it infers increasing in the time of analysis [23,28].

The proteomics community has divided online approaches into column-switching and multidimensional protein identification technology (MudPIT) systems (Figure 5C). MudPIT is a multidimensional chromatography setup proposed by Yates Group that uses a strong cationic exchange column (SCX) in 1D coupled directly to reversed-phase in 2D. Thus, the SCX column and the RP column are combined in one fused silica capillary with a glass needle directly connected to the mass spectrometer and act as an electrospray emitter due to voltage application in the bottom of the column (Figure 5C). For that, the sample is trapped first in the SCX phase. Different fractions of peptides are eluted from the SCX onto the RP phase by increasing salt steps. After the salt step, the peptides are eluted from the RP phase into the mass spectrometer using an acetonitrile gradient (Figure 5C and D). MudPIT has the limitation to clogged easily into glass needle tip from column interface to MS [29].

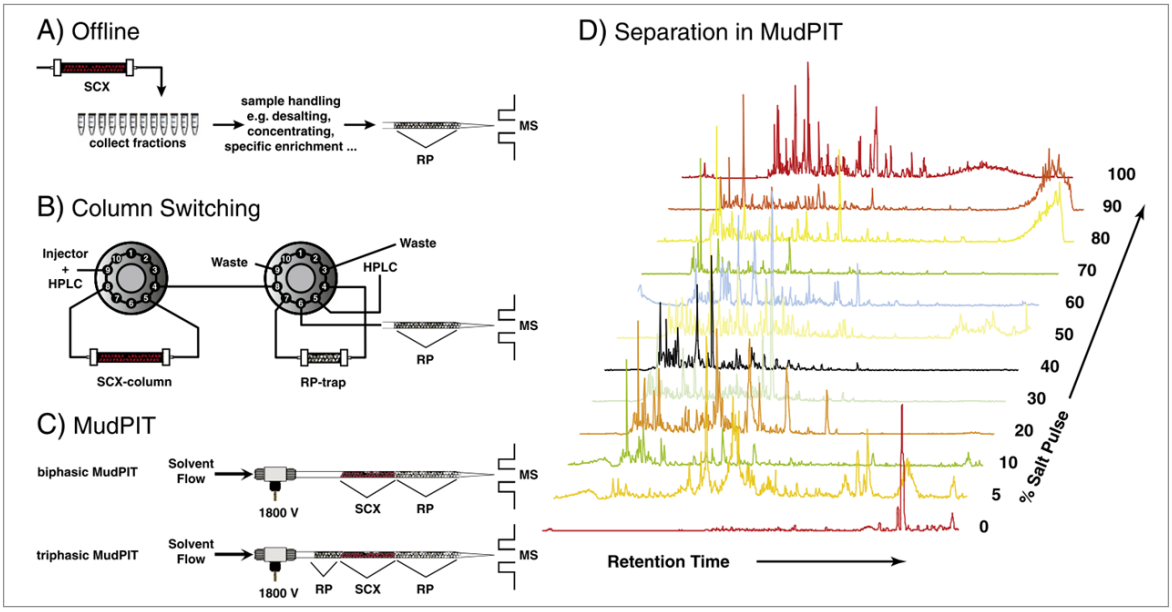


Figure 5. Different instrumental modes for two-dimensional liquid chromatography separations using SCX and RP as stationary phases. A) Off-line setup the sample is first separated by SCX, and fractions are collected by the handle or automatized**.** B) Online column switching setup. The sample is first loaded onto the SCX column and eluted stepwise onto the trap column. The sample is desalted and subsequently eluted onto the 2D, the analytical RP column followed by MS/MS analysis. C) MudPIT approach with SCX and RP stationary phases packed in one capillary also has the function of an electrospray tip for direct MS/MS analysis. In the triphasic setup, a different RP phase is packed before the SCX and functions as a trap for desalting the sample before SCX–RP-MS/MS. D) Typical multidimensional separation of a MudPIT-MS/MS analysis. Each color indicates the RP separation after one salt step. [Reprinted with permission from [29] Palma, S. D.; Hennrich, M. L.; Heck, A. J. R.; Mohammed, S. J. Proteomics, 2012, 75, pp 3791-3813 (https://doi.org/10.1016/j.jprot.2012.04.033). Copyright© (2021), Elsevier.]

The abbreviation LC-LC means that only relevant and properly chosen parts of the separation made in 1D are injected into 2D, and the rest of the eluate proceeds for disposal, which is linked to interferents or compounds that contribute to the matrix effect. Thus, LC-LC is often used when a greater resolution is required to examine a small segment of a particular peak from 1D, from a complex sample. In this case, the nomenclature of the technique is heart-cut, where only part(s) of interest(s) of separation in 1D is directed to 2D [23,28].

Another technique is named comprehensive multidimensional liquid chromatography. Here all fractions separated in 1D are submitted to 2D producing the analysis of all compounds in the sample, and consequently, all are of interest to the analyst. Generally, it has been used to mixtures with more than a thousand compounds where the goal is to characterize the sample completely. Thus, it has been used for studies on omics sciences, such as *i)* proteomics, *ii)* metabolomics, *iii)* lipidomics, *etc*.

The difficulties in the development of this technique are related to *i*) the compatibility of the mobile phases of the first dimension with the second dimension, *ii*) the resolution obtained in 1D should not be lost in the elution process for 2D, and *iii*) the size connections and columns should be planned to suitable elution, sensibility, separation in the 2D, mainly in online mode, due to the amount of fraction eluted be direct from 1D to 2D [23,24,26].

In terms of nomenclature/abbreviation when it refers to the heart-cut modality, the abbreviation is LC-LC. When it comes to the comprehensive modality, the abbreviation is LC×LC. The multiplication symbol means the products multiple of the peak capacity between 1D and 2D, which represents the separation area that has been usually represented as a rectangle [23,26,28]**.**

Currently, several works have been related in the literature to LC×LC and LC-LC modes for proteomics, with most of them based on SCX columns as the first dimension and RP column as the second dimension. Other growing strategies include RP columns in both the dimensions, where the mobile phase in the first dimension has high pH and the mobile phase in the second dimension has low pH. A third approach refers to the use of HILIC (hydrophilic interaction chromatography) columns as the first dimension and RP column as the second chromatographic dimension [30,31].

The high orthogonality of these methods: *i)* SCX×RP or SCX-RP; *ii)* RP×RP or RP-RP, and *iii)* HILIC×RP or HILIC-RP comes from different selectivity that each dimension can offer over partition phenomena. For example, the method *i)* have electrostatic interaction *vs* hydrophobic interaction or nonpolar interaction; in *ii)* conformational structure of biomolecules change in different pH between chromatographic dimensions generating different retention times, and *iii)* have polar *vs* nonpolar interactions, a full complementary and independent physical-chemistry mechanism. Some influential researchers in the field have reviewed substantially 2D LC modes, Table II summarizes some applications and interfaces used in 2D LC [30,31]**.**

Table II. Summary of some applications of 2D-LC to the proteomics analysis

Sample	Separation	Application	Hyphenation	Detection	Ref.
Peptides	Affinity×RP	Glycated proteins	Online (RP trap)	Orbitrap-MS	[32]
Peptides	SCX×RP	Proteins alterations in colon cancer	Offline	MS	[33]
Peptides	RP×RP	Proteome analysis of the methane producing archaeon *Methanosarcina mazei* strain Go1 cytosolic	Offline	Q Exactive	[34]
Peptides	RP×RP	Glycopeptides from Monoclonal Antibody	Offline	Orbitrap	[35]
Peptides	RP×RP HILIC×RP SCX×RP Other 13 LC×LC methods	Yeast cell lysate	Offline	UV and Triple TOF	[36]

Table II. Summary of some applications of 2D-LC to the proteomics analysis (Continuation)

Sample	Separation	Application	Hyphenation	Detection	Ref.
Peptides	SCX×RP RP×RP	Model of the brain of monkeys to study the characterization of molecular mechanisms of induced cerebral ischemia	Online (SCX and RP trap)	TripleTOF	[37]
Peptides	SCX×RP	Proteins in the jejunum tissues of enterotoxigenic *Escherichia coli*-infected piglets	Online	Q Exactive	[38]
Peptides	SCX×RP RP×RP	Characterization and quantitative proteomics mapping in cerebral infarcts	Online (SCX and RP trap)	Q-TOF and tripleTOF	[39]
Peptides	SCX×RP	Proteome analysis of S. cerevisiae	Online (RP trap)	FTICR-MS	[40]
Peptides	SCX × RP RP × RP HILIC × RP	Characterization of therapeutic monoclonal antibodies	Online (valve)	UV and QTOF-MS	[41]
Peptides	RP×RP	Detection and quantification of host-cell proteins in biotherapeutic drug substance	Online (valve)	Ion mobility and QTOF-MS	[42]
Protein	RP×RP	*E. coli* cell lysate	Online (RP trap)	LTQ-Orbitrap	[43]
Protein	SEC-RP	Endogenous membrane proteins	Offline	ETD Q-TOF	[44]
Protein	Affinity×RP	Analysis of glycated albumin	Online (RP trap column)	LTQ-Orbitrap	[45]
Protein	SEC-RP	Determination of degradants and small molecules in antibody-drug conjugate (ADC) solutions	Online (loop trap)	UV and MS	[46]
Protein	Mixed mode – RP; IEX-RP	Characterization of polysorbate surfactants in monoclonal antibody drug formulation	Online (loop trap)	CAD and MS	[47]
Protein	SAX × RP	Quantification of Gly m 4 protein in soybean	Online (RP trap)	UV and MS	[48]
Protein	SEC – RP(μchip)	Characterization of monoclonal antibody degradation products	Offline	Orbitrap-MS	[49]
Protein	IEX – RP SEC – RP	Characterization of monoclonal antibody variants	Online (RP trap and valves)	QTOF-MS	[50]

An overview over Table II infers that LC-LC-MS/MS method has been more used for monoclonal antibody or biomarker characterization, whereas LC×LC-MS/MS have been extensively applied for proteomes characterization. However, it does not seem to be a general rule for application choice, that can be inferred depending on the subject of study of the scientist. Besides, HILIC×RP has more challenges in the hyphenation because the mobile phase between dimensions is strictly incompatible. It is mean that loss of chromatography resolution between dimensions is easily achieved. For that, it has been used *offline* mode or sophisticated configuration using trap column, valves, or loops.

Tandem mass spectrometry

According to the theories of electromagnetism, charged particles (ions) in motion (accelerated) generate an electromagnetic field, the action of an external field, the use of radiofrequency (RF), and Direct Current (DC) or Alternating Current (AC) over the ions, allows their control. Consequently, particles subjected to acceleration and deflection (force) from the external electromagnetic field enable the physical phenomenon to measure the *m/z* of the ions. That means the type of force applied and adequate equation obtain a solution in *m/z*. Figure 6 shows an introductory block diagram for a typical tandem mass spectrometry instrument

for proteomics analysis. The hardware of the mass spectrometers have been changed continuously for the question of improvement of the instrument and probably by the strategy of the market of the companies.

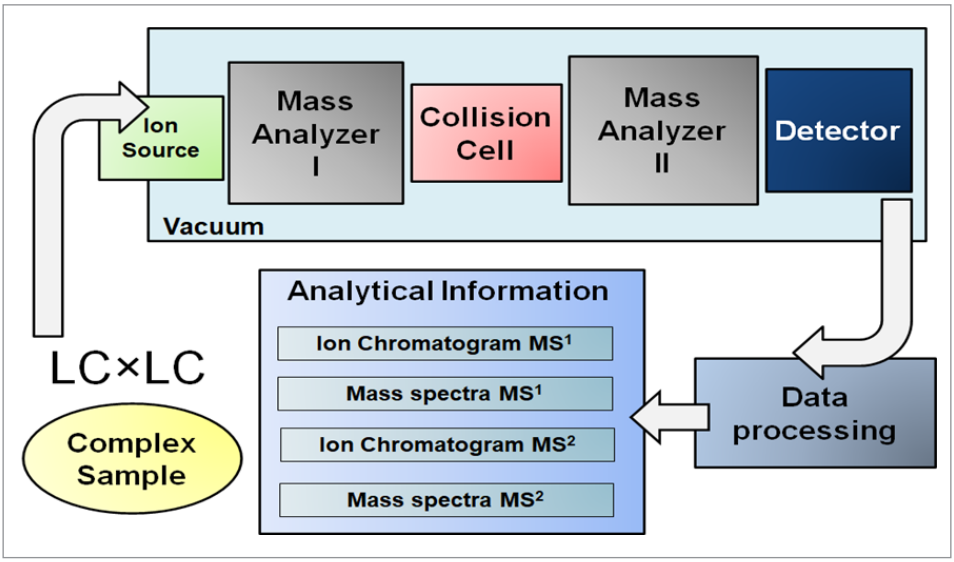


Figure 6. Block diagram for tandem mass spectrometer in proteomics analysis. After multidimensional liquid chromatography (LC×LC), the sample is injected into the ion source of mass spectrometry by an electrospray ion source. The Mass Analyzer I filter/separate more intense peaks from a mass range previously selected. Collision cells activate the molecules for their fragmentation. The Mass Analyzer II filter/separate more intense peaks from precursor ions fragmented. After detection, the data is processed as several Analytical Information for protein characterization, where MS^1 is the masses from Analyzer I, and MS^2 are the masses from fragments of the precursor ion.

Proteomics has driven the advance of mass spectrometry analysis and instrumentation. The mass accuracy combined with the resolution are the most important figures of merit that infer greater detection of species due to the discrimination between masses (m) for values < 1 ppm (10^{-6} m/Δm for suitable IUPAC definition). Instrumentation have undergone considerable advances in sensitivity, speed of acquisition, and quality to biomolecule fragmentation in recent years. The researchers have focused on developing *i)* sources of micro and nanoflow ionization to increase the sensibility, *ii)* innovations in mass analyzer shapes to increase resolution, *iii)* including their different hybrid configurations. That means using two mass analyzers in sequence; *iv)* optical devices to control the focus or deflection of the ion beams in the vacuum, using a suitable electromagnetic field to increase the sensibility, and *v)* new peptide and protein fragmentation techniques to improve identification [11,19].

The separation properties to ions submitted by an electromagnetic field and, differentiated by their mass charge ratio (*m/z*), make mass spectrometry a high-speed technique compared to chromatography since separation is governed by physicochemical separation of partition. However, chromatography remains the most efficient and cheapest technique in terms of separation power for the solution of complex samples for > 1000 analytes [18,26].

In general, proteins and peptides are polar, non-volatile, and thermally unstable. Because of these properties, one of the most significant scientific advances in the area was the introduction of soft ionization methods, without the random and abrupt degradation of the biomolecule, such as electrospray ionization (ESI) [51], matrix-assisted laser desorption ionization (MALDI) [52] and, recently, ionization by desorption and electrospray (DESI) [53]. In contrast to MALDI and DESI, the ESI ionization source produces ions in solution, an aspect that makes it compatible with liquid chromatography. In terms of sensitivity for mass spectrometry, a substantial gain was creating nanoelectrospray (nano-ESI) [54].

The nano-ESI do not use gases (N_2 or He) for sample desolvation because it is a low flow rate technique (less than 20 nL min^{-1}). This low flow allows to shape of microdroplets with a larger surface area and a smaller drop radius, contributing to the Coulombic explosion and consequent increase of sensitivity due to the lower dilution of the analytes in the mobile phase.

The usual nanoflow of the coupling of the LC to nano-ESI is 250 nL min^{-1}. There are two factors that essentially need to work in together: *i)* chromatographic efficiency according to the parameters of the *van Deemter* equation in the nano chromatographic column (75 µm i.d.), and *ii)* efficient aerosol formation of the biomolecules into the ionized state (ionization), to reach the vacuum chamber of the mass spectrometer. It has been shown that in the ESI process non-covalent bonds (biologists' nomenclature) or molecular interactions (chemists' nomenclature) between biomolecules are kept. The versatility of the technique allows the ionization and infusion of protein complexes in the mass spectrometer. Thus, it is possible to analyze the quaternary structure of small proteins. That infers studies on the protein conformations in their native shapes [10,11].

In the mass analyzer, the physical phenomenon to measure the *m/z* of the ions occurs (Figure 6). Currently, the analyzers used in proteomics or other complex bioanalytical demands focus on couple hybrid systems between *i)* quadrupole (Q), *ii)* time of flight (TOF), *iii)* linear ion trap (LIT), *iv)* Orbitrap, and *v)* Fourier Transform Ion Cyclotron Resonance (FTICR). Hybrid systems in mass spectrometry obtain better analytical figures of merits for detecting ions from a complex mixture. The hybrid configuration increases the possibilities to control, filter, and separate the ions; it allows fragmentation of molecules, which increases information for the characterization of unknown chemical species.

Among all mass analyzers, FTICR offers the highest resolution. However, the Orbitrap analyzer has also a high resolution and delivers best results in sequential mass spectra (MS/MS), with lower maintenance costs. Orbitrap and FTICR use the Fourier transform signal processing to convert the time domain signal from the frequency of the ionic current into *m/z* spectra [8,11,19].

The hybrid configuration of the ion trap coupled to Orbitrap has been the tool of choice for biomolecular studies from samples of high complexity, mainly the studies driven by shotgun and bottom-up proteomics. The ion trap is considered a mass analyzer of high yield in proteomics. It has significant versatility because *i)* has high capacity to store ions, *ii)* provides enrichment of sensitivity, *iii)* shows excellent range of mass selection, *iv)* scans at high speed, *v)* features efficient service cycle (high-duty cycle), *vi)* moderate mass resolution (full width at half height - FWHH - 2000); *vii)* mass accuracy around 100 ppm (1×10^{-4} m/Δm), *viii)* is robust, and *ix)* has simple architecture, what favors miniaturization [8,55].

The linear trap quadrupole (LTQ) is made of four hyperbolic rods positioned symmetrically on a 4 mm radius between them. Each rod is divided into three long axial sections 12, 37, and 12 mm (Figure 7A). In each section has a discrete DC level. The geometry was first simulated by the Simion technique, predicting the possibility of confining ions along the axis of the central section of the device, avoiding possible distortions of the electromagnetic field. The ability of this configuration to avoid distortion of the field is shown in the simulation of Figure 7B [55].

Although the routine operation of the LTQ requires further details in the explanation, the physical phenomenon to confinement of ion occurs due to the potential rotation applied in the central section, together with the application of discrete DC in each segment of the analyzer, as described by Figure 7C. Thus, when the positive ions are attracted to the opposing poles, in a short time, the negative poles are converted into positive poles causing the repulsion of the positive ions. The temporal and spatial change from positive to negative potential generates an oscillating, reproducible, and symmetrical potential surface (Figure 7D), causing the capture or storage of ions in vacuum.

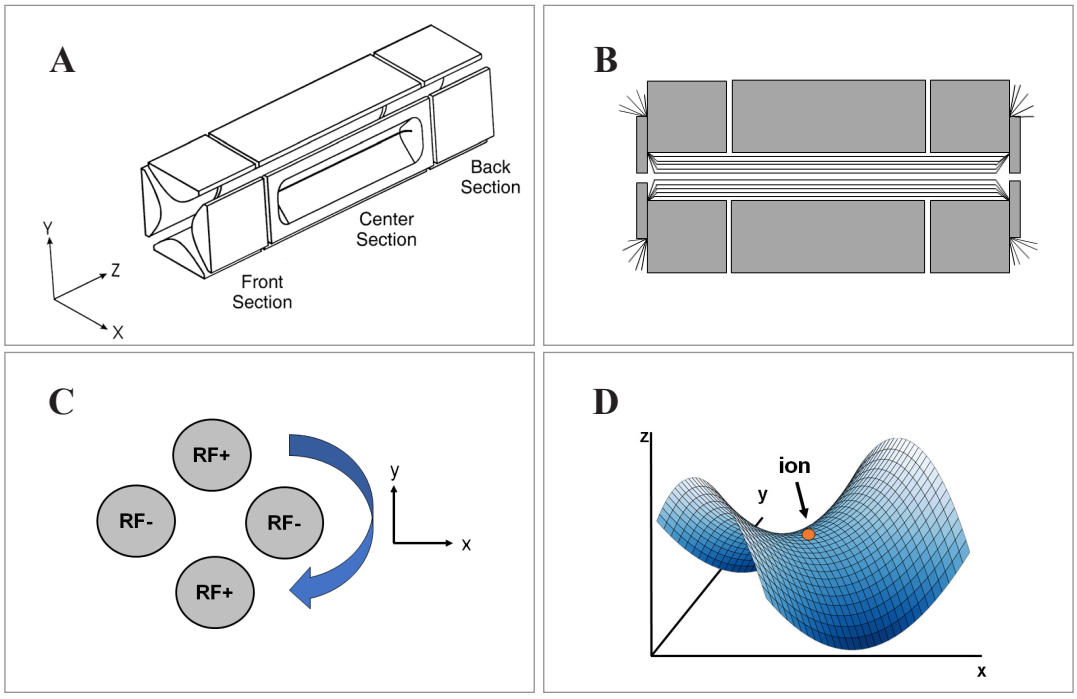


Figure 7. Summary of the operation of the linear trap quadrupole mass analyzer. A) The basic characteristic of the analyzer; B) Representation of the Simion simulation of the excitation resonance of the electromagnetic field without distortion; C) The radiofrequency (RF) applied varies depending on the time and potential. In a symmetrical and synchronized way, the positive potential at a given moment is converted into a negative potential at a second moment. The final display resembles a circular signal/potential rotation. [Reprinted (adapted) with permission from reference [55] Schwartz, J. C.; Senko, M. W.; Syka, J. E. P. J. Am. Soc. Mass Spectrom., 2002, 13, pp 659-669 (https://doi.org/10.1016/S1044-0305(02)00384-7). Copyright© (2021) American Chemical Society. Further permission related to the material excerpted should be directed to the ACS]. D) An instantaneous moment of the field generated by the quadrupole or surface of the potential, as a function of an applied RF. The orange circle represents an ion on the surface of the potential. [Reprinted (adapted) with permission from reference [56] March, R. E. Encyclopedia of Analytical Chemistry, 2012, p 4 (https://doi.org/10.1002/9780470027318.a6015.pub2). Copyright© (2021) John Wiley & Sons, Ltd.]

The origin of the Orbitrap analyzer began through the proof concept that a central spindle-like electrode could generate a purely harmonic potential in the direction of the z-axis. This was first devised by Kingdon in 1923, proven by Knight in 1981 by the Simion simulation, as shown in Figure 8A and applied by Makarov in 2000, as Kingdon's ideal trap, which would later be called Orbitrap [19,57].

The Orbitrap analyzer, also considered an ion trap, like the FTICR and the LIT, is configurated of a barrel-like anode electrode in a coaxial shape (external) that involves the cathode electrode in the shape of a spindle-like (central). The geometry confers a harmonic potential, ion oscillation stability, and purely governed by the electrostatic field (Figure 8B). The electrical voltage is applied axially between the central electrode, providing the stored ions with a stable orbital path and oscillation in the axial direction between the electrodes. Therefore, Orbitrap uses the oscillation frequency of the confined ions along the central electrode to determine its *m/z* ratio. These properties of the Orbitrap are the origin for the high resolution of the equipment. In contrast, the scanning speed of the *m/z* is considered moderate. As a rule, the use of high resolution affects the scanning speed and vice versa [19,57].

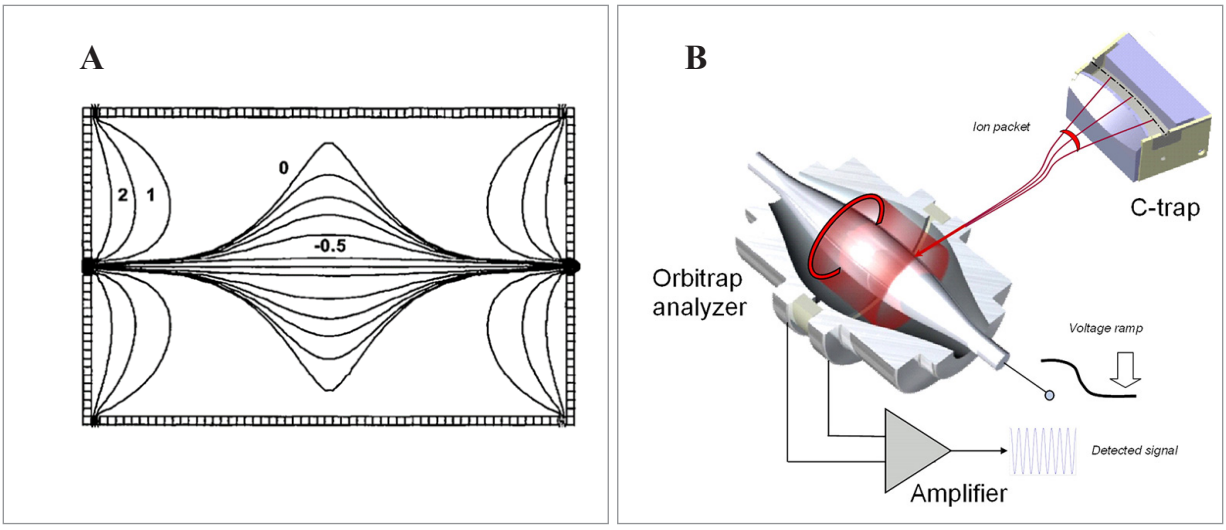


Figure 8. Summary of the operation of the Orbitrap mass analyzer. A) The Simion graphs of equipotential lines for ideal parameters of the Kingdon trap. The numbers refer to the potentials of the contour curves. Check that the central contours resemble the trajectory of the ions in Figure B. [Reprinted with permission from reference [19] Perry, R. H.; Cooks, R. G.; Noll, R. J. Mass Spectrom. Rev., 2008, 27, pp 661-699 (https://doi.org/10.1002/mas.20186). Copyright© (2021) John Wiley & Sons.] B) A sectional view of the Orbitrap analyzer. Through the C-trap the ions are injected into the Orbitrap at a point where z = 0 and perpendicular to the z-axis, where axial radiofrequency oscillations of the ions begin, without the need for additional excitation. The red cylinder is the area of the ions' trajectory. [Reprinted with permission from reference [58] Zubarev, R. A.; Makarov, A. Anal. Chem., 2013, 85, pp 5288-5296 (https://doi.org/10.1021/ac4001223). Copyright© (2013) American Chemical Society.]

The challenges to mass spectrometry analyzers concern intact protein analysis. So, scientists have invested in developing new FTICR cell harmonization, high-field Orbitrap geometries, and advances in signal processing. For that, the base unit resolution has recently been extended to proteins with masses of 150 kDa, using the FTICR or Orbitrap analyzers [59]. Due to the higher resolution of FTICR, it has been the analyzer of choice for top-down proteomics. However, the hybrid system LTQ-Orbitrap has proved to be a successful strategy.

In general terms, the quality of ion fragmentation determines the successful identification of peptides and proteins. Fragmentation in mass spectrometry activates the molecule, with the purpose to produce biomolecular "fingerprints" of a diverse set of molecules. The goal of all activation methods is essentially the same: to deposit energy in the ions to cause cleavages in chemical bonds reproducibly and to produce interpretable ion fragments, which means to reveal structural information or the sequence of the molecule of interest [60].

The fragmentation techniques used in proteomics are based on *i)* collision energy transfer (collision-induced dissociation - CID, high energy collision dissociation - HCD, electron capture dissociation – ECD, and electron transfer dissociation - ETD, surface-induced dissociation - SID; ion-ion reactions) or *ii)* photon-based (infrared multiphoton dissociation - IRMPD and Ultraviolet photodissociation - UVPD) [7,60].

Fragmentation methods by collision energy transfer are more useful for peptides with lower molecular weight and less efficient for peptides with high molecular weight and intact proteins or species containing labile PTMs, such as phosphopeptides.

The electron-based fragmentation methods generate fragmentation patterns of larger molecules that favor the interpretation of a species with labile PTMs, such as phosphorylation and glycosylation. Thus, the fragmentations type ECD and ETD have efficient results in the characterization of intact proteins [11,59].

SID is the alternative that provides the highest energy to gas-phase collision methods, in which the ions are activated and fragmented after colliding with a surface (which works how a large target). In addition to its ability to generate rich fragmentation patterns, for many classes of ions, SID has also been used to characterize the structure of protein complexes due to its high energy transfer. In photodissociation techniques, ions accumulate energy through the absorption of one or more photons. Consequently, the fragmentation of the ionic species is obtained [60].

In particular, two fragmentation methods have contributed considerably to the advances in top-down proteomics, ETD, and ultraviolet photodissociation UVPD. Currently, they are the most efficient fragmentation methods for intact proteins, especially when used in combination [10,59].

CID/CAD collision-based methods are the most common fragmentation techniques in proteomics. These generally provide fragmentation at the amide bond of the peptide/protein. Thus, ions type *b* and *y*- are obtained. The HCD method provides fragmentations that result in the formation of ions of type *a*- and *x*-. Electron-based methods, such as ECD and ETD, generally result in the cleavage of the N-C bond and produce fragments of ions type *c*- and *z*- [7,11,59].

The analytical challenges of proteomics

Although there have been considerable advances in techniques for the separation and identification of biomolecules, the analysis of intact proteins from a complex mixture remains a challenge to analytical chemistry because of the extreme complexity of large-sized proteins, post-translational modifications, and physicochemical properties. Proteins have all possibilities of chemical interactions, in terms of intramolecular and intermolecular. That infers analyzes with low reproducibility in the analytical methods and techniques. Besides, proteins have a high dynamic range of protein expression, spanning many orders of magnitude ($>10^{10}$). Additionally, proteins have conformational heterogeneity resulting from post-translational modifications (PTMs), besides expressed in a diversity of species in qualitative and quantitative terms. Therefore, our ability to understand a proteome through the analysis of intact proteins is currently limited [59].

Analytical chemistry has advanced significantly for the analysis of small molecules. Because of the less complexity, the chemical behavior of small molecules in analytical separation and detection systems is more conclusive for successful analysis. Certainly, shotgun and bottom-up proteomics have gained knowledge of analytical techniques for the analysis of small molecules. Consequently, the shotgun has provided greater characterization of the proteome emerging to expressive numbers in identifying proteins, never reported before. However, the analysis of a proteome from a complex mixture of several proteins digested into peptides increases the dependence on the accuracy of bioinformatics tools. The bioinformatics strategy alone does not have great chances to succeed in terms of the typical analytical figures of merits. That infers that the sequence of peptides into proteins in some cases brings certain bias. The main drawback is that peptides can assume a math property of combinatory analysis in the sequencing step. That infers some peptides can be sequenced the wrong way to some proteins, consequentially increase the number of identification of proteins that, in reality, does not exist. Thus, shotgun proteomics is a relative technique for protein identification, as well as for quantification. Also, protein coverage is desired to reach 100%, a challenging aspect for liquid chromatography and high-resolution mass spectrometry because of the misunderstood loss of peptides in the analysis process [13,61]**.**

The high orthogonality and selectivity of LC×LC have an essential influence on the proteomics results, mainly due to the decrease in sample complexity in the separation, providing a better detection and biomolecular characterization in MS/MS. However, LC×LC is not able to separate all compounds from proteomes. Therefore, two or more compounds with similar retention time and molar mass (isobarics) can be considered the same precursor ion in the m/z range, and they are co-sequenced together, mainly in shotgun proteomics. This drawback is named “chimera” spectra. Thus, the results from the isolation and simultaneous fragmentation of two or more different molecular ions reduce the score values and the number of identifications of bioinformatics tools due to unidentifiable fragment ions derived from contaminating parent ions.

Bioinformatics scientists have developed alternatives to lessen the effect chimera through the statistical treatment of mass spectra. Additionally, it becomes essential to develop high-resolution mass spectrometers or strategies such as ion mobility-MS/MS, to increase the rate of ion isolation and consequent improvement in the detection of MS.

Another common problem in mass spectrometer applications is ionic suppression because some peptides or proteins can ionize more efficiently than others, causing that other biomolecules have an inefficient ionization. Thus, the first one can make ionic suppression phenomena over the second one that affects the sensibility of analysis implicating in not identifying bioinformatics tools.

Yet, the main challenges continue to be top-down and native mass spectrometry proteomics because the detection limit and sensitivity of the mass spectrometer for intact proteins are much lower than for peptides. In any type of MS instrument, the sensitivity decreases drastically with the increase of mass weight. Today, it is still relatively difficult to analyze intact proteins more massive than 70 kDa. Moreover, as the molecular mass of the protein increases, the tertiary structure of proteins becomes more difficult to disrupt, which thereby limits the MS/MS fragmentation efficiency of intact proteins. Thus, most of the top-down applications focused on proteins less than 50 kDa, and there are very few applications to date on larger proteins (>100 kDa) [56].

In any proteomic technique (bottom-up, middle-down, or top-down), the ideal condition to increase sensitivity and to achieve the lowest limit detection or quantification for 2D-LC are basically: *i)* the highest orthogonality of separation; *ii)* gradient using a nano-flow scale for less amount of mobile phase (that infers lower dilution of each chromatography peak and leads to great Coulombic explosion in the MS); *iii)* capillary chromatography columns, usually 75 μm of inner diameter and 3 μm of size particle; *iv)* reduced plate height following *van* Deemter equation, usually 250 nL min^{-1}. Likewise, in mass spectrometry provides: *i)* efficient ionization for the most suitable gas-phase state of all molecular ions to enter in the electromagnet field driving to the vacuum chamber of MS; *ii)* a suitable interval of time storage ions in the analyzers with high velocity to analysis and fragmentation in the sequent analyzer; *iii)* fragmentation quality that depends on technique and type of biomolecule analyzed; *iv)* high resolution, and *v*) high sensitivity. Usually, these two last parameters are the intrinsic conditions of the mass analyzer type of choice, which means how much the analyzer differentiates the background noise from the analytical signal. Usually, they are linked to an electromagnetic theory from physics, such as summarized in Figures 7 and 8.

Mathematical modeling and chemometrics tools to improve proteomics analysis

Mathematical modeling is based on the mathematical model concept to representing a complex phenomenon or incomprehensible subject. Therefore, it brings ways simpler and more palpable to understand complex data using simulations. The construction of a model requires multidisciplinary knowledge and the ability to make sophisticated guesses when getting information, testing, etc. The steps of mathematical modeling and troubleshooting can be summarized in Figure 9 [62].

Once the mathematical model has been built, it can be applied for studying the modeled phenomenon. The most important is to check if the model is coherent to represent genuinely the phenomena that occur in the problem investigated, for that some adjust in the equation(s), proving tests, and validations must be evaluated. If the mathematical model is robust, it can be used to investigated similar problems. In chemistry, simulation can bring faster and cheaper solutions, which means that it is unnecessary to do many experiments to observe phenomena because simulation can be used as the first step to refine the experiments [62].

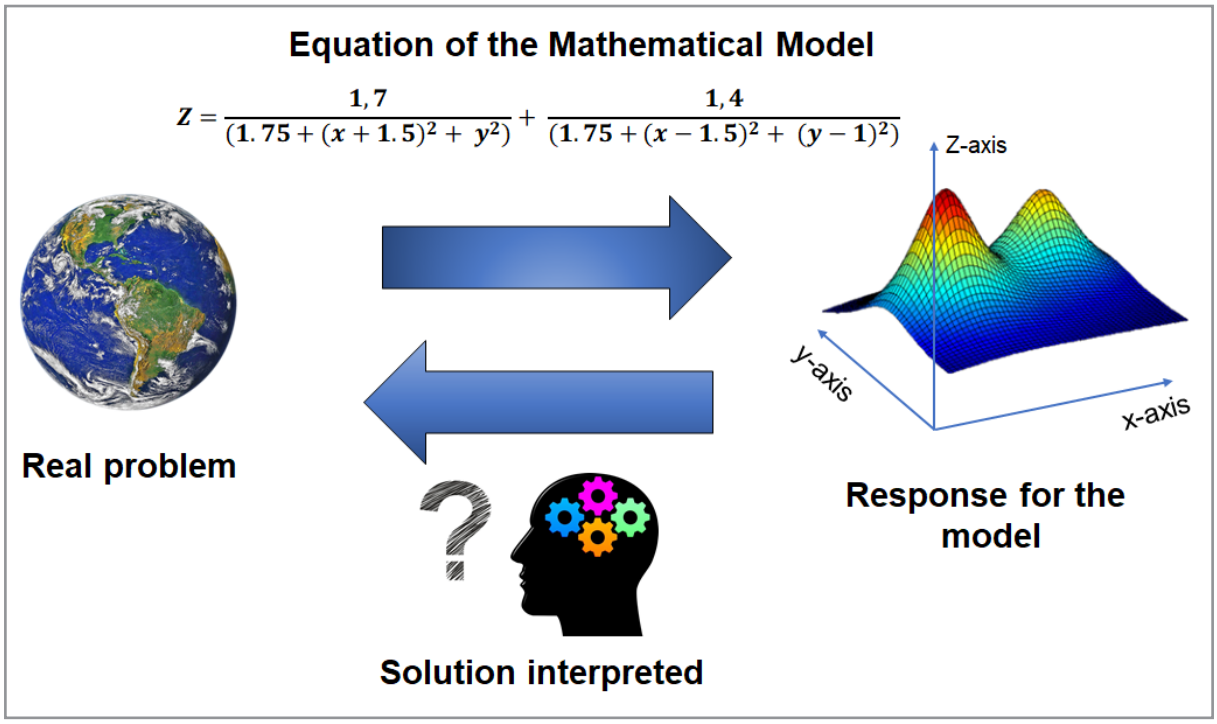


Figure 9. The process of mathematical modeling. First, we must identify the problem that is trying to solve by modeling. Next, it is necessary to understand which building blocks (equations) have to be included in the model. At this step, we define the most critical variables and quantities of them, and we also think of any relevant background assumptions, simplifications, and so forth.

Chemometrics is a science-based tool that uses mathematical concepts to simplify complex chemical data or optimizations. For example, using the Design of Experiments (DoE), an input data analysis, the most used chemometrics tool, it is possible to evaluate several parameters to find two or three parameters most significant on an analytical method to be optimized. Although DoE is very used in the analytical chemistry field in proteomics it is very incipient with few papers published [63,64].

Another chemometric tool used, in this case, in output data, to proteomics is principal components analysis (PCA). It is useful to simplify a complex data analysis to bring relevant information to interpretation. PCA uses the concept of dimensionality reduction by projecting of new axis (PCs). For example, in a proteomics analysis with more than 1,000 proteins for each sample analyzed, PCA allows us to plot 2D or 3D graphs that separate or put together sample groups. Thus, separate groups have different chemical information, and groups in the set have similar chemical information. Without this strategy, it is practically impossible to understand the most important chemical information of different samples analyzed. Another very well used chemometric tool used in proteomics is Hierarchical Cluster Analysis (HCA). Here different samples form clusters until all of them in the dataset are linked together in a hierarchical tree [65–67].

Other useful chemometrics tools can be used in proteomics analysis include multivariate calibration, K^{th} Nearest Neighbor (k-NN), Soft Independent Modeling of Class Analogy (SIMCA), Linear Discriminant Analysis (LDA), Partial Least Squares – Discriminant Analysis (PLS-DA), Partial Least Squares – Regression (PLS-R), Orthogonal Partial Least Squares – Discriminant Analysis (OPLS-DA), Parallel Factor Analysis (PARAFAC), PARAFAC 2, and Artificial Neural Networks (ANN). Some of these tools have been applied currently in proteomics analysis in food and biological questions. However, application of chemometrics tools in proteomics is still incipient. Although chemometrics is an excellent strategy to obtain relevant information from complex samples, it must be used with caution because, before its application, a study on the mathematical and statistical treatment must be considered. In general terms, the consensus on the previous information of the sample must have a link to the chemical information the chemometrics

has provided. The detailed description of all of these useful tools would demand a complementary review, which is not the goal on this one. However, Table III summarizes the main applications of chemometrics tools in proteomics data analysis [67–69]**.**

Table III. Summary of chemometrics studies applied in proteomics data analysis

Field	Sample	Chemometrics tools used								Ref.
		DoE	PCA	HCA	PLS-DA	LDA	PLS-R	OPLS-DA	ANN	
Food Chemistry	Meat		✓							[70]
Food Chemistry	Avocados		✓	✓	✓	✓	✓	✓		[71]
Food Chemistry	Meat		✓	✓	✓					[72]
Food Chemistry	Cucumber		✓	✓				✓		[73]
Food Chemistry	Meat		✓					✓		[74]
Food Chemistry	Shrimp		✓					✓		[75]
Food Chemistry	Biactive peptides							✓		[76]
Analytical Chemistry	Mix of proteins				✓					[77]
Analytical Chemistry	Mix of proteins		✓	✓						[78]
Analytical Chemistry	*Saccharomyces cerevisiae*	✓								[64]
Analytical Chemistry	*Saccharomyces cerevisiae*	✓								[63]
Health	Cutaneous Leishmaniasis		✓							[79]
Health	Ovarian cancer								✓	[80]

In the last years, a chemometric strategy based on DoE has been introduced, named as Chemical Mathematical Model to maximize protein sequence coverage for shotgun proteomics. Thus, two significant parameters were statistically evaluated from the whole LC×LC-MS/MS platform [81,82]**.** These parameters show more representative to increase shotgun proteomics coverage. Besides, the evidence shows that performance is strongly linked to the chemical nature of the peptides, for example, hydrophobicity. Thus, the origin of the chemical approach of the model is revelated. Figure 10 shows the behavior of the surface of response in different methods to improve identification of proteoforms in shotgun and bottom-up proteomics analysis, once it allows greater coverage of proteins. Note that different instrumental conditions are necessary to detect different hydrophobicity of peptides. Lastly, an optimization condition from a complex system (LC×LC-MS/MS) can be amenable to simplification into mathematical models due to *Pareto Principle* [81,82]**.**

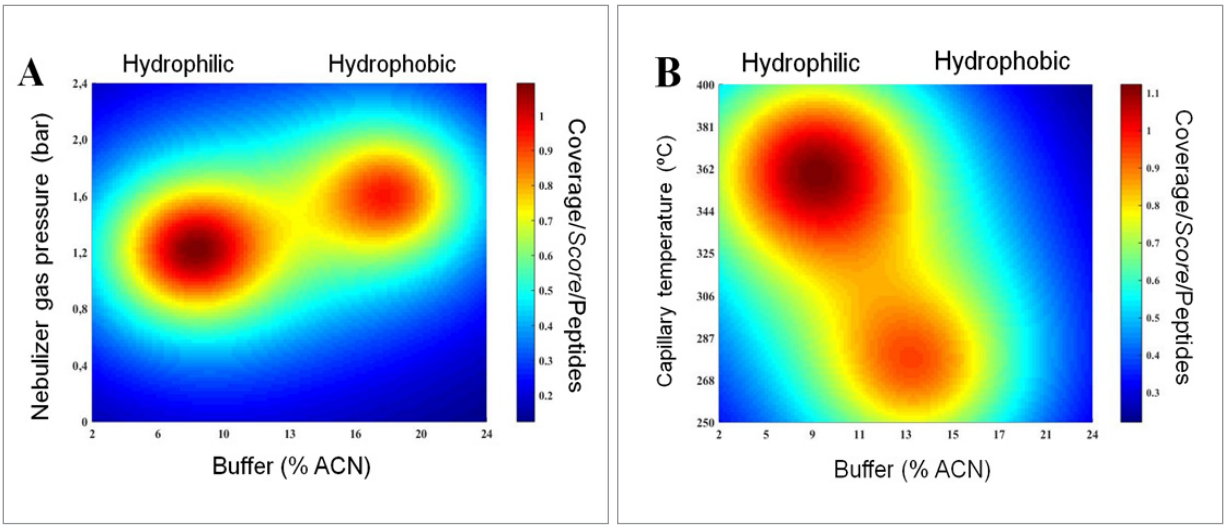


Figure 10. Two Chemical Mathematical Model to maximize protein sequence coverage for shotgun proteomics. A) Digested bovine serum albumin (BSA) sample to evaluate the optimal conditions of SCX-RP-Q-ToF. B) Digested yeast proteins to evaluate the optimal conditions of MudPIT-LTQ-Orbitrap. The x-axis is the significant parameter from 2D-LC and the y-axis is the significant parameter from ESI-MS/MS. Areas in red means larger peptides identifications through evaluation of three responses i) Coverage of Proteins ii) Score, and iii) the number of peptides. [Reprinted (adapted) with permission from reference [82] Batiston, W. P. Exploring the fundamentals of liquid chromatography and mass spectrometry for integration between proteomics, microfluidics, and chemometrics. Doctoral thesis, 2020. Sao Carlos Institute of Chemistry, University of Sao Paulo, Sao Carlos, SP, Brazil (https://doi.org/10.11606/T.75.2020.tde-27082020-142027).]

Finally, bioinformatics has emerged as an imperative tool for proteomics analysis and is essential in any proteomics technique (bottom-up, middle-down, or top-down). The most important subject of study of bioinformatics is the algorithm. An algorithm is a finite sequence from executable actions that gets a solution for a specific problem. As the algorithm are precise, unambiguous, mechanical, efficient, and correct procedures it is important to a deep understanding of the figures of merit in analytical chemistry for proteomics. Among the main outcomes from bioinformatics, we can benefit from reproducible analysis, precision, accuracy, chemical behaviors of the biomolecule and its fragmentation pattern, as well as understanding the random or systematic errors. Because an algorithm only can interpret a pattern or well-defined behavior, a proteomic analysis with significant random errors could infer some bias in the results from algorithm interpretation.

CONCLUSIONS

In terms of theoretical perspectives on the top-down and native proteomics, they would have conditions suitable to identify with accuracy proteins. However, there are tremendous analytical challenges to overcome the direct identification of proteins with >70 kDa to mass spectrometry. One of the main drawbacks sounds inevitable; the structural diversity of proteins when submitted to analytical methods drive different chemical structures under the same condition of analysis, what substantially decreases the reproducibility of the methods. Besides the expressive number of species to the identification and the challenges to get a good fragmentation in mass spectrometry for biomolecules above 50 kDa. Although of the several limitations of peptides analysis, consequently driven to relative identification of proteins. We can infer that shotgun and bottom-up proteomics must persist in studying the massive scale of analysis from proteomes using LC×LC-MS/MS, because of great simplicity and very well-established analysis ways. A light at the end of the tunnel for overcoming the drawbacks to separations limitations in the intact proteins could be the exploration of new stationary phases for high resolution and sensitivity in nanoflow liquid chromatography, such as monolithic columns or open tubular columns. Besides, in mass spectrometry, the understanding of new strategies and sophisticated ion control, increases resolution, and new fragmentation modes could provide more evidence to improve proteomics analysis. However, the development of analytical instruments demands long time, elevated costs, and skilled personnel. Such bottlenecks are not interesting

to most of the scientific community, which can put the field in a stagnation, even if it is of high interest to analytical instrument companies. Smart strategies can improve analytical figures of merit in proteomics, like mathematical modeling, chemometrics tools, and continuous advances in bioinformatics.

Conflicts of interest

The authors declared having no conflicts of interest.

Acknowledgements

The São Paulo Research Foundation (FAPESP), [grant number 2015/16025-8] to WPB is gratefully acknowledged. Moreover, INCTBio is kindly acknowledged for the grants from FAPESP [grant number 2014/50867-3] and Council for Scientific and Technological Development (CNPq) [grant number 465389/2014-7].

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Brazilian Journal of Analytical Chemistry
2021, Volume 8, Issue 31, pp 74-88
doi: 10.30744/brjac.2179-3425.AR-50-2020

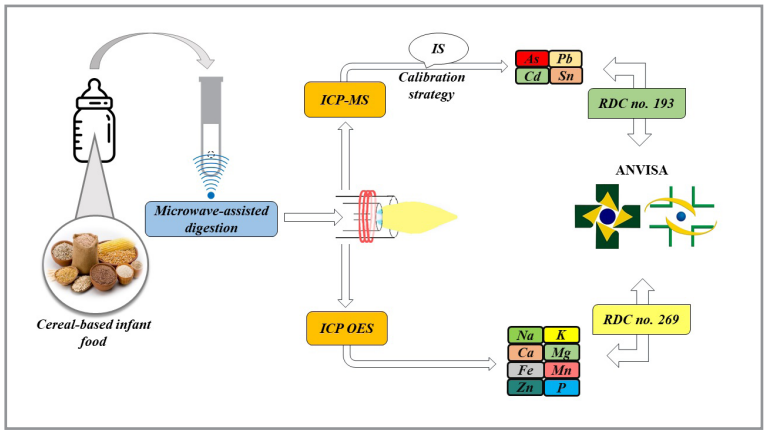
BrJAC

ARTICLE

Determination of Macro, Micro and Toxic Elements using Argon-Based Plasma Spectroanalytical Methods in order to support Brazilian Regulations on Inorganic Constituents in Infant Foods

Marcelo Larsen de Lima Tozo, Fernanda Costa Pinheiro, Joaquim Araújo Nóbrega*

Group for Applied Instrumental Analysis, Department of Chemistry, Federal University of São Carlos
P.O. Box 676, São Carlos, SP, 13560-270, Brazil



Children's cereal-based foods and supplements are widely consumed by newborn and infant population. The Resolution RDC no. 193/2017 of the Brazilian National Health Surveillance Agency (ANVISA) established maximum tolerated limits (MTL) for toxic elements in infant foods aiming to control health risks. Complementarily, the determination of macro and microelements based on values of recommended daily intake (RDI) is regulated by Resolution RDC no. 269/2005. Two analytical procedures were developed using inductively coupled plasma mass spectrometry (ICP-MS) for determination of As, Cd, Sn and Pb and inductively coupled plasma optical emission spectrometry (ICP OES) for determination of Ca, Fe, K, Mg, Mn, Na, P and Zn attending both Brazilian regulations. Microwave-assisted acid digestion of solid samples was carried out in closed vessels using 7 mol L^{-1} HNO_3. Two rice flours reference materials as well as addition and recovery experiments were applied to check accuracy. Recoveries ranged from 70 to 128% for ICP OES and from 82 to 128% for ICP-MS using internal standardization. The analytical procedure presented LOQs lower than maximum limits allowed by both regulations. Fifteen samples of cereal-based infant foods were analyzed and all analyte concentrations were lower than the respective MTLs recommended by RDC no. 193, except to Pb concentration in one milk flour sample. According to the RDI established by RDC no. 269 and the average concentrations of Ca, Fe, K, Mg, Mn, P and Zn in samples, it was possible to assign a minimum mass value of food that meets the recommendations of the Brazilian resolution for different population groups.

Keywords: Sample preparation, ANVISA Resolution 193, ANVISA Resolution 269, ICP-MS, ICP OES.

Cite: Tozo, M. L. L.; Pinheiro, F. C.; Nóbrega, J. A. Determination of Macro, Micro and Toxic Elements using Argon-Based Plasma Spectroanalytical Methods in order to support Brazilian Regulations on Inorganic Constituents in Infant Foods. *Braz. J. Anal. Chem.,* 2021, *8* (31), pp 74–88. doi: http://dx.doi.org/10.30744/brjac.2179-3425.AR-50-2020

Received 15 September 2020, Revised 21 December 2020, 2nd time Revised 17 January 2021, Accepted 19 January 2021, Available online 23 February 2021.

INTRODUCTION

Good nutrition is vital for human health. In this sense, it is known that cereal grains are important in human health and also a good source of energy and dietary fibers as well as proteins and essential elements, especially for children and newborns during development phase [1]. Despite the nutritional important roles of cereal grains in the human diet, cereal-based foods, commonly present in the diet of children and newborns, are a possible source of exposure to contaminants, such as As, Cd, Pb and Sn, also known as potentially toxic elements (PTEs) [2-7].

Contamination by some PTEs, even in low concentrations, can be harmful to health, causing diseases and irreversible damage to the body. Because children differ regarding their physiology and metabolism, the contamination risk can be more severe [8,9]. Exposure to environmental threats at these sensitive stages of the child's life may negatively influence the growth and development and also cause irreversible damage, such as injury to the liver, kidneys, bones, lungs, central nervous system, cardiovascular diseases and even cancerous effects [10].

Associated with the control of PTEs in foods, the interest in the determination of As, Cd, Sn and Pb in infant foods is also related to the fulfillment of Brazilian legislations, including the Resolution RDC no. 193, of December 2017, which establishes Maximum Tolerated Limits (MTLs) of these contaminants in infant foods and cereal-based lactating foods [11]. In addition to RDC no. 193, the Resolution no. 269, of September 2005, recommends the determination of macro and microelements based on values of recommended daily intake (RDI) of proteins, vitamins and minerals for individuals in different population groups [12].

According to Damodaran et al. [13] the variation in the deficiency of some elements, rare or practically nonexistent in the human body, is due to socio-economic issues and geographical divisions. However, plant-based foods are the main sources of macro and microelements to satisfy nutritional needs. In biological systems, elemental nutrients are separated into two categories: macroelements (elements in higher concentrations in human body, such as Ca, K, Mg, Na and P) and microelements (elements present in lower concentrations in human body, but which perform important functions for normal metabolism of human beings, such as Co, Cr, Cu, Fe, Mn and Zn) [3,13].

Spectrochemical methods with atomization-excitation-ionization in argon plasmas are widely used for elemental determination providing multi-element analysis, high sensitivity, robustness and relatively simple operation of equipment [14-16]. Due to the high sensitivity and low detection limits argon-based plasma methods are intensely used for determination of inorganic contaminants at trace concentrations in several types of food samples [15-17]. Additionally, the combination of microwave-assisted digestion and closed vessels has clear advantages compared to traditional acid digestion using conventional heating and open vessels in terms of better recoveries for volatile elements, lower contamination, lower volume of reagents, better reproducibility and a better working environment [18].

In this context, this study proposed a microwave-assisted digestion procedure using dilute nitric acid solutions for determination of As, Cd, Sn and Pb using ICP-MS and Ca, Fe, K, Mg, Mn, Na, P and Zn using ICP OES in several types of children's foods and supplements in order to meet simultaneously both Brazilian resolutions: RDC no. 193 and RDC no. 269. Instrumental strategies for correcting spectral interferences as well as calibration methods for non-spectral interferences were evaluated in order to improve accuracy and precision of the analytical procedure using either ICP-MS or ICP OES. Fifteen samples of cereal-based infant foods were analyzed and all analyte concentrations were lower than the respective MTLs recommended by RDC no. 193, except to Pb concentration in one milk flour sample. According to the RDI from RDC no. 269 and the average concentrations of Ca, Fe, K, Mg, Mn, P and Zn in samples, it was possible to assign a minimum mass value of food that meets the recommendations of the Brazilian resolution for four different population children groups.

MATERIALS AND METHODS

Samples and sample preparation

Fifteen samples of children's foods and supplements (coded from A1 to A15) were analyzed. Samples A1, A2 and A3 were purchased in commercial establishments (as normally sold in commercial packaging) in São Carlos, SP, Brazil, and all other samples were supplied by the Laboratory Exata located in Jataí, GO, Brazil also as they are normally sold. Samples, types and their nutritional indication are presented in Table I. These samples were produced by several food companies (Danone, Maisena, Nestle, Sustagen and Yoki) in four Brazilian states. One sample came from Buenos Aires, Argentina.

Table I. Samples of children's foods and supplements and their food indication according to the commercial label of each food

Sample	Type of infant food	Indication
A1	Infant Cereal – Rice	Children over 6 months
A2	Corn Starch with Rice Flour	Children over 2 years
A3	Rice Cream	Children over 2 years
A4	Milk Flour	Children over 2 years
A5	Infant Formula	Infants from 0 to 6 months
A6	Milk Flour	Children over 2 years
A7	Infant Cereal – Rice and Oats	Children over 6 months
A8	Infant Cereal – Corn	Children over 6 months
A9	Infant Formula	Infants over 5 months
A10	Infant Cereal – Multicereals	Children over 6 months
A11	Sustagen	Children over 6 months
A12	Infant Formula	Infants over 9 months
A13	Powdered Milk	Children over 1 year
A14	Powdered Millk	Children over 1 year
A15	Infant Formula	Infants from 0 to 6 months

Masses of approximately 500 mg of each sample were weighed in perfluoroalkoxy-alkane (PFA) digestion vessels and digested in a single reaction chamber (SRC) system (UltraWave™, Milestone, Sorisole, BG, Italy). Volumes of 150 mL of water and 5 mL of concentrated nitric acid were inserted into the SRC and the chamber was pressurized with nitrogen gas to 40 bar (99.9%, White Martins-Praxair, Sertãozinho, SP, Brazil) as recommended by the manufacturer. Volumes of 5 mL of HNO_3 in three different concentrations (1; 2 and 7 mol L^{-1}) were tested for digestion of food samples. The microwave heating program was applied as follows [18]: (1) 2.5 min to reach 140 °C, (2) 2.5 min hold at 140 °C, (3) 2.5 min to reach 180 °C, (4) 2.5 min hold at 180 °C, (5) 10 min to reach 220 °C, (6) 10 min hold at 220 °C. Temperature was controlled by an internal temperature sensor.

Subsequently, digests were diluted to 25.0 mL with distilled-deionized water and an aliquot of each solution was appropriately diluted with deionized water, followed by quantification by ICP OES (10-fold dilution) using external calibration and by ICP-MS (2-fold dilution) using internal standardization.

Reagents and standard solutions

Experiments were performed using HNO_3 (Synth, Diadema, SP, Brazil) purified in a sub-boiling distillation apparatus Distillacid™ BSB-939-IR (Berghof, Eningen, Germany) and ultrapure water with resistivity higher than 18.2 MΩ cm (Milli-Q® Bedford, MA, USA). All glass and polypropylene bottles were washed and kept in an acid bath (HNO_3 10% v/v) for 24 h. The standard solutions used for calibration and for addition and recovery experiments were prepared by adequate dilution of mono-elementar stock solutions containing 1000 mg L^{-1} of As, Ca, Cd, Fe, K, Mg, Mn, Na, P, Pb, Sn and Zn (Qhemis, Jundiaí, SP, Brazil) in 0.7 mol L^{-1} HNO_3 medium. The internal standards evaluated (ISs) were also prepared by adequate dilution of mono-elementar stock solutions containing 1000 mg L^{-1} of Ge, Pd and Y. These elements were evaluated as IS based on previous studies [18-20].

The concentrations for analytical calibration solutions used in ICP-MS were 0, 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 and 20.0 µg L^{-1} of As, Cd, Sn and Pb prepared in 0.14 mol L^{-1} HNO_3 medium. Addition and recovery experiments were performed at two levels of addition: 0.50 and 1.0 µg L^{-1} for all analytes in four different samples: A1 (Infant cereal of rice); A3 (Rice cream); A9 (Infant formula) and A13 (Powdered Milk). The standards were added before sample digestions. To correct for matrix effects, the evaluated ISs Ge, Pd and Y were added at 1.0 µg L^{-1} to analytical calibration solutions, analytical blanks, and sample digests. For addition and recovery experiments the ISs were added at same concentration for both addition levels.

For ICP OES, the concentrations for analytical calibration solutions were 0, 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10, 25 and 50 mg L^{-1} of Ca, Fe, K, Mg, Mn, Na, P and Zn prepared in 0.70 mol L^{-1} HNO_3 medium. Matrix effects were not observed using ICP OES and, consequently, ISs were not added. Addition and recovery experiments were not performed for ICP OES analysis because both reference materials have certified values for all analytes. The certified reference material (CRM) of rice flour NIST 1568a (National Institute of Standard and Technology, Gaithersburg, MD, EUA) and the reference material (RM) also of rice flour Agro AR-01/2015 (Embrapa Pecuária Sudeste, São Carlos, SP, Brazil) were used for optimization of experimental procedures and evaluation of accuracy for ICP OES and ICP-MS. For ICP-MS, addition and recovery experiments were also considered because both reference materials did not inform reference concentrations for Pb and Sn. CRM NIST 1568a has certified values for As and Cd. On the other hand, RM Agro AR-01/2015 only has certified value for As.

Instrumentation

Measurements were performed using an ICP OES model iCAP6000 (Thermo Fisher Scientific, Waltham, MA, USA) operated under robust conditions and axial viewing mode and an ICP-MS model Agilent 7800 Quadrupole (Agilent Technologies, Tokyo, JHS, Japan) without using collision cell (*standard mode*) or using collision cell technology (*He mode*) pressurized with He gas (99.999%, White Martins-Praxair, Sertãozinho, SP, Brazil) both applying the aerosol dilution strategy, i.e. the aerosol was diluted with argon under adjusted flow rates (aerosol dilution gas flow rate of 0.62 L min^{-1} and carrier gas flow rate of 0.40 L min^{-1}, thus 1.02 L min^{-1} of total flow rate [20]. Argon (99.999%, White Martins-Praxair) was used in all measurements for both instruments. Plasma operating conditions adopted in ICP-MS and ICP OES are presented in Table II.

Table II. Instrumental parameters adopted in ICP OES and Quadrupole ICP-MS

Instrumental Parameter	ICP OES	ICP-MS
RF applied power (kW)	1.20	1.55
Plasma gas flow rate (L min^{-1})	12	15
Auxiliary gas flow rate (L min^{-1})	0.5	1.0
Carrier gas flow rate (L min^{-1})	0.50	1.02
Carrier gas flow rate in *HMI mode* (L min^{-1})	NA	0.40

Table II. Instrumental parameters adopted in ICP OES and Quadrupole ICP-MS (Continuation)

Instrumental Parameter	ICP OES	ICP-MS
HMI gas flow rate (L min^{-1})	NA	0.62
Sampling depth (mm)	NA	8.0
He flow rate in collision cell (mL min^{-1})	NA	4.5
Integration time (s)	15	3.0
Nebulizer	V-Groove	Mira-Mist
Spray chamber	Cyclonic	Double-pass
Number of replicates	3	3
Analyte	**Emission Line (nm)**	**Isotope (*m/z*)**
As	NA	75
Ca	184.006	NA
Cd	NA	114
Fe	238.204	NA
K	769.896	NA
Mg	279.079	NA
Mn	259.373	NA
Na	330.237	NA
P	178.284	NA
Pb	NA	208
Sn	NA	120
Ge	NA	70
Pd	NA	104
Y	NA	89
Zn	202.548	NA

NA: Not applicable.

RESULTS AND DISCUSSION

Microwave-assisted sample preparation

The preliminary assessment of the digestion procedure was carried out visually since the goal was to reach digestion without residual solids for all analyzed samples. Residual solids were observed for samples digested using 1 and 2 mol L^{-1} HNO_3. Thus, a solution containing 7 mol L^{-1} HNO_3 was selected for further experiments since complete and clear digests were obtained for all samples without adding hydrogen peroxide. The sample preparation procedure using only 1+1 v/v dilute nitric acid solution is attractive because the use of hydrogen peroxide may sometimes imply in the addition of contaminants when not using a high purity reagent [16,18,21].

Analytical performance for ICP OES and ICP-MS

Limits of detection (LOD) and quantification (LOQ) were calculated considering standard deviation (SD) for 10 measurements of a blank solution divided by slope of analytical curve multiplied by 3 (LOD) and 10 (LOQ), and then multiplied by the dilution factor [22]. For ICP-MS, measured isotopes, mode of acquisition, linear correlation coefficient and LOQs are shown in Table III. The LOQs obtained for all analytes were lower than the respective MTL suggested by RDC no. 193 [11], inferring that the developed procedure has sufficient detectability to meet this resolution.

Table III. Maximum tolerable limits (MTL) and figures of merit for determination of As, Cd, Pb and Sn by ICP-MS using external calibration and internal standardization

Isotope	**$^{75}As^{+}$**	**$^{114}Cd^{+}$**	**$^{120}Sn^{+}$**	**$^{208}Pb^{+}$**
Acquisition mode	*He*	*Standard*	*Standard*	*Standard*
MTL[a] (mg kg^{-1})	0.15	0.050	50	0.050
External Calibration				
Sensitivity	956	2076	4464	4842
R^2	0.9999	0.9998	0.9999	0.9997
LOD (mg kg^{-1})	0.0031	0.00050	0.0069	0.0087
LOQ (mg kg^{-1})	0.010	0.0020	0.023	0.029
Internal Standardization				
Internal Standard	Y	Y	Y	Y
R^2	0.9993	0.9992	0.9994	0.9990
LOD (mg kg^{-1})	0.0038	0.00050	0.0076	0.0093
LOQ (mg kg^{-1})	0.013	0.0020	0.025	0.031

[a] Resolution no. 193, of December 12, 2017 [11].

For ICP-MS measurements, sample dilution is often necessary to keep the total dissolved solids (TDS) below 0.1% m/v. To overcome this limitation and improve the sensitivity, ICP-MS was operated using aerosol dilution strategy. This instrumental strategy enabled the introduction of digests with TDS of up to 0.5% m/v and residual acidity up to 1% v/v, which eliminates possible contamination associated with manual dilution, saves time and reduces the volume of waste compared to conventional dilution using a liquid diluent [18,20].

ICP-MS is susceptible to spectral and non-spectral interferences due to matrix effects associated with transport, nebulization, and/or energetic effects in argon plasma [15,16]. Consequently, internal standardization was used as calibration strategy to correct matrix effects in the determination of all analytes. Furthermore, the instrumental strategy of collision cell technology (CCT) with kinetic energy discrimination (KED) was adopted to correct spectral interferences when determining As. The CCT is an instrumental strategy used for removing spectral interferences caused by polyatomic species [23]. The collision cell mode was used only for determination of As, due to possible interferences caused in the mass/charge 75, such as $^{40}Ca^{35}Cl^{+}$ and $^{59}Co^{16}O^{+}$. For all other elements, adequate accuracy was obtained using the standard mode acquisition.

Measurements using ICP OES were performed in axial configuration for improving sensitivity. Table IV presents the reached limits of quantification (LOQ) for determination of Ca, Fe, K, Mg, Mn, Na, P and Zn using ICP OES.

Table IV. Analytical parameters for determination of Ca, Fe, K, Mg, Mn, Na, P and Zn by ICP OES using external calibration

Element	Sensitivity	R2	LOD (mg kg^{-1})	LOQ (mg kg^{-1})
Ca	1019	0.9999	3.5	12
Fe	95	0.9999	0.060	0.19
K	13077	0.9998	0.48	1.6
Mg	7306	0.9998	0.51	1.7
Mn	54727	0.9998	0.013	0.042
Na	1497	0.9999	8.7	29
P	129	0.9998	1.9	6.3
Zn	24067	0.9996	0.55	1.8

Evaluation of accuracy for ICP OES and ICP-MS procedures

For ICP-MS measurements, the accuracy of the developed analytical procedure was evaluated by addition and recovery experiments at two-levels (0.50 and 1.0 µg L^{-1}) applied to four samples with different compositions (infant cereal, rice cream, infant formula and powdered milk) and also by analysis of CRM NIST 1568a and RM Agro AR-01/2015, both composed by flour rice. The addition levels of spike experiments were performed based on the specific MTL for each analyte by means of RDC no. 193 [11] (Table III). Spikes were added before microwave-assisted digestion. Table V presents the recoveries obtained for addition and recovery experiments using external calibration and internal standardization. Considering the CRM and RM analyzed, only As (0.29 ± 0.03 and 0.112 ± 0.015 mg kg^{-1}, respectively) and Cd (0.022 ± 0.002 mg kg^{-1} for the CRM) presented certified values. For As, recoveries of 101% (0.30 ± 0.03 mg kg^{-1} of As for CRM) and 106% (0.12 ± 0.01 mg kg^{-1} of As for RM) were obtained for both materials. For Cd, only the CRM contained the certified content, and a 70% recovery was obtained (0.015 ± 0.001 mg kg^{-1} of Cd).

Table V. Addition and recovery experiments (recovery (%), RSD (%), n = 3) by ICP-MS using external calibration and internal standardization

Isotope	Adition (µg L^{-1})	External Calibration			
		A1	A3	A9	A13
$^{75}As^{+}$	0.5	51 (4)	79 (8)	84 (6)	86 (3)
	1	63 (7)	79 (2)	83 (2)	79 (4)
$^{114}Cd^{+}$	0.5	85 (7)	95 (9)	72 (2)	74 (6)
	1	83 (4)	91 (2)	73 (4)	75 (6)
$^{120}Sn^{+}$	0.5	88 (5)	90 (4)	105 (6)	82 (3)
	1	87 (7)	88 (1)	95 (9)	80 (7)
$^{208}Pb^{+}$	0.5	94 (8)	101 (9)	113 (6)	94 (7)
	1	89 (4)	103 (10)	94 (6)	93 (25)

Table V. Addition and recovery experiments (recovery (%), RSD (%), n = 3) by ICP-MS using external calibration and internal standardization (Continuation)

Isotope	Adition (μg L^{-1})	Internal Standardization			
		A1	A3	A9	A13
$^{75}As^{+}$	0.5	85 (6)	84 (4)	86 (4)	96 (4)
	1	83 (7)	86 (1)	82 (3)	90 (3)
$^{114}Cd^{+}$	0.5	91 (5)	108 (5)	82 (2)	85 (7)
	1	90 (4)	97 (3)	85 (6)	87 (5)
$^{120}Sn^{+}$	0.5	94 (6)	97 (5)	96 (7)	92 (4)
	1	94 (7)	94 (1)	87 (8)	91 (5)
$^{208}Pb^{+}$	0.5	102 (7)	104 (8)	97 (6)	98 (6)
	1	96 (3)	108 (11)	87 (9)	102 (23)

The choice of IS was evaluated by addition and recovery experiments and also by analysis of CRM NIST 1568a and RM Agro AR-01/2015. Among the ISs evaluated (Ge, Pd and Y), the best recoveries, ranging from 82 to 108%, were obtained for all analytes when using Y as IS, but satisfactory recoveries were also obtained when using $^{70}Ge^{+}$ as internal standard for $^{75}As^{+}$, $^{120}Sn^{+}$ and $^{208}Pb^{+}$, as also observed in previous studies [18,20]. Since RDC no. 193 [11] does not specify a validation parameter, recoveries ranging from 80 to 120% were considered satisfactory.

The improvement in recoveries using internal standardization can be explained due to the correction of matrix effects caused during transport and/or ionization of analytes. In general, it is accepted that a good IS should have physico-chemical properties similar to the analytes, such as similar isotopic mass between the IS and the analyte [24] or the first ionization energy [25]. However, recent studies have demonstrated divergences in the literature related to IS selection criteria in ICP-MS [19,20,26,27]. According to Olesik and Jiao [26,27] and Barros et al. [19,20] in current ICP-MS instruments is not always necessary to have analyte and IS with similar masses probably due to the different behavior of space charge effects in current ion lenses design. This new assumption might explain why a single IS, sometimes with highly different mass and physico-chemical parameters, is effective for correcting matrix effects, as also observed here.

For ICP OES measurements, recoveries ranged from 70 to 128% for the RM of rice flour Agro AR-01/2015 and from 85 to 123% for the CRM NIST 1568a inferring that the analytical procedure developed for determination of macro and micro elements in baby food samples using ICP OES is accurate. Some elements i.e., Ca, Na and K, presented recoveries slightly out of range considered quantitative (from 80 to 120%), however, for just one of the evaluated RMs. No significant matrix effects were observed for ICP OES measurements, i.e. matrix effects observed in ICP OES were reduced in comparison to ICP-MS. Therefore, internal standardization was not required. Table VI shows certified and determined contents obtained for both reference materials.

Along with the contents shown in Table VI, F-test with 95% of confidence and paired Student's *t*-test were applied to evaluate agreement between certified and determined values for each element in both reference materials. The t-test was performed assuming unequal variances for Zn on RM Agro and Fe, K, Mg and Na on NIST SRM and equal variances for the others elements according to the F-test previously made. Under the calculated conditions (n = 3), with 95% confidence ($t_{95\%}$ = 2.78), Mg and P had shown significant differences (3.19 and 3.08, respectively) for the RM Agro AR-01/2015, and also K and Zn had shown significant differences (36.4 and 5.44, respectively) for the CRM NIST 1568a. Adopting 99% confidence level ($t_{99\%}$ = 4.60), only K had shown significant difference for the CRM NIST 1568a.

Table VI. Certified and determined contents (mean ± standard deviation, mg kg^{-1}, n = 3), recoveries (%), Student's t-test obtained for RM Agro AR-01/2015 and CRM NIST 1568a using ICP OES

Element	RM Agro AR-01/2015			NIST SRM 1568a		
	Certified value	Determined value	*t* value	Certified value	Determined value	*t* value
Ca	90 ± 32	114 ± 3 (128)	0.75	118 ± 6	106 ± 1 (91)	1.97
Fe	17 ± 1	17.32 ± 0.05 (102)	0.32	7.4 ± 0.9	6.2 ± 0.3 (87)	1.26
K	1852 ± 319	1908 ± 20 (103)	0.18	1280 ± 8	1591 ± 3 (123)	36.4
Mg	1258 ± 116	1620 ± 20 (120)	3.08	560 ± 20	513 ± 3 (92)	2.32
Mn	62 ± 4	67.0 ± 0.6 (109)	1.24	20 ± 2	16.86 ± 0.07 (85)	1.57
Na	117 ± 28	82 ± 3 (70)	1.24	<LOD	<LOD	NA
P	3037 ± 184	3631 ± 28 (119)	3.19	1530 ± 80	1454 ± 15 (96)	0.93
Zn	19 ± 3	23 ± 1 (119)	1.26	19.4 ± 0.5	16.66 ± 0.6 (86)	3.51

NA: Not applicable.

Sample analysis

After optimizing the analytical parameters for determination of macro, micro and toxic elements using argon-based plasma spectrochemical methods, fifteen samples of infant foods and supplements were analyzed (Tables VII and VIII). For toxic elements (Table VII), the concentration ranges determined in the samples were 0.026 to 0.14 mg kg^{-1} for As and 0.029 to 0.097 mg kg^{-1} for Sn. For Cd, only two samples (A6 and A7 contained 0.002 and 0.009 mg kg^{-1} of Cd, respectively) contained concentrations higher than the respective LOQ. For Pb, only one sample (A6, 0.07 mg kg^{-1}) was higher than the LOQ established for Pb. However, all determined concentrations for these elements were lower than the limits proposed by RDC no. 193 [11], except to Pb for sample A6.

Table VII. Determination of As, Cd, Sn and Pb in samples of infant foods and supplements (mean ± standard deviation, mg kg^{-1}, n = 3) using ICP-MS

Sample	$^{75}As^{+}$	$^{114}Cd^{+}$	$^{120}Sn^{+}$	$^{208}Pb^{+}$
A1	0.14 ± 0.02	<0.0020	<0.025	<0.031
A2	<0.013	<0.0020	<0.025	<0.031
A3	0.1333 ± 0.0005	<0.0020	<0.025	<0.031
A4	<0.013	<0.0020	<0.025	<0.031
A5	<0.013	<0.0020	0.03 ± 0.01	<0.031
A6	<0.013	0.002 ± 0.001	<0.025	0.07 ± 0.03
A7	0.09 ± 0.01	0.009 ± 0.001	<0.025	<0.031
A8	<0.013	<0.0020	<0.025	<0.031
A9	<0.013	<0.0020	0.097 ± 0.002	<0.031
A10	<0.013	<0.0020	<0.025	<0.031
A11	<0.013	<0.0020	<0.025	<0.031
A12	<0.013	<0.0020	0.060 ± 0.005	<0.031

Table VII. Determination of As, Cd, Sn and Pb in samples of infant foods and supplements (mean ± standard deviation, mg kg^{-1}, n = 3) using ICP-MS (Continuation)

Sample	$^{75}As^{+}$	$^{114}Cd^{+}$	$^{120}Sn^{+}$	$^{208}Pb^{+}$
A13	0.027 ± 0.002	<0.0020	<0.025	<0.031
A14	0.026 ± 0.007	<0.0020	<0.025	<0.031
A15	<0.013	<0.0020	0.085 ± 0.009	<0.031

Table VIII. Determination of Ca, Fe, K, Mg, Mn, Na, P and Zn in samples of infant foods and supplements (mean ± standard deviation, mg kg^{-1}, n = 3) using ICP OES

Sample	Ca	Fe	K	Mg	Mn	Na	P	Zn
A1	2577 ± 21	254 ± 5	922 ± 15	175 ± 3	7.5 ± 0.2	1210 ± 12	1833 ± 11	107 ± 6
A2	167 ± 7	109 ± 5	232 ± 8	23 ± 2	1.16 ± 0.05	87.5 ± 0.4	376 ± 17	52 ± 2
A3	41 ± 2	208 ± 3	1134 ± 79	238 ± 7	9.8 ± 0.2	84 ± 2	1228 ± 35	115.5 ± 0.2
A4	2492 ± 36	130 ± 2	5414 ± 153	425 ± 7	73 ± 0.07	854 ± 20	2677 ± 36	36 ± 1
A5	4559 ± 118	56 ± 1	7534 ± 219	343 ± 12	0.94 ± 0.02	1147 ± 41	2713 ± 82	46 ± 2
A6	2424 ± 55	118 ± 4	5915 ± 18	522 ± 9	10.3 ± 0.4	808 ± 11	3004 ± 28	40 ± 1
A7	3663 ± 81	302 ± 15	1610 ± 25	265 ± 10	11.0 ± 0.2	2112 ± 29	3015 ± 74	141 ± 6
A8	2635 ± 79	268 ± 14	997 ± 30	60 ± 2	1.49 ± 0.07	1681 ± 62	1820 ± 57	129 ± 29
A9	5771 ± 107	73 ± 1	11882 ± 474	624 ± 8	0.31 ± 0.02	1862 ± 13	3923 ± 62	49.6 ± 0.4
A10	2456 ± 33	344 ± 11	1539 ± 21	220 ± 3	5.6 ± 0.1	1211 ± 16	1927 ± 16	179 ± 38
A11	2909 ± 80	187 ± 9	3244 ± 62	575 ± 37	22 ± 2	991 ± 34	2511 ± 89	108 ± 9
A12	6000 ± 156	77 ± 2	10252 ± 234	584 ± 19	0.40 ± 0.01	1593 ± 44	3653 ± 86	52 ± 1
A13	8726 ± 184	3.6 ± 0.4	19164 ± 246	771 ± 19	0.080 ± 0.009	2772 ± 77	7699 ± 164	29 ± 1
A14	13475 ± 212	36 ± 11	18818 ± 548	784 ± 21	1.0 ± 0.2	2680 ± 63	8073 ± 181	94 ± 3
A15	2854 ± 180	42 ± 2	7767 ± 638	527 ± 31	0.69 ± 0.05	1128 ± 56	1769 ± 108	46 ± 3

Higher concentrations of As were observed for rice-based food samples (A1, A3 and A7), which can be correlated with the known accumulation of As in rice. Unlike other cereals, rice is generally grown in flooded soils (anaerobic conditions with excess water) that provide mobilization of As and, consequently, a high accumulation of this element in the plant [28]. On the other hand, high Sn concentrations were observed for samples A5, A9, A12 and A15, all classified as infant formula. This behavior can be correlated with the metallic packaging of these formulations, known as one of the main sources of contamination of inorganic Sn [7].

For determination of macro and microelements (Table VIII), the concentration ranges determined in infant foods and supplements samples were 41 to 13475 mg kg^{-1} for Ca; 3.6 to 344 mg kg^{-1} for Fe; 232 to 19164 mg kg^{-1} for K; 23 to 784 mg kg^{-1} for Mg; 0.080 to 11.0 mg kg^{-1} for Mn; 84 to 2772 mg kg^{-1} for Na; 376 to 8073 mg kg^{-1} for P, and 29 to 179 mg kg^{-1} for Zn.

According to the recommended daily intakes (RDI) from RDC no. 269 [12] and the average concentrations of Ca, Fe, K, Mg, Mn, P and Zn in samples, it is possible to assign a minimum mass value of food that meets the recommendations of the Brazilian resolution for different population groups. To do so, the average concentrations of the analytes determined in the samples were grouped according to children's food indication and then a mean value was calculated for each element. Table IX shows the recommended daily intake (RDI), minimum mass of food that meets the RDI for each analyte according to the population group (infants and children) considering the mean concentrations calculated based on indication. It was observed that the intervals of minimum mass of food for infants from 0 to 6 months, infants from 7 to 11 months, children from 1 to 3 years old and children from 4 to 6 years old, are respectively from: 4 to 83 g, 38 to 161 g, 9 to 559 g, and 37 to 609 g, respectively. Considering a 5 g portion of sample, it was possible to calculate an average percentage of recommended daily intake (%RDI) for the foods indicated for infants from 0 to 6 months of 6% for Ca; 91% for Fe; 10% for K; 6% for Mg; 136% for Mn; 11% for P; and 8% for Zn. For a 30 g portion of sample, it was also possible to calculate an average %RDI for the foods indicated for infants from 7 to 11 months, children from 1 to 3 years old and children from 4 to 6 years old ranged from 21 to 28% for Ca; 72 to 89% for Fe; 5 to 19% for K; 17 to 20% for Mg; 22 to 35% for Mn; 19 to 29% for P; and 51 to 80% for Zn. These results evidence the high nutritional potential of these foods.

Table IX. Mean concentration, recommended daily intake (RDI), minimum mass of food (g) and average percentage of recommended daily intake for infants (0-6 months and 7-11 months) and children (0-3 years and 4-6 years) according to the chemical element

Element	Mean concentration (mg kg^{-1})				RDI[a] (mg day^{-1})			
	0-6 months	7-11 months	1-3 years	4-6 years	0-6 months	7-11 months	1-3 years	4-6 years
Ca	3707	3716	3779	4103	300	400	500	600
Fe	49	215	178	162	0,27	9	6	6
K	7651	4349	5363	6240	400	700	3000	3800
Mg	435	358	369	405	36	53	60	73
Mn	1	7	13	11	0.003	0.6	1.2	1.5
Na	1138	1523	1317	1380	N/A	N/A	N/A	N/A
P	2241	2669	3106	3211	100	275	460	500
Zn	46	109	94	87	2.8	4.1	4.1	5.1
Element	**Minimum mass of food (g)**				**%RDI**			
	0-6 months	7-11 months	1-3 years	4-6 years	0-6 months	7-11 months	1-3 years	4-6 years
Ca	81	108	132	146	6	28	23	21
Fe	6	42	34	37	90	72	89	81
K	52	161	559	609	10	19	5	5
Mg	83	148	163	180	6	20	18	17
Mn	4	87	9	136	135	34	33	22
Na	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
P	45	103	148	156	11	29	20	19
Zn	61	38	44	59	9	80	69	51

[a]Resolution no. 269, of September 22, 2005 [12].

Dairy products, such as milk, yogurt and cheese, are the most Ca-rich foods in Western diets. Although grains are not particularly rich in calcium, the use of calcium-containing additives in these foods accounts for a substantial proportion of the calcium ingested by people who consume large amounts of grains [29]. In the analyzed samples, Ca occurred in higher quantities in samples A13 and A14: 8726 and 13475 mg/kg, respectively.

Manganese presents the lowest concentrations determined for all samples. Manganese (Mn) is an essential element, but it can also be toxic when in high concentrations. The essentiality of Mn is reflected in national and international policies on formulas and foods for children, which stipulate minimum concentrations of Mn. Infant formula regulations have not yet been adjusted to reflect this growing body of research on neurotoxicity of Mn; while the maximum content of Mn is regulated for baby formulas in some jurisdictions, others do not establish a maximum content for Mn in formulas for children and babies [30].

Samples A1, A7, A8, A10 and A11 had the highest concentrations of Fe. As bovine milk is a poor source of bioavailable Fe, its use is not recommended for babies under 1 year. Inadequate early intake of cow's milk is associated with an increased risk of Fe deficiency anemia. Pediatric societies have concluded that babies who are not or are only partially fed human milk should receive an Fe-enriched formula. Supplementation is also recommended for premature babies, as their Fe stores are low [30].

Comparison with other methods

Due to the importance of monitoring elemental concentrations in infant foods, procedures for determination of macro, micro and toxic elements in children's food have been developed [31-33] Table X shows a comparison of different procedures for those determinations, highlighting a similar type of sample, cereal (porridge) rice-based, and studied elements among the cited papers [31-33].

Table X. Some procedures for determination of macro, micro and toxic elements in infant food samples

Type of children's food	Method	Element	Calibration strategy	Sample preparation	Concentration range in cereal rice-based (mg kg^{-1})	Reference
Infant food rice-based	ICP-MS	As, Ca, Cd, Fe, K, Mg, Mn, Na, Pb, Sn, Zn	No calibration strategy evaluated	0.3-0.5 g sample mass. Microwave digestion (concentrated HNO_3) and dilution with distilled-deionized water	As: 0.08 ± 0.02 Ca: 4454 ± 1797 Cd: 0.007 ± 0.005 Fe: 65 ± 32 K: 4024 ± 809 Mg: 535 ± 104 Mn: 0.9 ± 0.1 Na: 2756 ± 758 Pb: 0.18 ± 0.02 Sn: 0.05 ± 0.04 Zn: 35 ± 9	[31]

Table X. Some procedures for determination of macro, micro and toxic elements in infant food samples (Continuation)

Type of children's food	Method	Element	Calibration strategy	Sample preparation	Concentration range in cereal rice-based (mg kg^{-1})	Reference
Infant formula and infant food	ICP-MS	As, Ca, Cd, Fe, Mg, Mn, Pb and Zn	No calibration strategy evaluated	1 g sample mass. Microwave-assisted digestion (2 mL 65% HNO_3 + 3 mL deionized water) and dilution with 20% HNO_3	As[a]: 33.0 ± 0.6 Ca: 38.0 ± 0.6 Cd[a]: 1.70 ± 0.04 Fe: 1.2 ± 0.6 Mg: 127 ± 1 Mn: 3.16 ± 0.01 Pb[a]: 1.2 ± 0.1 Zn: 1.50 ± 0.01	[32]
Infant food and other rice-based products	ICP-MS	As, Cd, Fe, Mn, Pb and Zn	Use of IS (In, Y, Ge, Sc) but without prior analysis	0.15 g sample mass. Digestion block (1.5 mL of 65% HNO_3 + 1 mL of H_2O_2 30%) and dilution with distilled-deionized water	As: 0.10 ± 0.04 Cd: 0.012 ± 0.007 Fe: 20 ± 94 Mn: 11 ± 4 Pb: 0.03 ± 0.02 Zn: 85 ± 46	[33]
Infant food, infant formula and children's supplements	ICP-MS and ICP OES	As, Cd, Sn, Pb, Ca, Fe, K, Mg, Mn, Na, P and Zn	Use of IS (Y, Ge, Pd) with prior analysis	0.5 g sample mass. Microwave-assisted digestion (5 mL of 7 mol L^{-1} HNO_3) and dilution with distilled-deionized water	As: 0.09 ± 0.01 Cd: 0.009 ± 0.001 Pb: <0.031 Sn: < 0.025 Ca: 3663 ± 81 Fe: 302 ± 15 K: 1610 ± 25 Mg: 265 ± 10 Mn: 11.0 ± 0.2 Na: 2112 ± 29 P: 3015 ± 74 Zn: 141 ± 6	This study

[a]Concentration in (μg kg^{-1}).

Similarly to the proposed procedure in reference [32], the procedure here developed also is based on a less concentrated nitric acid solution. According to these studies, concentrations of toxic elements (As, Cd and Pb) in cereal-based samples do not pose a risk to this consumer group, despite highlighting the importance of monitoring these elements to prevent health problems. This conclusion is also true to the samples here analyzed.

CONCLUSIONS

In this study, a tailored microwave-assisted sample preparation for supplements and infant foods using only 7 mol L^{-1} nitric acid solution was developed. Precise and accurate determinations of As, Cd, Sn and Pb were performed using ICP-MS and Ca, Fe, K, Mg, Mn, Na, P and Zn using ICP OES. ICP-MS presents wide range of concentrations, however, thinking about laboratories that do not have the option of performing

analyzes by ICP-MS, since it is a more expensive instrumental method, we also evaluated the ICP OES performance. ICP OES can be considered a suitable analytical method for applying the Resolution no. 269 since it is less expensive and contamination issues during sample preparation are less critical when compared to ICP-MS. For ICP-MS, the combination of instrumental strategies based on aerosol dilution and CCT as well as the use of the calibration strategy with internal standardization, allowed minimum dilution of digests and the introduction of digests with TDS of up to 0.5% m/v and residual acidity of up to 1% v/v. Considering the limits for As, Cd, Sn and Pb recommended by RDC no. 193 and the RDI for Ca, Fe, K, Mg, Mn, P and Zn recommended by RDC no. 269, the developed analytical procedure using ICP-MS and ICP OES presented sufficient detectability to meet both Brazilian resolutions.

Acknowledgments

The authors are grateful to the "Programa Institucional de Bolsas de Iniciação Científica", CNPq, UFSCar (PIBIC, CNPq, UFSCar) and the "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq, 128923/2019-9, 141634/2017-0, 305201/2018-2 and 428558/2018-6) for fellowships and financial support. This study was financed in part by the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil" (CAPES) – Finance Code 001. Instrumental support provided by Agilent Technologies (São Paulo, SP, Brazil), Nova Analítica / Thermo Scientific (São Paulo, SP, Brazil), and Milestone (Sorisole, BG, Italy) is gratefully acknowledged. The authors also would like to express their gratitude to the "Instituto Nacional de Ciências e Tecnologias Analíticas Avançadas" (CNPq, Grant No. 573894/2008-6 and FAPESP, Grant No. 2014/50951-4) and to "Embrapa Pecuária Sudeste" - National Bank for Economic and Social Development (BNDES - 2117020010607).

Conflicts of interest

All authors declared that they have no conflict of interest.

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Brazilian Journal of Analytical Chemistry
2021, Volume 8, Issue 31, pp 89–102
doi: 10.30744/brjac.2179-3425.AR-56-2020

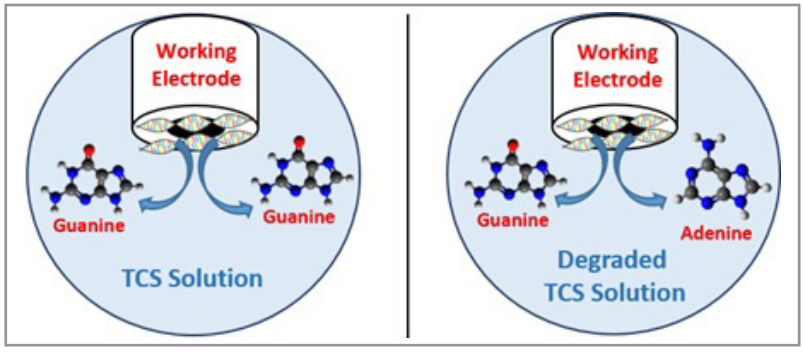
BrJAC

ARTICLE

Triclosan: Electrochemistry, Spontaneous Degradation and Effects on Double-Stranded DNA

Elizaura Hyeda Carvalho Silva[1], Ilanna Campelo Lopes[1*], Evellin Enny Silva Bruzaca[1], Paulina Andréa Viana de Carvalho[1,2], Auro Atsushi Tanaka[1,3*]

[1]Departamento de Química, Centro de Ciências Exatas e Tecnologia, Universidade Federal do Maranhão, CEP 65080-805, São Luís, MA, Brazil
[2]Instituto Federal de Educação, Ciência e Tecnologia do Maranhão, CEP 65363-000, Zé Doca, MA, Brazil
[3]Instituto Nacional de Ciência e Tecnologia de Bioanalítica, Caixa Postal 6154, CEP 13083-970, Campinas, SP, Brazil



Triclosan (TCS) is an antiseptic agent widely used mainly in personal care products and an important contaminant, which degrades in the environment causing toxicity on health, including negative effects on DNA. In this context, an electrochemical investigation of TCS in aqueous solution was studied by voltammetric techniques. The TCS underwent irreversible oxidation in a pH-dependent process, leading to the formation of two reversibly oxidized and pH-dependent oxidation products. An oxidation mechanism for TCS and its oxidation products in neutral aqueous medium was proposed. Besides that, the TCS spontaneously degraded into supporting eletrolytes with 3.4 ≤ pH ≤ 12.04 over the incubation time and the degraded TCS in solution was detected by electrochemical and spectrophotometric techniques. A higher degradation of TCS was observed in alkaline medium. In addition, the interaction in situ of this anti-microbial with DNA was investigated using dsDNA incubated solutions and dsDNA electrochemical biosensor, by voltammetry. TCS and degraded TCS interacted with dsDNA causing the condensation of the double helix structure, release of guanine (by TCS and degraded TCS) and adenine (by degraded TCS) bases from dsDNA and a possible intercalation of degraded TCS in the polynucleotide chain. No dsDNA oxidative damage was detected.

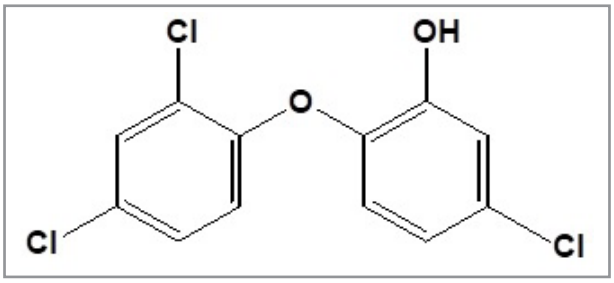
Keywords: Triclosan; Contaminant degradation; Electrochemical oxidation; DNA interaction studies.

Cite: Silva, E. H. C.; Lopes, I. C.; Bruzaca, E. E. S.; de Carvalho, P. A. V.; Tanaka, A. A. Triclosan: Electrochemistry, Spontaneous Degradation and Effects on Double-Stranded DNA. *Braz. J. Anal. Chem.,* 2021, *8* (31), pp 89–102. doi: http://dx.doi.org/10.30744/brjac.2179-3425.AR-56-2020

Received 7 October 2020, Revised 3 December 2020, 2nd time Revised 20 January 2021, Accepted 20 January 2021, Available online 08 March 2021.

INTRODUCTION

Pharmaceutical and personal care products have gained great attention in the last decades due to their persistent character in the environment [1]. Triclosan (TCS, 5-chloro-2-(2,4-dichlorophenoxy)-phenol) (Scheme 1) [2], a common contaminant in the environment [3], is a synthetic, lipid-soluble and broad spectrum antibacterial agent widely [4] used as addition ingredient in personal care products, medical, household, veterinary, and daily consumer products [5]. Among these, the chemical can be found in numerous professional and consumer goods [1] such as toothpaste and soaps [6], deodorants and cosmetics [7], kitchenware and plastic food containers [8].



Scheme 1. Chemical structure of TCS.

TCS is quite harmful to the water environment as it has chronic toxicity [9]. It is known to undergo several types of partitioning and degradation process in the aquatic environment, including direct photochemical degradation [10]. When degraded, TCS can produce derivatives of great toxicity such as chlorophenols and dioxins, which are highly toxic to aquatic organisms (fish, algae, bacteria and protozoa) [11–13]. Furthermore, previous reports related that chloroform, extremely toxic intermediate product generated by TCS, was found in the free-chlorine-mediated oxidation of TCS drinking water [14]. It has also been reported that this pollutant can exert estrogenic and androgenic effects on the human body [15] and adverse effects on endocrine function in animals [16]. In the last few decades, it has been shown that TCS is biologically linked to antibiotics, leading to the possibility of inducing resistance to these drugs [17,18]. Evidence also pointed that exposure to an extreme amount of TCS can cause skin irritation, immunotoxic and neurotoxic reactions in humans [19].

The great concern on the toxicity of TCS and its metabolites on the environment and human health has led several researchers to study the negative effect of this biocide on DNA. TCS was recognised as a potential carcinogen by the United States Environmental Protection Agency (US EPA) in 2008 [20]. Degradation products from TCS are also known to be toxic and carcinogenic such as dioxin photoproducts [21] and chloroform which is classified by the US EPA as a probable human carcinogen [22].

Although there are several studies related to exposure to TCS and the adverse effects on human and environmental health, there is still a peculiar concern related to its harmful effects, mainly in relation to its carcinogenic potential, due to the lack of knowledge about the underlying mechanisms of these effects. TCS was exposed to HaCaT cells, an immortalized keratinocyte cell line from human skin, and evidenced TCS-induced genomic damage in human keratinocytes, leading to disturbances in proliferation rates [23]. When evaluating levels of global DNA methylation (GDM) in human hepatocytes, the results indicated that TCS reduced the levels of GDM and down-regulated the methylated DNA-binding domain proteins (MBD2 and MBD3), and MeCP2 gene expression by increasing 8-OHdG (8-hydroxy-2-deoxyguanosine) levels and inhibiting the mammalian DNA methyltransferase (DNMT1) activity in human hepatocellular carcinoma cells line (HepG2 cells) [24]. In addition, an investigation study of the association between TCS exposure and male fertility shown that this exposure can increase the percentage of sperm with abnormal morphology [25]. TCS is also associated with the development of liver tumours in rodents [26]. Additionally, this common biocide altered DNA methylation in zebrafish exposed during embryogenesis as well as related genes expression [27] and reduced significantly the reproduction of earthworms *Eisenia*

fetida exposed to TCS, causing DNA damage and increase of expression levels of the Hsp70 gene of earthworms [28].

In view of the problem of the potentially toxic effects of TCS, several studies have been presented in the literature, using different methodologies for detection and determination of this compound [29–32]. Studies on degradation of TCS have also been reported as well as the investigation of the behaviour of antimicrobial agent in DNA. However, a complete methodology on electrochemical behaviour of TCS and its spontaneous degradation in aqueous solution has not been explored. Moreover, DNA based electrochemical biosensors became a very viable alternative for evaluating the interaction of this compound with DNA. They have been successfully utilized to investigate the interaction of small molecules with DNA and, compared to other methods, these sensors offer high sensitivity in the detection of small perturbations in the double helix chain and understanding the mechanisms of oxidative damage to DNA [33]. Our research group has used these biosensors in some previous studies to investigate the in situ interaction of anticancer drugs with DNA [34,35].

Thus, the aims of this work were (i) to study the electrochemical behaviour of TCS in aqueous solution by voltammetry (ii) to monitor the chemical degradation process of this antimicrobial agent in aqueous medium by voltammetric and spectrophotometric techniques and (iii) to investigate the interaction of TCS with DNA using DNA electrochemical biosensor and DNA incubated solutions.

MATERIALS AND METHODS

Reagents and solutions

TCS (Irgasan, 5-chloro-2-(2,4-dichlorophenoxy)phenol, ≥ 97.0% HPLC, CAS number: 3380-34-5), deoxyribonucleic acid sodium salt from calf thymus (dsDNA, type I, fibers , CAS number: 73049-39-5), polyguanylic acid potassium salt (poly[G], lyophilized powder, CAS number: 54684-83-2) and polyadenylic acid potassium salt (poly[A], CAS number: 26763-19-9) were purchased from Sigma-Aldrich.

Stock solutions of 0.1 mM TCS were prepared in NaOH-H_2O (25:75, v/v) and 340 μg mL^{-1} dsDNA was prepared in deionized water. Real concentration of dsDNA was calculated by the multiplication of the experimentally UV-Vis spectrophotometric absorbance found by the conversion factor (1u $A_{260\ nm}$ = 50 μg mL^{-1} of dsDNA) [36]. Stock TCS solutions were kept wrapped in aluminium foil to protect them from the light. Both stock solutions were stored in the fridge (4 °C) until further utilization.

The supporting electrolyte solutions of different pH values used in the experiments were prepared according to the literature [37]: CH_3COOH/CH_3COONa (pH 3.4 to 5.4), Na_2HPO_4/NaH_2PO_4 (pH 6.1 to 8.1), $Na_2B_4O_7$/NaOH (pH 9.2 to 10.2), Na_2HPO_4/NaOH (pH 11.2) and KCl/NaOH (pH 12.0). The pH measurements were carried out using a Metrohm 827 pH Lab pH-meter (Switzerland) with a Metrohm combined glass electrode.

All solutions were prepared using reagent-grade chemicals and purified water from a Millipore Milli-Q system (resistivity 18.2 MΩ cm, conductivity ≤ 0.1 μS cm^{-1}).

Procedures

Electrochemical measurements

Voltammetric experiments were performed using an Autolab PGSTAT 128N potentiostat in combination with GPES 4.9 Software (Eco Chemie B. V., Utrecht, The Netherlands). Cyclic voltammetry (CV), differential pulse (DP) and square wave (SW) voltammetry measurements were carried out using a glassy carbon electrode (GCE, d = 5.0 mm) as working electrode, a Pt wire counter electrode and an Ag/AgCl (3 M KCl) electrode as reference, in a one-compartment electrochemical cell of 10 mL capacity.

The GCE was polished using diamond spray (particle size 1 μm, Kemet International Ltd, UK) before every electrochemical assay. After polishing, the electrode was rinsed thoroughly with Milli-Q water. Following this mechanical treatment, the GCE was placed in buffer supporting electrolyte and various voltammograms were recorded until a steady state baseline voltammogram was obtained.

Buffer solutions were bubbled with high purity N_2 (White Martins) for 10 minutes before CV measurements.

DP voltammograms presented were baseline-corrected using the moving average application with a step window of 2 mV included in the GPES version 4.9 software. This mathematical treatment improves the visualization and identification of peaks over the baseline without introducing any artefact. Nevertheless, this mathematical treatment of the original voltammograms was used in the presentation of all experimental voltammograms for a better and clearer identification of the peaks.

Spectrophotometric measurements

Spectrophotometric measurements were fulfilled in a spectrophotometer UV-Vis Varian Cary 50 (running with Cary Win UV software). Spectra were measured in a quartz glass cuvette (optical path of 1 cm) from 200 to 400 nm.

TCS Degradation in solution

The evaluation of TCS degradation in aqueous solution was studied spontaneously at different time periods at room temperature, by voltammetric and spectrophotometric measurements. For the first technique, 0.01 mM TCS solutions (prepared in supporting electrolytes with $3.4 \leq pH \leq 12.04$ and left to degrade chemically) were incubated for 24 h, 30 and 64 days and then analysed. 0.0625 mM TCS in buffer solutions ($3.4 \leq pH \leq 9.20$) were left to degrade for 30, 64 and 70 days and after measured by UV-Vis spectrophotometry. After each analyse, for both techniques, degraded TCS solutions were transferred to glass flasks and stored until further utilization.

TCS-DNA Interaction studies

The interaction of DNA with TCS and degraded TCS was explored using dsDNA, poly[G] or poly[A] incubated solutions and dsDNA biosensor, and performed by DP voltammetry.

Incubated solutions - Solutions of 100 μg mL^{-1} dsDNA, poly[G] or poly[A] with 5 μM TCS or degraded TCS were prepared and incubated in 0.1 M acetate buffer pH 4.5 at different time period: 0, 24, 48 and 72 h (TCS-dsDNA; TCS-poly[G]; TCS-poly[A]) and 0, 24, 48, 72 e 96 h (degraded TCS-dsDNA; degraded TCS-poly[G]; degraded TCS-poly[A]). The degraded TCS solution was left to degrade in 0.1 M acetate buffer pH 4.5 for 40 days. DP voltammograms were recorded in the solutions above described after each incubation period. Control solutions of dsDNA, poly[G] or poly[A] in 0.1 M acetate buffer pH 4.5 were also made and analysed under similar conditions as TCS and degraded TCS incubated with dsDNA, poly[G] or poly[A] solutions.

dsDNA biosensor - The dsDNA biosensor was prepared by dropping consecutively three aliquots of 50 μL of 100 μgmL^{-1} dsDNA solution in the electrode surface. The biosensor was dried under a constant flux of N_2 and rinsed in deionised water. After that, it was immersed and left to incubate for 1, 24, 48, 72 and 96 h in 5 μM degraded TCS (95 days of degradation in buffer pH 4.5) in 0.1 M acetate buffer pH 4.5. For each incubation time a new dsDNA biosensor was prepared. Besides that, biosensors were washed with deionised water and were ready to be run in the supporting electrolyte 0.1 M acetate buffer pH 4.5. Others dsDNA biosensors were built under similar conditions (without incubations in degraded TCS) and subsequently measured in buffer solution as controls.

Control solutions and biosensors were made in order to certify that the changes observed in the voltammograms were only due to the interaction effects of the TCS and degraded TCS with dsDNA, poly[G] or poly[A].

These two procedures were adapted from the literature [35].

RESULTS AND DISCUSSION

TCS Electrochemical oxidation

Cyclic and square wave voltammetry

The electrochemical behaviour of TCS was first investigated by CV in a fresh solution of 0.01 mM TCS in 0.1 M phosphate buffer pH 7.0. Independently of the scan direction (oxidation and reduction) only one

well-defined anodic peak was observed, in agreement with previous reports [38-40]. Thus, the potential range was selected to positive values for further studies.

The cyclic voltammogram obtained in a fresh solution of 0.01 mM TCS in 0.1 M acetate solution pH 3.4 (Figure 1A) showed the TCS oxidation peak at E = + 0.82 V (peak T_{1a}). No one reverse peak was observed when reversing the scan direction. This shows that the oxidation process of TCS is irreversible [38–40]. In addition, during the first scan, two small reduction peaks appeared (peaks T_{2c} and T_{3c}) at E = + 0.35 V and E = + 0.23 V, respectively. These products were oxidized, on the second scan, at E = + 0.40 V and E = + 0.25 V (peaks T_{2a} and T_{3a},). Both peaks are related to the formation of two oxidation products of TCS, which undergo reversible redox process. In addition, there was a decrease of peak T_{1a} current due to the adsorption of TCS and/or its oxidation products. The similar behaviour was observed in all electrolytes with different pH values.

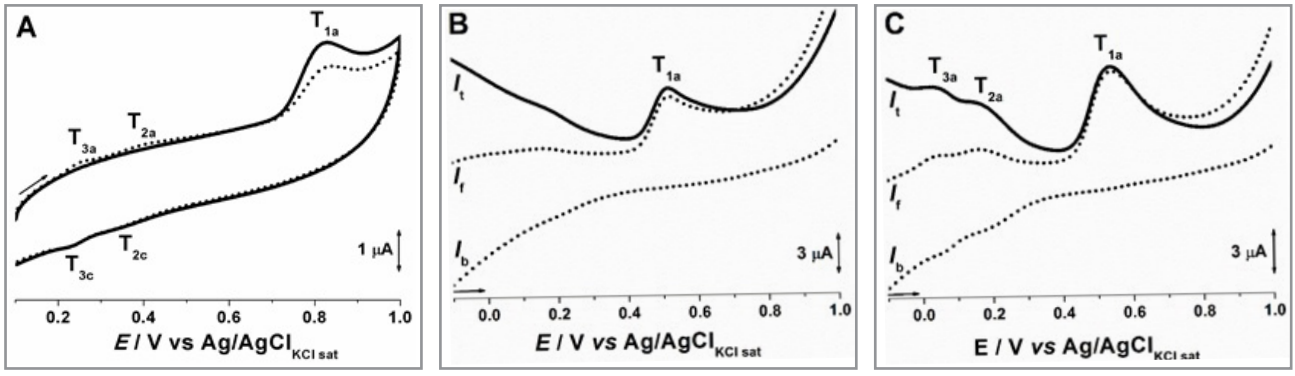


Figure 1. (A) Cyclic voltammograms in a fresh solution of 0.01 mM TCS in 0.1 M acetate solution pH 3.4: (—) 1st and (•••) 2nd scans; Step potential of 3 mV and scan rate of 50 mV s^{-1}. SW voltammograms in a fresh solution of 0.01 mM TCS in 0.1 M phosphate buffer pH 7.0: (B) 1st and C) 2nd scans; Frequency of 50 Hz, potential increment of 2 mV and pulse amplitude of 50 mV.

SW voltammetry experiments were also recorded in a fresh solution of 0.01 mM TCS over a wide pH range. Figure 1B showed the oxidation of the TCS on first scan at *E* = + 0.51 V (peak T_{1a}) in 0.1 M phosphate buffer pH 7.0. Furthermore, the forward component (I_f) presented one oxidation peak at the same potential and current intensity as the total current (I_t), while for the backward component (I_b) no cathodic peak was observed. Therefore, the irreversibility of this reaction was confirmed. On the second scan, the two oxidation peaks at *E* = + 0.17 V (peak T_{2a}) and *E* = + 0.04 V (peak T_{3a}) appeared as shown in Figure 1C. Products formed at T_{2a} and T_{3a} peaks are reversibly reduced, since oxidation and reduction peaks for each one - observed in the I_f and I_b - showed the same potentials and currents values. All three peaks presented the same behaviour seen by CV measurements.

Differential Pulse Voltammetry

The electrochemical behaviour of 0.01 mM TCS based on its effect of pH in several electrolytes was investigated by DP voltammetry. The voltammograms were obtained immediately after addition of TCS in each electrolyte. Figure 2A shows the voltammetric profile for TCS (peak T_{1a}) recorded in 3.4 ≤ pH ≤ 12.0. The dependence of the peak T_{1a} potential and current versus pH is presented in Figure 2B. The experiments obtained in pH ≤ 9.2 buffer solutions showed their oxidation potentials were shifted to more negative values with increasing pH. The slope of the dotted line of – 55 mV per pH unit for this peak shows that the mechanism of oxidation process involves the same number of electrons and protons [41]. The number of electrons (*n*) involved in the reaction was estimated using the equation 1 [42], where R = 8.314 J mol^{-1} K^{-1}, T = 298 K and F = 96500 C mol^{-1}.

$$W_{1/2} = 3.52RT/nF \quad \text{(Equation 1)}$$

The value of $W_{1/2}$ (peak width at half heigth of peak T_{1a}) have been found to be 126 mV. Thus, for this process the transfer of one electron and one proton occurred in its oxidation mechanism. In pH > 9.2 buffer solutions, the peak T_{1a} potential remained constant, indicating that the oxidation process is independent of the pH value and TCS and/or TCS oxidation products undergo chemical deprotonation after the determining step. Thus, the $pK_a \approx 9.2$ for TCS was estimated. Furthermore, the current intensity of the peak T_{1a} decreased with increasing pH, showing a maximum current in pH 3.4.

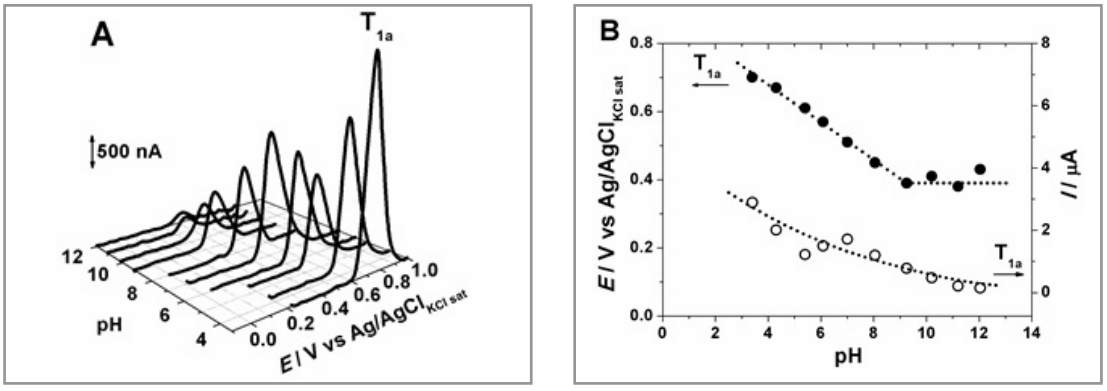


Figure 2. Baseline-corrected DP voltammograms obtained in a fresh solution of 0.01 mM TCS as a function of pH: A) Plot of 1st scan; B) Plot of (●) E_p and (○) I_p of peak T_{1a} vs. pH. Pulse amplitude of 50 mV, pulse width of 70 ms, step potential of 2.5 mV, interval of time of 500 ms and scan rate of 5 mV s^{-1}.

Consecutives voltammograms obtained in each supporting electrolyte showed a decrease in the peak T_{1a} current with the increase in the number of scans, and the peak potential shifted to more positive potential values. This behaviour indicates that the electroactive area on the electrode surface has been reduced due to the adsorption of the TCS and/or its oxidation products on it. Moreover, after the second scan two oxidation peaks (peaks T_{2a} and T_{3a}) appeared as in CV and SW voltammetry. On the third scan, both peaks T_{2a} and T_{3a} currents increased due to the formation of more oxidation products adsorbed on the electrode surface, as illustrated in Figure 3 for TCS in 0.1 M acetate buffer pH 5.4 solution.

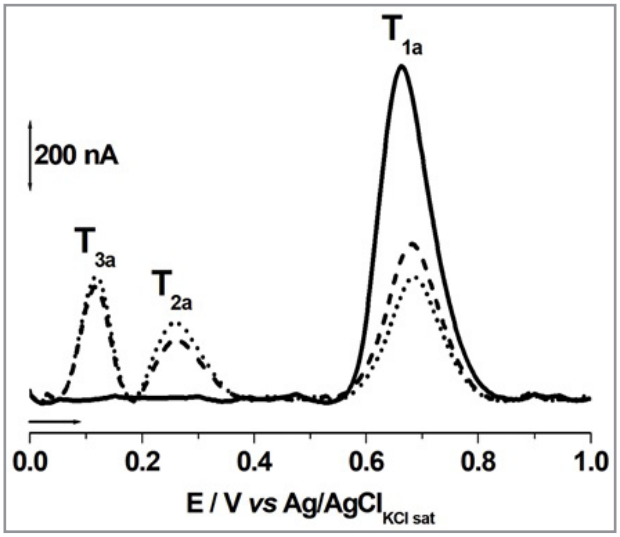


Figure 3. Baseline-corrected DP voltammograms obtained in a fresh solution of 0.01 mM TCS in 0.1 M acetate buffer pH 5.4: (▬) 1st, (▪▪▪) 2nd and (•••) 3rd scans. Pulse amplitude of 50 mV, pulse width of 70 ms, step potential of 2.5 mV, interval of time of 500 ms and scan rate of 5 mV s^{-1}.

The effect of pH on the oxidation potential of peaks T_{2a} and T_{3a} were performed for the second scan in all supporting electrolytes (Figure 4). For 3.4 ≤ pH ≤ 9.2 the potential of both peaks was dependent of pH, i.e., shifted to less positive values with increasing pH (Figure 4A). The slope of the dotted line of 62 mV (peak T_{2a}) and 56 mV (peak T_{3a}) show that both oxidation processes involve the transfer of the same number of electrons and protons. Since the values of $W_{1/2}$ = 57 mV for peak T_{2a} and $W_{1/2}$ = 59 mV for peak T_{3a} the mechanisms of each process take the loss of two electrons and two protons. The intensity of peaks current versus pH presents a maximum of current in pH 3.4 (peak T_{2a}) and pH 7.0 (peak T_{3a}) (Figure 4B).

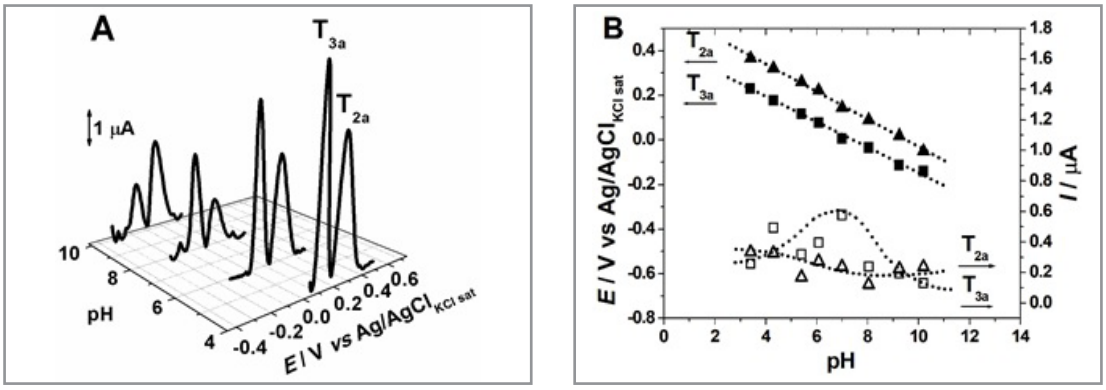
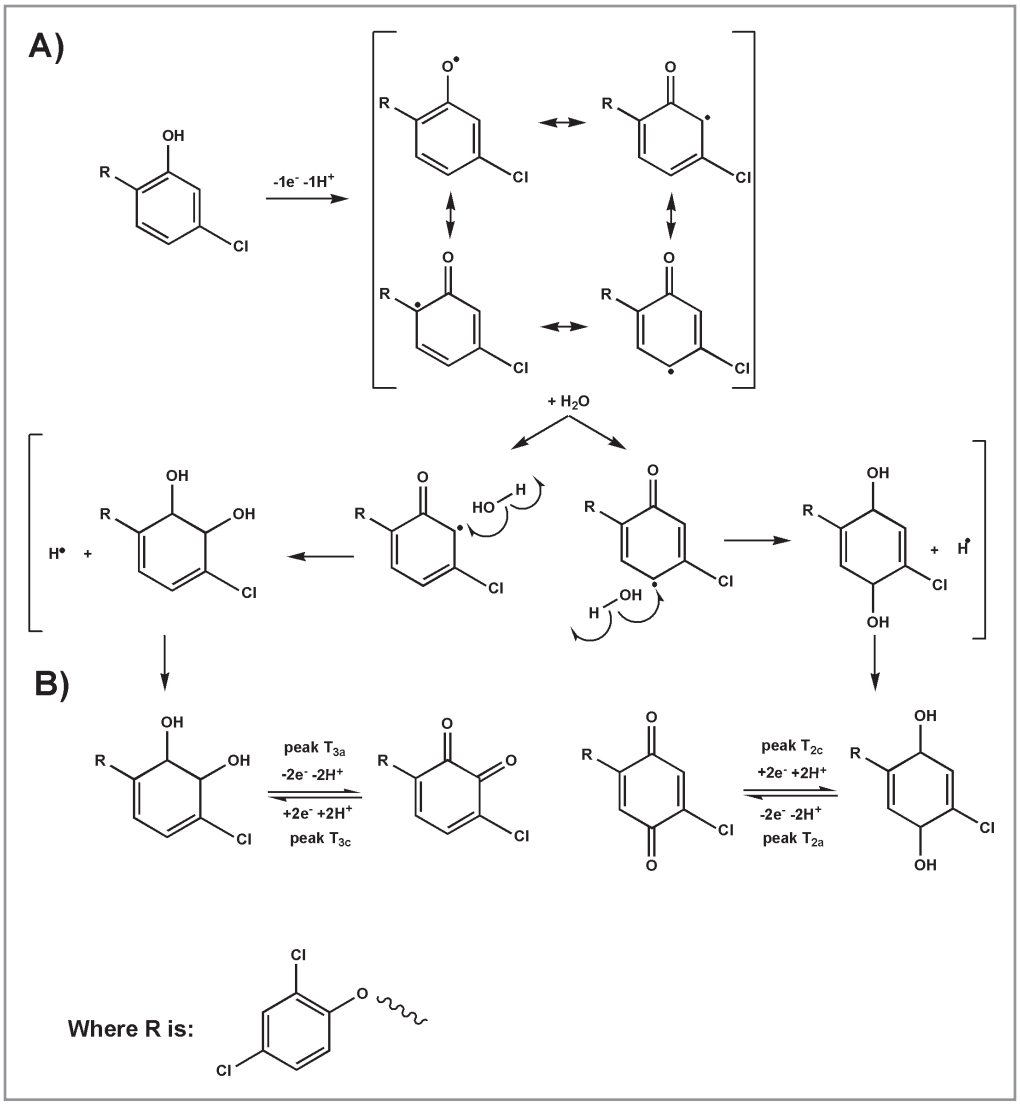


Figure 4. Baseline-corrected DP voltammograms obtained in a fresh solution of 0.01 mM TCS as a function of pH: A) Plot of 2nd scan; B) Plot of E_p of peaks (▲) T_{2a} and (■) T_{3a} and I_p of peaks (∆) T_{2a} and (□) T_{3a} vs. pH. Pulse amplitude of 50 mV, pulse width of 70 ms, step potential of 2.5 mV, interval of time of 500 ms and scan rate of 5 mV s^{-1}.

Oxidation mechanism of TCS and its oxidation products

Based on information obtained from previous results, a mechanism of oxidation of TCS and its oxidation products in neutral aqueous solution was proposed (Scheme 2).

As the oxidation of TCS, peak T_{1a}, involves the transfer of one electron and one proton, the reaction occurs in the phenolic hydroxyl group of TCS, yielding a phenoxy radical [38,40,43–45], which is stabilized by resonance (Scheme 2A). Among the resonance hybrids, two most stable forms are probably attacked by water through a chemical oxidation reaction, where a hydroxyl group is incorporated into each intermediate, forming two reversible oxidation products of TCS: 2-chloro-5-(2,4-dichlorophenoxy)-[1,2]-benzoquinone (peak T_{3a}) / 2-chloro-5-(2,4-dichlorophenoxy)benzene-1,2-diol (peak T_{3c}); and 2-chloro-5-(2,4-dichlorophenoxy)-[1,4]benzoquinone (peak T_{2a}) / 2-chloro-5-(2,4-dichlorophenoxy)benzene-1,4-diol (peak T_{2c}), in two electrons and two protons reaction each (Scheme 2B). The redox products (peaks T_{2a}-T_{2c}) were also detected as oxidation products of TCS using GC/MS and RRLC-MS/MS techniques [43,44].



Scheme 2. Proposed mechanism for oxidation of TCS (A) and its oxidation products (B) in neutral aqueous solution.

TCS Degradation process

Voltammetry analysis

The TCS degradation was first investigated by DP voltammetry in order to investigate its chemical degradation in aqueous solution. The degradation process of TCS was observed due to changes in the voltammetric profiles of TCS over the incubation time.

Firstly, the oxidation behaviour of TCS in a fresh solution of 0.01 mM TCS in 0.1 M acetate solution pH 3.4 was investigated. Voltammograms recorded showed the peak T_{1a} at E = + 0.69 V (Figure 5A). After 24 h of incubation time, the intensity of this peak current decreased considerably when compared with the peak current for TCS obtained previously. After 30 and 60 days in a buffer solution, the decrease in peak T_{1a} current was proportional to the incubation time. This behaviour corresponds to the decrease in the concentration of TCS in solution and it is attributed to the spontaneous degradation of this antimicrobial compound [10,46,47]. No one further peak was observed, indicating that the TCS degradation product(s) formed in solution were not electroactive under conditions studied.

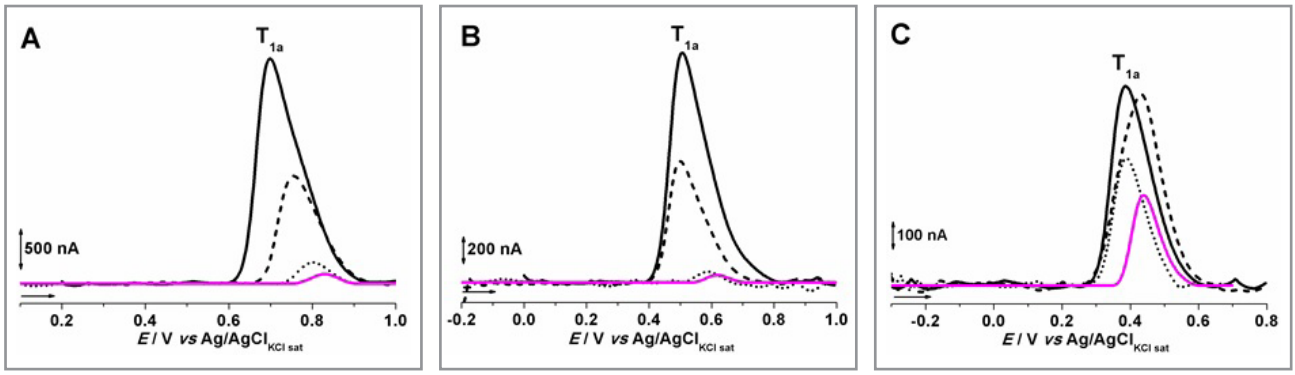


Figure 5. Baseline-corrected DP voltammograms obtained in a fresh solution of 0.01 mM TCS (▬) and after 24 h (▪▪▪), 30 (•••) e 64 days (▬) of incubation in: (A) acetate solution pH 3.4; (B) phosphate buffer pH 7.0 and (C) borax buffer pH 9.2. Pulse amplitude of 50 mV, pulse width of 70 ms, step potential of 2.5 mV, interval of time of 500 ms and scan rate of 5 mV s^{-1}.

The behaviour for TCS described above was observed in all supporting electrolytes investigated. Figure 5B and C presented the voltammograms obtained in a neutral and basic medium, respectively.

UV-Vis Spectrophotometry analysis

The TCS degradation in aqueous solution was also investigated by UV-Vis spectrophotometry. The spectrum registered in a fresh solution of 0.0625 mM TCS in 0.1 M acetate solution pH 3.4 showed one absorption band with a maximum at λ = 280 nm (Figure 6A) in agreement with the literature [46]. A progressive decrease of absorbance was observed after longer incubation times (30 and 64 days), and no other band appeared. After 70 days, the spectrum registered showed the total disappearance of the band.

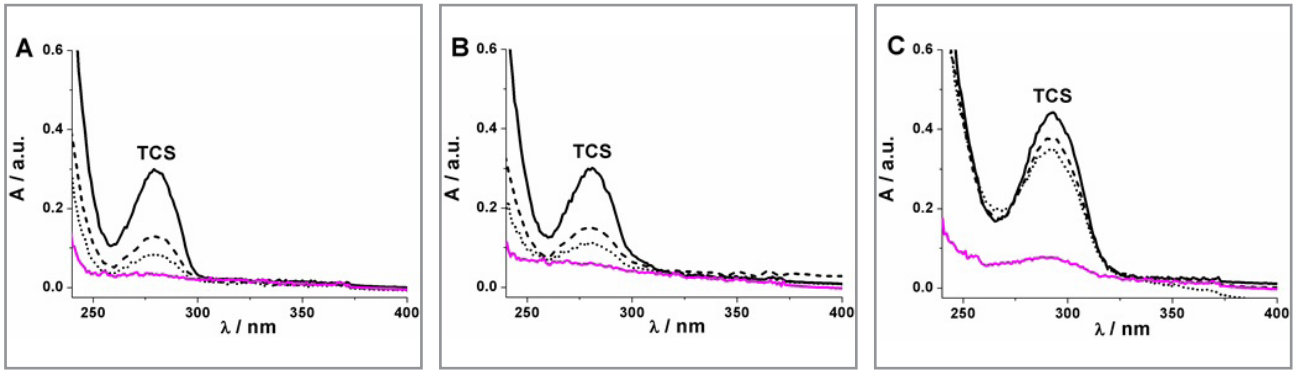


Figure 6. UV-Vis absorption spectra for 0.0625 mM TCS in: (▬) 0 h and after (▪▪▪) 30, (•••) 64 and (▬) 70 days of incubation in: (A) acetate solution pH 3.4; (B) phosphate buffer pH 7.0 and (C) borax buffer pH 9.2.

The same behaviour was observed for TCS in 0.1 M phosphate buffer pH 7.0 and 0.1 M borax buffer pH 9.2 (Figure 6B and C). However, in alkaline conditions, the TCS absorption band shifted to λ = 291 nm [46]. Furthermore, after 70 days of incubation, one absorption band was still detected in basic medium.

Previously, it has been reported that the TCS has an absorption band at λ = 280 nm for the molecular form and at λ = 292 nm for the anionic form in a study carried out on the direct phototransformation of TCS in surface waters [48]. In addition, the anionic form has higher molar absorptivity than the molecular form and is found in greater amounts in alkaline solutions. This was observed in our experiments (Figure 6C) which shows higher absorption bands for the TCS and their displacement for a longer wavelength when compared to solutions with lower pH values. TCS is also long known to be more photochemically labile under basic conditions [10] and the transformation of its anionic form under sunlight is much faster

than the transformation of the molecular form [48]. Thus, in this work, we can state that the spontaneous degradation of TCS observed in the electrolytes studied was more pronounced in alkaline medium.

The spectrophotometric experiments are in accordance with DP voltammetry, showing that the TCS undergoes chemical degradation in aqueous solution.

Effects of TCS on DNA

The effects of TCS on DNA were studied through the interaction in situ of TCS and degraded TCS with dsDNA by DP voltammetry, in order to investigate conformational changes in the structure of dsDNA caused by antibacterial agent and/or degradation products.

Interaction of TCS and degraded TCS with dsDNA, POLY[G] and POLY[A] Incubated Solutions

The interaction of TCS and degraded TCS with dsDNA was first investigated in incubated solutions as a function of the incubation time (Figure 7A and B).

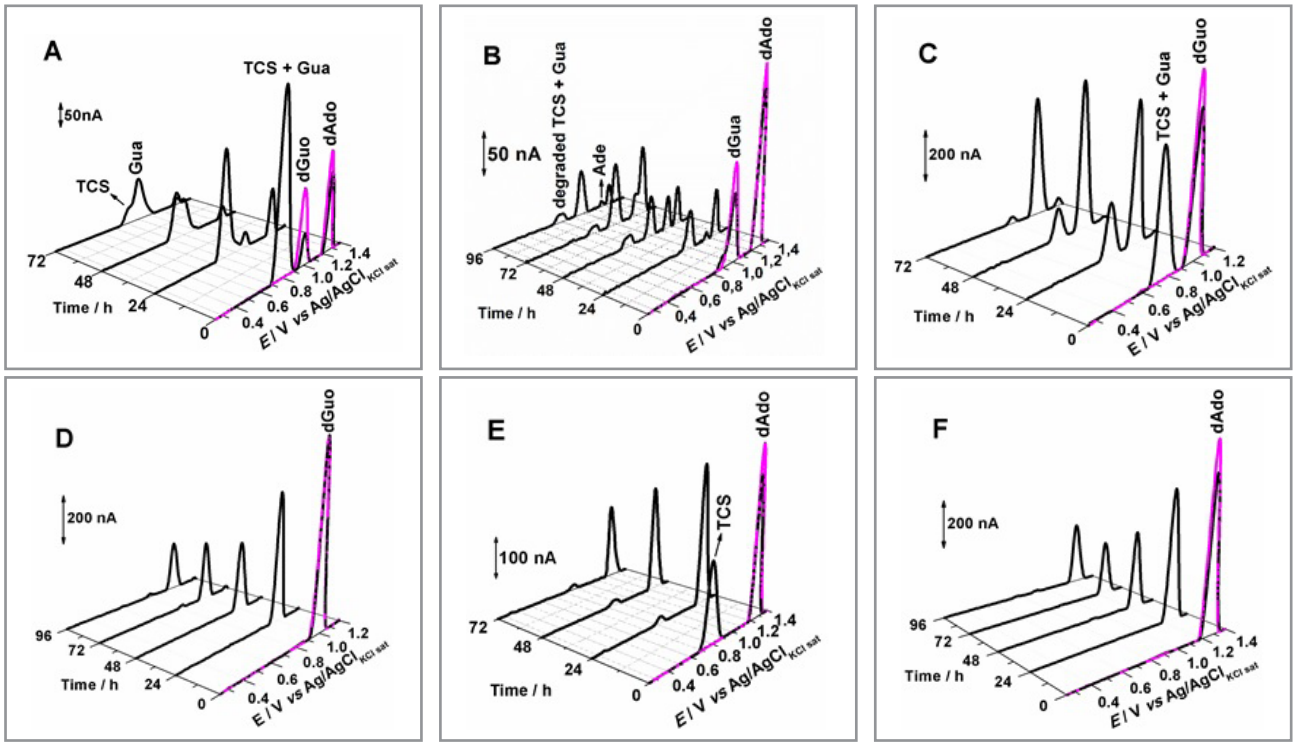


Figure 7. Baseline-corrected DP voltammograms obtained in 0.1 M acetate buffer solution pH 4.5: (—) control solutions and (—) incubated solutions as: (A) 5 μM TCS + 100 μg mL^{-1} dsDNA; (B) 5 μM degraded TCS + 100 μg mL^{-1} dsDNA; (C) 5 μM TCS + 100 μg mL^{-1} Poly[G]; (D) 5 μM degraded TCS + 100 μg mL^{-1} Poly[G]; (E) 5 μM TCS + 100 μg mL^{-1} Poly[A] and (F) 5 μM degraded TCS + 100 μg mL^{-1} Poly[A]. Pulse amplitude of 50 mV, pulse width of 70 ms, step potential of 2.5 mV, interval of time of 500 ms and scan rate of 5 mV s^{-1}.

100 μg mL^{-1} dsDNA solution was registered in 0.1 M acetate buffer pH 4.5 as a control and presented the desoxyguanosine (dGuo) peak, at E = + 0.98 V, and desoxyadenosine (dAdo) peak, at E = + 1.25 V, [49] (Figure 7A). Right after mixing 5 μM TCS with dsDNA in solution, a marked decrease in dsDNA peaks was observed. This behavior indicates that TCS interacted with dsDNA causing condensation of the double helix chain, leading to the difficulty in oxidizing nitrogenous bases on the electrode surface. A further peak at E = + 0.78 V was detected in the voltammogram, corresponding to the oxidation of TCS and possible free guanine (Gua) mixed in solution, as both are oxidized separately to similar potentials, E = + 0.78 V and E = + 0.85 V [34], respectively. Over incubation time dsDNA peaks drastically decreased and

after 48 and 72 h the dGuo peak disappeared. In addition, the TCS peak decreased, due to its degradation in solution, and the Gua peak stood out, due to more Gua formation in solution. However, no oxidative damage to dsDNA was observed in the voltammograms, since the biomarkers of guanine (8-oxoGua) and adenine (2,8-oxoAde), at *E* = + 0.45 V, [35] was not detected under these conditions.

For the degraded TCS-dsDNA solution interaction, the study showed the dsDNA peaks at *E* = + 0.99 V (dGuo) and *E* = + 1.26 V (dAdo) for the control experiment (100 μg mL^{-1} dsDNA solution) (Figure 7B). Soon after adding 5 μM degraded TCS to the dsDNA solution, dsDNA peaks decreased showing an interaction of degraded TCS with the double helix structure. After longer incubation times, new peaks were seen at + 0.79 V and + 1.16 V, corresponding to the oxidation of (degraded TCS + Gua) and free adenine (Ade) [34] released from dsDNA, respectively. No peak associated to 8-oxoGua and/or 2,8-oxoAde biomarkers was detected in these experiments.

In order to obtain more information about the interaction of TCS and degraded TCS with dsDNA occurred, investigations involving homopolynucleotides (poly [G] and poly [A]) in incubated solutions were conducted at different incubation times (Figure 7C and D) and (Figure 7E and F), respectively.

100 μg mL^{-1} poly[G] solution in 0.1 M acetate buffer pH 4.5 was run as a control and exhibited one peak at *E* = + 1.01 V, corresponding to the oxidation of dGuo residues, which decreased with increasing incubation time with 5 μM TCS (Figure 7C). One new peak occurred at *E* = + 0.74 V immediately after adding TCS to the buffer. This peak corresponding to the oxidation of (TCS + Gua) in solution, which reduced after longer incubation times, due to the TCS degradation. On the other hand, the voltammogram registered for 100 μg mL^{-1} poly[A] solution as a control showed one peak at *E* = + 1.26 V, related to the oxidation of dAdo residues (Figure 7E). This peak decreased with increasing incubation time in 5 μM TCS solution and the TCS peak at *E* = + 0.88 V was detected in all voltammograms. Moreover, no 8-oxoGua or 2,8-oxoAde peak was observed under these conditions, indicating that no dsDNA oxidative damage was detected.

In Figure 7D, a solution of 100 μg mL^{-1} poly[G] in 0.1 M acetate buffer pH 4.5 (control) showed the dGuo peak at *E* = + 0.99 V. Between 24 and 96 h of incubation with 5 μM degraded TCS this peak decreased successively. For the degraded TCS-poly[A] solution interaction, the voltammogram revealed the dAdo peak at *E* = + 1.25 V (Figure 7F) which also decreased over the incubation time with 5 μM degraded TCS, compared to the peak for the control poly[A] solution. No oxidative damage to dsDNA was noticed.

Interaction of degraded TCS with dsDNA biosensors

Degraded TCS-dsDNA interaction was also investigated by incubating dsDNA biosensors in 5 μM degraded TCS solutions in different interaction times (Figure 8).

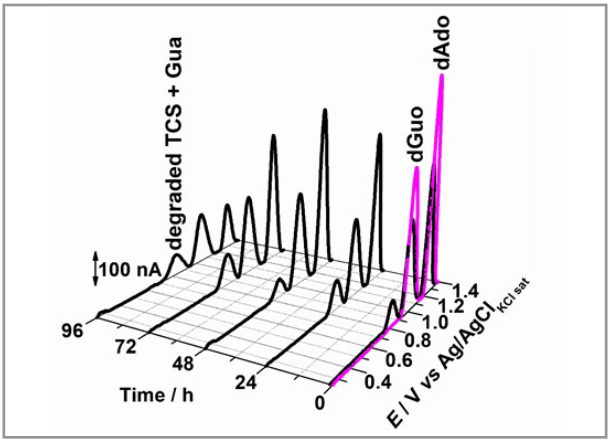


Figure 8. Baseline-corrected DP voltammograms obtained in 0.1 M acetate buffer pH 4.5: 100 μg mL^{-1} dsDNA biosensor (—) control and incubated with: (A) 5 μM degraded TCS (—). Pulse amplitude of 50 mV, pulse width of 70 ms, step potential of 2.5 mV, interval of time of 500 ms and scan rate of 5 mV s^{-1}.

For the control biosensor dsDNA, the nucleosides peaks were detected at *E* = + 1.02 V (dGuo) and *E* =+ 1.28 V (dAdo) (Figure 8). These peaks decreased during the incubation period, indicating the degradation products of TCS interacted with dsDNA leading to the condensation of double helix molecule. After 1 h of interaction an additional peak was seen at *E* = + 0.84 V, associated to the oxidation of (degraded TCS + Gua). It is known that electrode surface is completely covered by dsDNA film and biosensor response is due only to the interaction of the compound with dsDNA, consequently preventing non-specific adsorption [50]. However, in this case, this peak is indicative of a possible intercalation of degraded TCS in the DNA structure, since the peak current practically remains constant until 96 h of incubation instead of increasing due to the increased concentration of the dsDNA released Gua. No oxidative damage to dsDNA was observed, since the 8-oxoGua and 2,8-oxoAde biomarkers was not detected.

These results are in agreement with the analysis obtained using dsDNA incubated solutions.

CONCLUSIONS

This research provides a complete electrochemical study of antimicrobial compound TCS at a glassy carbon electrode, as well as a novel study of its spontaneous degradation in aqueous solution. TCS undergoes irreversible electrochemical oxidation through the transfer of one electron and one proton and with the formation of two oxidation products reversibly oxidized, involving the loss of two electrons and two protons each. A remarkable chemical degradation of TCS in a time-dependent manner was observed in all electrolytes studied with faster degradation in alkaline medium. This work also explains novel evidences that the TCS and degraded TCS interact in situ with dsDNA, leading to the condensation of the structure of the DNA molecule and release of nitrogenous bases: guanine (by TCS and degraded TCS) and adenine (by degraded TCS). Furthermore, the degraded TCS possibly intercalated in the double-stranded DNA. However, no oxidative damage to dsDNA by TCS or its degradation product(s) was detected under the experimental conditions shown. Thus, this research brings interesting findings that may lead to a clearer understanding of the action mechanism of TCS in the DNA in the future.

Conflicts of interest

The authors declare that have no conflicts of interest to disclose.

Acknowledgements

We sincerely thank funding agencies: FAPEMA fellow-Brazil (BIC-02375/14), CNPq fellow-Brazil (313627/2013-4), CNPq-Brazil (103256/2014-8), CAPES-Brazil (Finance Code 001), CNPq (Project 400578/2013-1) and INCT de Bioanalitica (Project CNPq/INCT 465389/2014-7).

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Brazilian Journal of Analytical Chemistry
2021, Volume 8, Issue 31, pp 103–116
doi: 10.30744/brjac.2179-3425.AR-08-2021

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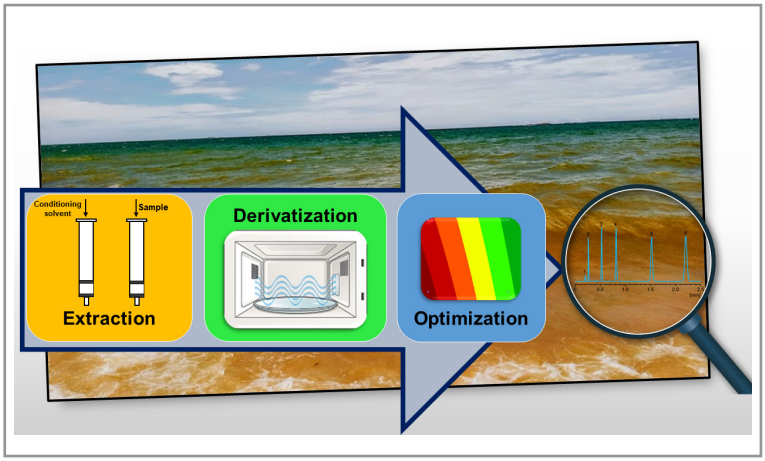
ARTICLE

Emerging Contaminants in Aqueous Matrices Determined by Gas Chromatography-Mass Spectrometry

Emerson Luis Yoshio Hara[1], Barbara Gomes Souza Soares[1], Adriano Lucas Paiva dos Santos[1], Bruno José Gonçalves da Silva[1], Gilberto Abate[1], Kelly Cavalcanti Machado[2], Beatriz Isabella Cestaro[1], Rafael Garrett Dolatto[1], Marco Tadeu Grassi[1]*

[1]*Departamento de Química, Grupo de Pesquisa em Química Ambiental, Universidade Federal do Paraná, Rua Francisco H. dos Santos, 100, Jardim das Américas, Curitiba, Paraná, 81531-980, Brazil*

[2]*Instituto Carlos Chagas/Fundação Osvaldo Cruz Paraná, Rua Professor Algacyr Munhoz Mader, 3775, Cidade Industrial de Curitiba, Curitiba, PR, 81310-020, Brazil*



Reports on the determination of emerging contaminants (EC) in aqueous samples have been increasingly common. Due to the low levels of concentration of the analytes as well as the complexity of this matrix, the analysis is done preferably by liquid chromatography (LC). Owing to the polar character of most of the EC determination by gas chromatography is deprecated. One way to overcome this barrier is through derivatization, which, in some cases, can be a lengthy step, presents risks to the analyst as well as to the environment due to the toxicity of the derivatizing agent, and, thus, ends up favoring the use of LC. An analytical protocol was developed in this work to increase the efficiency of derivatization in a shorter reaction time for the determination of ibuprofen, 4-octylphenol, 4-nonylphenol, triclosan, bisphenol A, diclofenac, estrone, 17-β-estradiol, estriol, coprostanol, and cholesterol. The proposal then was to carry out the silylation reaction of the analytes with the aid of a domestic microwave oven. The results indicated that the use of the device provided an increase in the efficiency of the reaction, due to the homogeneous heating of the solution. Besides, there was a significant decrease in the derivatization time of the analytes from 30 min to 5 min. Additionally, through a design of experiments (DOE), it was possible to perceive the influence of some instrumental parameters of GC-MS, such as temperature, pressure intensity, and pressure pulse time in the injector on the detectability of the investigated analytes. This study allowed a satisfactory separation of the analytes and an average increase in their areas of up to 35%. These aspects made it possible to obtain an analytical method with limits for the

Cite: Hara, E. L. Y.; Soares, B. G. S.; dos Santos, A. L. P.; da Silva, B. J. G.; Abate, G.; Machado, K. C.; Cestaro, B. I.; Dolatto, R. G.; Grassi, M. T. Emerging Contaminants in Aqueous Matrices Determined by Gas Chromatography-Mass Spectrometry. *Braz. J. Anal. Chem.*, 2021, *8* (31), pp 103–116. doi: http://dx.doi.org/10.30744/brjac.2179-3425.AR-08-2021

Received 15 January 2021, Revised 22 April 2021, Accepted 26 May 2021, Available online 02 June 2021.

detection and quantification of EC between 0.03-11.00 ng mL^{-1} and 0.10-33.35 ng mL^{-1}, respectively, and uncertainties below 9%. The developed method was applied in the determination of the analytes in coastal seawater and the determined concentrations varied from 0.24 ng L^{-1} for estriol and 43.60 ng L^{-1} for cholesterol. Thus, the improvement of the silylation reaction, combined with the strategy of instrumental optimization, proved to be simple, efficient, and fast, as well as being a comparable alternative to liquid chromatography.

Keywords: Design of experiments, Chromatographic analysis, Derivatization, Surface water, Sample preparation.

INTRODUCTION

For more than two decades, compounds known as EC have attracted the attention of society, researchers, and environmental agencies. This is due to the significant persistence in the environment and the ability to disturb the biota's physiology, in addition to these substances being associated with bioaccumulation and biomagnification processes in the food chain [1,2]. Currently, approximately 700 compounds are classified as EC, are subdivided into the following classes: drugs, steroids and hormones, personal care products, surfactants, flame retardants, agricultural inputs, and industrial additives [3–5]. Although these products are essential for maintaining the lifestyle of today's society, the widespread use, inadequate disposal, and sanitary deficiencies cause them to occur in the environmental compartments, that is, in the air, in the soil, and in particular water [6–9].

In aquatic systems such as raw and treated sewage, surface water, groundwater, and water for public supply, the concentrations of EC reported in the literature can vary from pg/L to µg/L [10]. Due to the low concentrations at which they are detected and the complexity of the analysis, several extraction strategies are used for this purpose. Among them, liquid-liquid extraction (LLE) [11] and solid-phase extraction (SPE) [12], miniaturized strategies such as solid-phase microextraction (SPME), sorting by rotating disk (RDSE) [13] and passive sampling to assess bioavailability via polar organic chemical integrative sampler (POCIS) [14]. For the simultaneous quantification of several classes of EC, instrumental techniques, such as liquid phase (LC) [15–18] and gas phase (GC) chromatography, both hyphenated with mass spectrometers are the most used. However, the physical-chemical characteristics of the compounds, such as water solubility, polarity, boiling points, high molar masses, and low volatility [19] favor the use of LC in reverse phase [20] compared to GC. Additionally, GC determination requires a derivatization step that increases the length of the procedure, or the majority may not be suitable for all target compounds [21].

Although derivatization is an additional step in the analysis, the use of GC-MS can be interesting, due to the lower cost of equipment for implantation in routine laboratories and lower solvent consumption compared to the elution used in LC-MS [13,22]. In this sense, the literature provides some examples of how GC can be successfully used to determine EC. In a Brazilian metropolis, tap water samples were analyzed to determine 11 compounds classified as EC (caffeine, bisphenol A, 4-octylphenol, 4-nonylphenol, cholesterol, estrone, 17β-estradiol, 17α-ethinylestradiol, progesterone, coprostanol, and stigmasterol). The extraction was performed via SPE and determination by GC-MS, being possible to quantify in the samples stigmasterol (0.34 ± 0.13 µg L^{-1}), cholesterol (0.27 ± 0.07 µg L^{-1}), caffeine (0.22 ± 0.06 µg L^{-1}), and bisphenol A (0.16 ± 0.03 µg L^{-1}) [21].

In another study, the persistence, mobility, and bioavailability of 32 different types of EC were investigated at a sewage treatment plant that empties into a lake in Sweden [14]. The compounds were selected to contemplate a wide range of hydrophobicity (biocides, fragrances, organophosphates, plasticizers, surfactants, polycyclic aromatic hydrocarbons, food additives, rubbers, and polymers). In Germany, an analytical protocol was developed to determine polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), phthalate esters, nonylphenols, bisphenol A and selected steroid hormones in various types of aqueous samples such as snow and wastewater [23]. In both studies, the polar compounds were

evaluated after a derivatization step, by silylation reaction, using the derivative BSTFA, which is widely cited for this purpose [22,24,25].

Derivatization consists of the chemical modification of the analytes to obtain products with characteristics compatible with the technique used for quantification. For EC, which has chemical groups with a polar character such as carboxylic acids, thiols, hydroxyls, amines, amides, aldehydes, and enolizable ketones, derivatization becomes mandatory for the viability of using GC-MS [26].

Based on this strategy, extraction from aqueous matrices and determination by GC-MS of 16 EC including parabens, hormones, anti-inflammatories, triclosan, and bisphenol was studied by Arismendi et al. [27]. Through the design of experiment (DOE) and response surface methodology (RSM), the authors optimized the analyte silylation. The best conditions established were the volume of the derivatizing agent (MTSFA) of 70 µL, the temperature at 80 ºC, and a reaction time of 35 min. Although this study reached attractive LOD values (0.02-0.16 µg L^{-1}), the derivatization reaction time is still long, probably due to the low efficiency for the solution to reach thermal equilibrium with a heating plate. To overcome this limitation, some studies suggest the use of microwave irradiation to improve the efficiency of heating the reaction medium [28]. However, this strategy has not yet been applied to EC derivatization for determination by GC-MS.

Because of this, the main objective of this work was to establish a derivatization protocol for EC aiming at the determination of EC in aqueous matrices, assisted by microwaves, aiming to prioritize the increase of sensitivity and analytical frequency, concomitantly with the reduction of time and energy consumption. Besides, a chromatographic method was evaluated using DOE seeking the optimization of instrumental parameters of the GC-MS. For this purpose, the parameters injector temperature, time, and pressure pulse intensity were studied employing factorial design (2^3) and by response surfaces methodology. Finally, the developed protocol was applied to determine the target compounds in samples of coastal seawater.

MATERIALS AND METHODS

Reagents, solvents, and analytical standards

All solvents used (methanol, chloroform, ethyl acetate, acetonitrile, dichloromethane, acetone, and hexane) were purchased from brands such as JT Baker (USA), Merck (Germany), Panreac (Spain), and the like, always with high purity or degree HPLC. The water used in the experiments was obtained via reverse osmosis in a Permution system, model RO 510 (Brazil) and then, in a Merck Millipore model Simplicity (Germany) purifier. The compounds investigated in this work were estriol, 17-β-estradiol, estrone, ibuprofen, 4-octylphenol, 4-nonylphenol, triclosan, bisphenol-A, cholesterol, coprostanol, and diclofenac.

In addition to these, bisphenol-A-D16, ibuprofen-D3, and 17-β-estradiol-D3 were used as internal deuterated (IS) standards. All analytical standards and IS were purchased in individual flasks, with purity greater than 95% and supplied by Sigma-Aldrich (Germany), as well as the derivatizing N, O-Bis (trimethylsilyl) trifluoroacetamide with trimethyltrichlorosilane (BSTFA-TMCS 99:1 v/v).

Derivatization of emerging contaminants

Two procedures were evaluated to compare the efficiency of the analyte derivatization. In both, the addition of 1.0 mL of working solution in a concentration of 1.0 ng mL^{-1} in a 2.0 mL vial was carried out. All methanol (solvent in which the analytes were prepared) was evaporated on a Christ vacuum rotary evaporator (model RVC 2-18 CD, Germany) at 60 °C for approximately 10 min. After the solvent was completely dried, 20 µL of BSTFA-TMCS was added.

In the first protocol, the vial was sealed and heated in the GC-MS oven at 70 °C for 5 min, and in another, the vial was heated for 30 min (n=3). After heating, the vial was removed from the oven, allowed to cool, and its content was suspended in 1.0 mL of hexane and the solution was injected into the GC-MS. In the second method, the vial was sealed, and the derivatization reaction was carried out in a domestic microwave oven, operated at a power of 840 W, for 5 min. At the end of this step, the vials were removed from the microwave oven and when they reached room temperature, the volume was made up to 1.0 mL with hexane and injected into the GC-MS (n=3).

Chromatographic method

The selection of the initial instrumental parameters was performed in a GC-MS Shimadzu, model TQ8040 (Japan) equipped with automatic sampler Shimadzu, model Palm AOC-5000 Plus (Japan). All injections in the GC were performed in a volume of 1.0 µL. The injector was operated at 300 °C in splitless mode and the chromatographic column was an Agilent, model DB-5ms (USA): 30 m long, 0.25 mm thick, and internally coated with a 0.25 µm thick film containing 5% phenyl and 95% dimethyl-polysiloxane. As carrier gas, helium with 99.999% purity and flow rate of 1.0 mL/min was supplied by the company White Martins (Brazil). Three chromatographic column heating programs (n=3) were evaluated, as shown in Table I.

Table I. Heating programs evaluated in the GC

Program 1			**Program 2**			**Program 3**		
Ratio (°C/min)	**T (°C)**	**t (min)**	**Ratio (°C/min)**	**T (°C)**	**t (min)**	**Ratio (°C/min)**	**T(°C)**	**t (min)**
-	100	2	-	100	2	-	100	2
12	310	3	15	160	2	10	310	7
Run time: 22.5 min			10	210	0	Run time: 30 min		
			15	280	0			
			10	300	1			
			15	320	4			
			Run time: 26 min					

The mass spectrometer was operated in the 50 to 500 *m/z* full scan mode (electron ionization, 70 eV) and the mass analyzer is a quadrupole type. The transfer line and the ion source remained at a constant temperature at 300 °C and 250 °C, respectively.

Optimization of instrumental parameters of GC-MS

To increase the detectability of the compounds, through the investigation of adjustments of the instrumental parameters of the GC-MS, a DOE was carried out considering three factors studied at two levels (2^3), in addition to 1 central point (n=2), totaling 18 experiments. For that, the parameters of the previous section (chromatographic method) were maintained, and the variables investigated were injector temperature (1), duration of the application of the pressure pulse in the injector (2), and the intensity of the pressure pulse (3), as shown in Table II.

Table II. Instrumental parameters of the GC-MS investigated in factor design 2^3

		Level		
	Parameters	**(-)**	**0**	**(+)**
1	Injector T (°C)	200	250	300
2	Pressure pulse time (min)	1	3	5
3	Pressure pulse (kPa)	10	80	150

Quality Assurance (QA) and Quality Control (QC)

The analytical curves were obtained using the linear regression method, from the correlation between the concentration of each analyte, versus the ratio values between the peak area of the analyte and the internal standard. The analytical curves were prepared at seven concentration levels: 5, 10, 20, 40, 80, 120 and 160 ng mL^{-1} (n=3). The IS concentration, bisphenol-A-D16, ibuprofen-D3, and 17-β-estradiol-D3, was kept constant at 100 ng mL^{-1}. The preparation and injection of the solutions were carried out daily to minimize the effects of solvent and analyte volatilization.

The limits of detection (LOD) and quantification (LOQ) were calculated based on the merit parameters of the analytical curves, obtained in triplicate, from the equations LOD=3.3(s S^{-1}) and LOQ=10(s S^{-1}). Where "s" corresponds to the standard deviation estimate of the linear coefficient of the analytical curves and "S" the mean value of the sensitivity or slope.

To assess repeatability, a solution containing a mixture of analytes in a concentration of 100 ng mL^{-1} was prepared and 5 injections were made on two consecutive days. After that, the RSD of the peak areas of the analytes was calculated.

The accuracy was evaluated through recovery tests, performed with 1.0 L of coastal seawater in amber flasks where aliquots of the solution with the mixture of the EC standards were transferred in the concentrations of 50 ng L^{-1} and 100 ng L^{-1} (n=3). The flasks were manually shaken, and then solid-phase extraction (SPE) was performed. In addition, two blanks were made with sea water.

Extraction of emerging contaminants from water by SPE

EC extraction was performed using the SPE method. For this purpose, cartridges were used for extraction in solid-phase Oasis HLB (Hydrophilic/Lipophilic-Balance) purchased from Waters Co. (USA), containing 500 mg of sorbent phase and with a capacity of 6 mL. Then, the cartridges were connected to a manifold processing system consisting of a vacuum-resistant glass chamber [29] connected to a Tecnal oil-free vacuum pump, model TE-0581.

The extraction procedure was performed as follows: first, the sorbent phase of the cartridges was conditioned with two 3.0 mL aliquots of methanol, followed by two 3.0 mL aliquots of ultrapure water. Then, the cartridges were connected to the manifold through PTFE Sulpeco tubes, model Visiprep (USA) to allow the continuous passage of the entire sample volume (1.0 L) through the sorbent phase.

This system allowed us to work continuously and cleanly, since all the tubes and connections used are made of materials with a low capacity of organic compounds, thus minimizing problems of contamination and memory effect. Then, the total volume of the sample was eluted through the cartridge, under vacuum, under a controlled flow of 6 mL/min. After the end of the extraction, the cartridges were packed in aluminum foil and kept under refrigeration (4 °C), until elution and chromatographic determination.

The analyte elution was also performed with the aid of a manifold. Two 2.5 mL aliquots of methanol were added to the SPE cartridges, followed by a 2.0 mL aliquot of acetonitrile. The eluates were collected in 10 mL test tubes and taken to the rotary vacuum evaporator for complete drying of the solvents. Then, derivatization, suspension in 1.0 mL of hexane, and injection into the GC-MS were performed.

Method application

Seawater samples (n=3) were collected at point S19°56'43.7" W40°08'44.2", located on the southeast coast of Brazil, state of Espírito Santo in the city of Aracruz, whose population is approximately 100 thousand inhabitants and the sewage system reach 80% of the population [28].

All samples were placed in 1.0 L amber bottles and kept refrigerated (4 °C) for a maximum of 7 days until they were prepared and analyzed. The analysis of the samples followed the procedure using SPE.

RESULTS AND DISCUSSION

Derivatization of EC

Derivatization is a crucial procedure for the determination of EC by GC-MS [30] and in this work, the silylation reaction was used. Also, the protocol that uses the derivative BSTFA reagent, less susceptible to steric effects in larger molecules, such as hormones, was selected. The reaction consists of chemically modifying the EC structure, via a bimolecular nucleophilic substitution mechanism (SN2) [26]. The hydrogen atom present in hydroxyl and EC-linked functional groups (ECOH) is replaced by the radical trimethylsilyl, $(CH_3)_3SiX$, according to Equation 1:

$$ECOH + XSi(CH_3)_3 \rightarrow ECOSi(CH_3)_3 + HX \qquad \text{Equation 1}$$

This gives the analytes greater volatility due to the drastic reduction in intermolecular hydrogen interactions, improved affinity for the stationary phase of the chromatographic column, and greater thermal stability. The reaction yield is favored, kinetically, and thermodynamically, by the time and adequate heating of the reaction medium [26].

Considering the balance shift in the direction of product formation it is interesting for applications in methods that precede the chromatographic determinations. In this sense, Figure 1 presents a comparative graph of the efficiency results of the derivatization performed by heating the solution containing the EC in the GC oven for 30 min and in the microwave oven for 5 min.

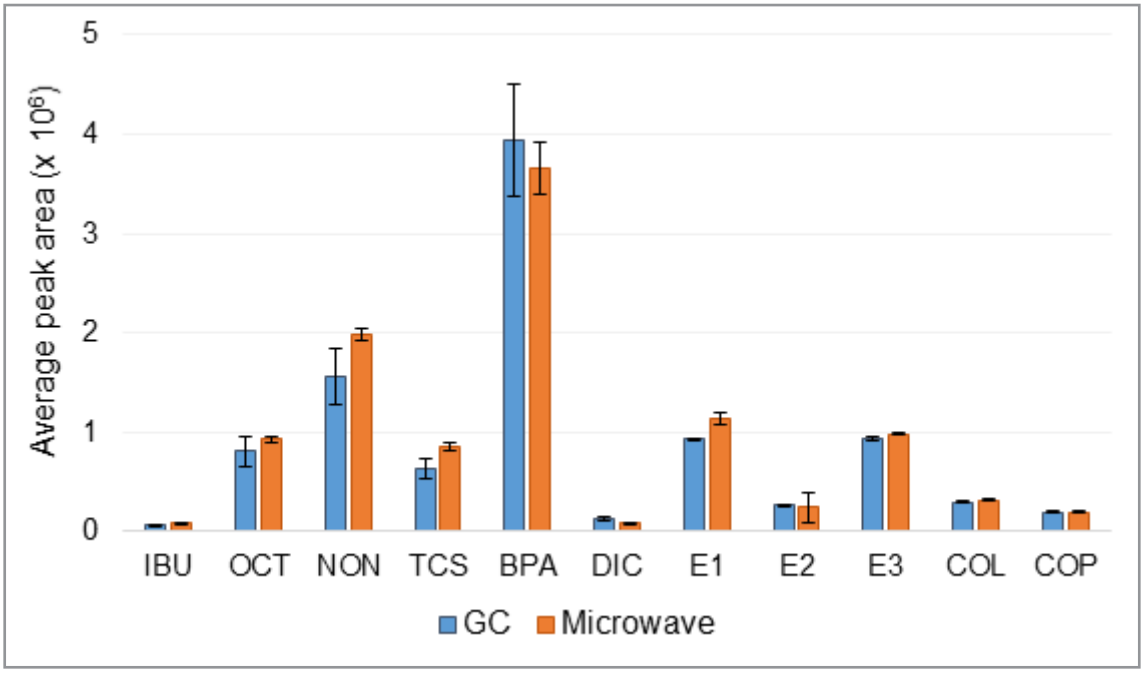


Figure 1. Comparison of the efficiency of derivatization reactions, from a solution of EC in a concentration of 1.0 µg/mL, in the GC oven for 30 min and a domestic microwave oven for 5 min: IBU: ibuprofen; OCT: 4-octylphenol; NON: 4-nonylphenol; TCS: triclosan; BPA: bisphenol-A; DIC: diclofenac; E1: estrone; E2: 17-β-estradiol; E3: estriol; COL: cholesterol; COP: coprostanol.

Analyzing the mean values of the peak areas of the analytes in Figure 1, it is possible to verify the efficiency of the derivatization process for both the reaction in the GC oven and the domestic microwave since the values of the analyte areas are well next. However, if we take 4-nonylphenol (NON) as an example, the average peak area of the compound was 1.6 x 10^6 AU in the GC oven, while for the domestic microwave the value was 2.1 x 10^6 AU, that is, on average there was an increase of 31%. Similar behavior

was observed for four other compounds (IBU, TCS, E1, and E3), and for the other analytes, the standard deviation estimate values (Figure 1) were statistically equal, or slightly higher, as verified for DIC.

It should be noted that another solution containing the analytes was also heated in the GC oven for 5 min. However, the heating time was not enough to promote the derivatization reaction of the analytes, because in none of the chromatograms the peaks of the compounds were detected. Thus, this procedure was interrupted.

Probably the superior performance of the microwave is associated with the effective heating of the system containing reagents and analytes. The increase in temperature occurs in molecules that have an appropriate permanent dipole moment and absorb radiation through rotational excitation [31]. This occurs in the BSTFA-TMCS solution and to a lesser extent in the glass walls of the vials, which may contain adsorbed moisture, contributing to increasing the effectiveness of the thermal process. On the other hand, in the GC oven, more time is needed to achieve thermal equilibrium, that is, the uneven heating of the reaction medium decreased the reaction yield.

Because of obtaining higher average areas in a microwave oven, this was selected for the other stages of the work, especially due to the shorter reaction time (5 min) and the characteristics of this equipment, such as simplicity of operation, cost, and wide availability. Besides, the lowest time and energy expenditures were considered, aiming at the development of less energy-intensive protocols. It should be noted that in some situations the use of domestic microwave ovens in the laboratory is not recommended, especially for safety reasons [31]. On the other hand, when possible, its use requires caution and modifications are important to adapt the device for each purpose [32].

GC-MS optimization

Three temperature schedules were evaluated in the GC-MS to decrease the running time, associated with an efficient resolution of the analytes. Figure 2 shows the mean values of the analyte peak areas that were obtained from the GC heating ramps, as shown in Table I.

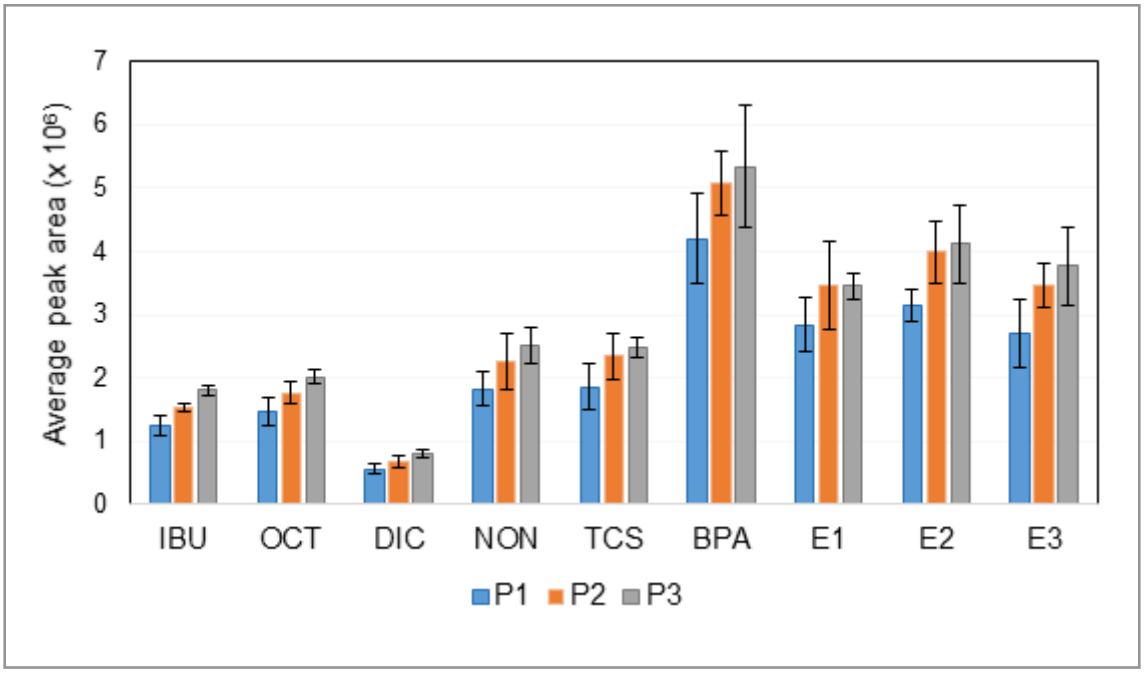


Figure 2. Mean values of the peak areas of the analytes, referring to a solution of the EC in a concentration of 1.0 µg/mL, obtained from the GC heating programs.

Temperature gradient 1 showed smaller peak areas, on average, compared to 2 and 3. Comparing the three schedules, according to standard deviation estimation bars, it is possible to notice that 1-2 and 2-3 are statistically similar. Only 1-3 differ, within the limits of their standard deviation estimates.

Regarding ramp 2, the heating rate was increased from 10 °C/min to 15 °C/min, aiming at the faster separation of the compounds. According to Sequinel et al. [33], for there to be gains in the quality of the separation and the speed of the analysis, the increase in the column temperature must be more expressive, combined mainly with a column of shorter length and greater flow of the carrier gas. However, the increase in the heating rate caused the chromatographic run time to decrease to 26 min, but there was no statistically significant gain in the mean values of the analyte peak areas.

On the other hand, program 3 exhibited peak areas, on average, 22% above other programs. This may be related to the lower heating rate of the column applied in program 3 (10 °C/min), while in program 1 the ratio was 12 °C/min. In this case, it can be considered that the decrease in the heating ratio by 3 provided an improvement in the chromatographic resolution, however, the analysis time increased, as expected [34]. For these reasons, schedule 3 was maintained for the other stages of this work.

With the preliminary instrumental conditions, it was possible to obtain the fragmentation patterns of the analytes, in terms of their mass/charge ratios (*m/z*). Table III presents some physical-chemical parameters of the compounds and the respective m/z selected for quantification and confirmation. The chromatographic data were obtained through the individual injection of the solutions of each EC derivatized at a concentration of 1.0 μg mL^{-1} (n=3).

Table III. Physico-chemical parameters and m/z ratios used in the quantification and confirmation of EC

Compound	Class	Molar mass (g mol^{-1})	Boiling point (°C)	log Kow	Quantification ion (*m/z*)	Confirmation ion (*m/z*)
Ibuprofen	Pharmaceutical	206	319	4.0	160	233; 263
4-octylphenol	Surfactant	206	315	5.5	179	278
4-nonylphenol	Surfactant	220	330	5.8	179	292
Triclosan	Personal Care	290	344	4.8	200	346; 359
Bisphenol A	Plasticizer	228	400	3.3	357	-
Diclofenac	Pharmaceutical	296	412	4.1	214	242; 276
Estrone	Estrogen	270	445	3.1	342	257; 218
Estradiol	Estrogen	272	445	4.1	416	285; 326
Estriol	Estrogen	288	469	2.5	296	311; 414
Coprostanol	Sterol	389	475	8.8	215	355; 445
Cholesterol	Sterol	387	480	8.7	329	369; 458

In the investigation of instrumental parameters of the GC-MS, splitless injections are recommended for low volume samples (between 1.0 μL and 3.0 μL) and analytes whose concentrations are in the order of pg L^{-1} to μg L^{-1} [35].

Because of the low concentrations of compounds reported in the literature in natural waters, it was necessary to use the splitless injection mode and to evaluate the most appropriate temperature of the injector in order not to degrade the analytes as well as sufficiently volatilize the derivatized EC [36]. Also, another parameter evaluated was the internal pressure of the injector during sample injection.

The pressure was applied for a while during the injection and then returned to the default value. Thus, there was an increase in the transition speed of the injector compounds to the column, which in turn led to a reduction in the band widening and the narrowing of the EC peaks [37].

Considering these aspects, it is important to note that the 18 experiments were carried out randomly and that for each experiment, a solution containing the mixture of the analytes was prepared. From the integration of the peak areas of each contaminant and the mathematical treatment proposed by the geometric mean of the areas, it was possible to analyze the effects of each parameter.

Thus, Figures 3A and 3B make the interpretation of the results intelligible. In Figure 3A, the response surfaces referring to the time of application of pressure in the injector versus the temperature of the injector, and the pressure applied in the injector versus the injector T are shown.

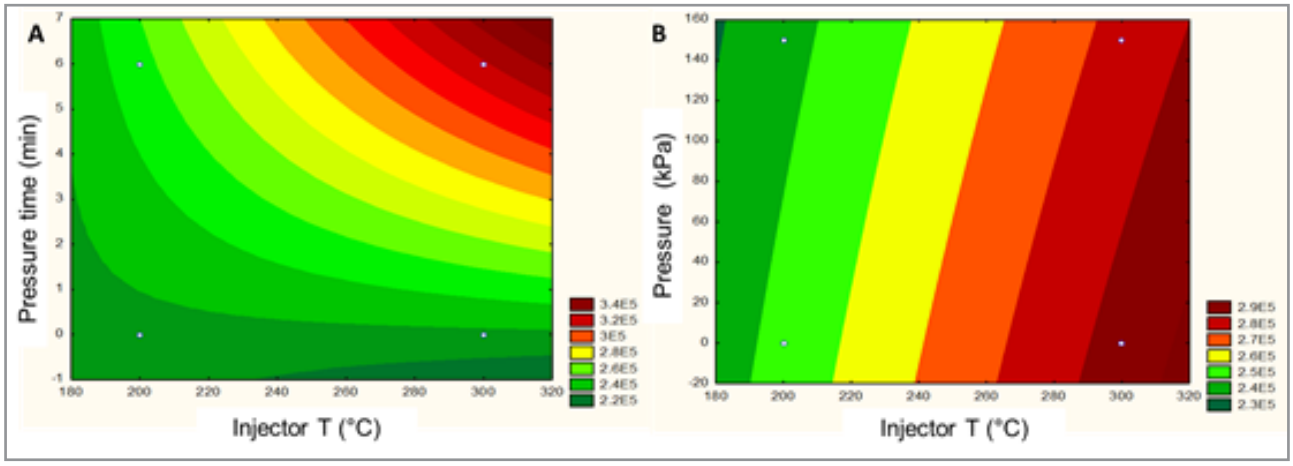


Figure 3. Response surfaces regarding the combination of pressure time versus injector temperature (A) and pressure versus injector temperature (B).

The more intense red colors indicate the parameters that can provide greater gains in responses, as observed in the abscissa where the injector temperature (>280 °C) is represented. In the ordinates, the intensity of the applied pressure is represented.

In Figure 3B, it is possible to notice that the lower pressures (<80 kPa) coincide with the more intense shades in red. Thus, it was decided to use a temperature of 300 °C and 80 kPa in the other stages of the work. In Figure 3A, it is possible to observe the response surface regarding the effect of the injector temperature with the time of application of the pressure pulse.

In the same way, it appears that the red colors are more intense and correspond to both the longest application times of the pressure pulse (> 5 min) and the highest temperatures. For the remaining stages of this work, we chose to use 7 min. To investigate the significance of the effects and possible interactions between the variables, the results were evaluated using the Pareto diagram, shown in Figure 4.

Figure 4 shows a Pareto chart that corroborates the results presented in Figures 3A and 3B. Thus, considering minimum Student´s t values or tc = 2.31 and at a 95% confidence level, the effects of “pressure application time”, injector temperature, and pressure intensity are significant, that is, the optimization of these parameters can change the detectability of the compounds positively.

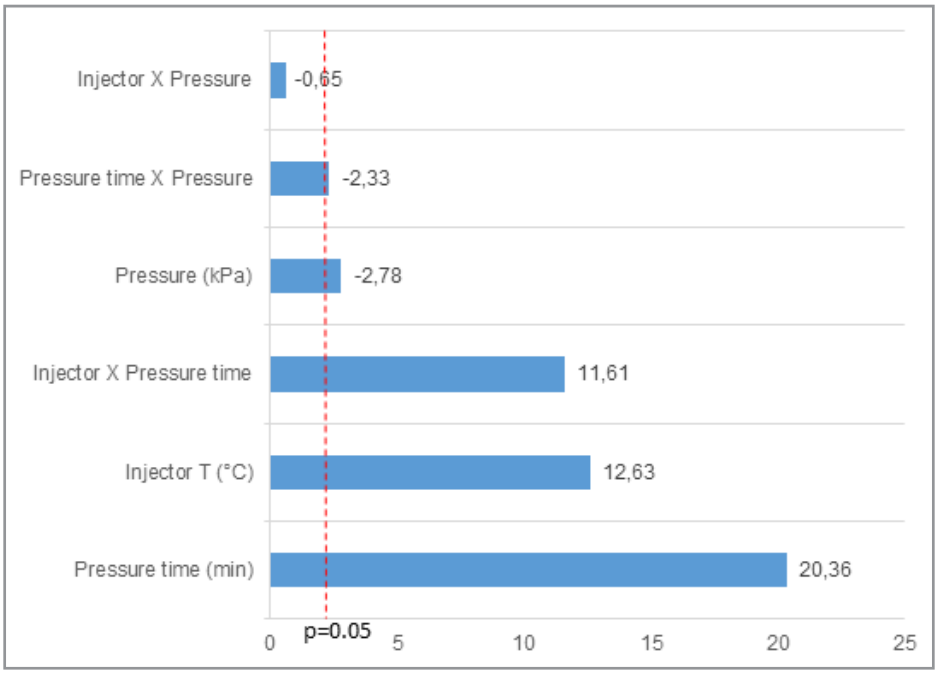


Figure 4. Pareto chart presents the effects of the parameters of the design of experiments.

Likewise, it can be inferred that the application of the pressure pulse for a longer period, associated with a higher injector temperature, enabled an increase in the peak areas of the analytes.

This is evident since both effects are much higher than the tc value. Besides, an interaction between these two factors was verified, favoring the volatilization efficiency of the compounds and the transfer of masses to the chromatographic column. Although the pressure intensity does not show significant significance, it can be considered that the intermediate values of pressure intensity are sufficient for adequate separation of the analytes.

The optimization of the instrumental parameters of the GC-MS with the aid of the DOE 2^3, enabled gains in the areas of the EC peaks between 15% (DIC) to 35% (NON). Thus, this increase in the sensitivity of the instrumental technique is crucial in determining the compounds since they are present in the environment, typically in the order of ng/L.

QA/QC

Table IV presents the values of the coefficients of determination, detection limit, and limit of quantification.

Table IV. Determination coefficients (R^2) And instrumental LOD and LOQ values

	IBU	OCT	NON	TCS	BPA	DIC	E1	E2	E3	COP	COL
R^2	0.9991	0.9994	0.9990	0.9990	0.9995	0.9993	0.9960	0.9999	0.9989	0.9996	0.9963
LOD ng mL^{-1}	2.40	0.51	1.16	1.33	1.33	11.00	0.03	0.15	0.16	0.11	0.11
LOQ ng mL^{-1}	7.23	1.55	3.53	4.04	4.02	33.35	0.10	0.45	0.48	0.35	0.35

IBU: ibuprofen; OCT: 4-octylphenol; NON: 4-nonylphenol; TCS: triclosan; BPA: bisphenol-A; DIC: diclofenac; E1: estrone; E2: 17-β-estradiol; E3: estriol.

The value of R^2 allows estimating the linearity of the analytical curve, because the closer to 1.0, the smaller the dispersion of the set of experimental points and the lower the uncertainty of the determination coefficients [38,39]. Thus, it was possible to conclude that the values presented in Table IV are satisfactory and meet the requirements established in the literature.

Based on this, analytical curves were obtained in triplicates and their parameters (intercept and slope) used to evaluate the performance of the protocol.

The values of the limits of detection and quantification were considered satisfactory considering that a GC-MS was used and thus requires a previous derivatization step to determine such values. Despite this, the values are comparable to those in the literature even when an LC-MS [40–42] and GC-MS [13] were used.

Repeatability represents the agreement between the results of successive measurements of the same method performed under the same measurement conditions. Thus, the analyst, the preparation of the standard solutions, the injections, and the equipment were maintained throughout the work. Figure 5 shows the graph of the results regarding repeatability. In this procedure, in total, 10 injections were performed from a single vial.

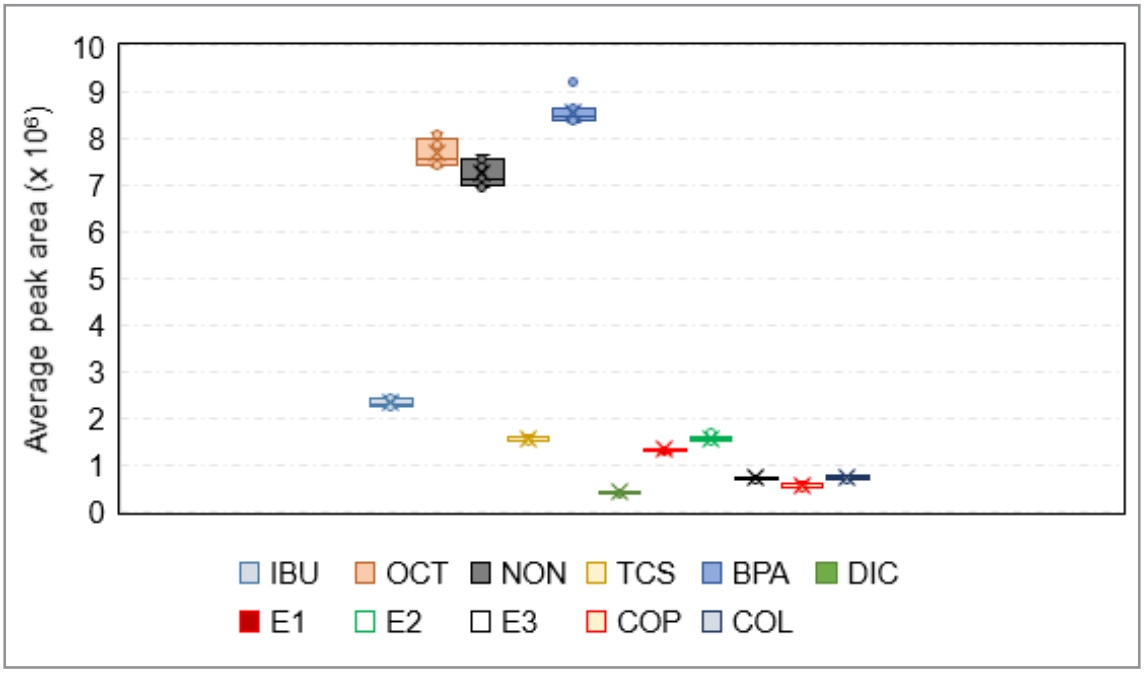


Figure 5. Distribution of analytes against repeatability: IBU: ibuprofen; OCT: 4-octylphenol; NON: 4-nonylphenol; TCS: triclosan; BPA: bisphenol-A; DIC: diclofenac; E1: estrone; E2: 17-β-estradiol; E3: estriol; COP: coprostanol; COL: cholesterol.

The results show a satisfactory resolution of the analytes as well as a reduced variability in the peak area values of each analyte. The RSD values varied between 3% and 9%. In this sense, it was possible to conclude that the procedure was satisfactory due to the low variability of peak area values.

In order to carry out a systematic and more relevant study from an analytical point of view, we chose to carry out a recovery study using coastal seawater. From an environmental perspective, the analysis of this matrix is even more significant in a country like Brazil that has a major deficiency in sewage treatment. It is important to note that almost 27% of the Brazilian population lives close to the coast, that is, there are more than 50 million people who have their tailings thrown in the Atlantic, mostly without any type of treatment [43].

The recovery study for the seawater sample fortified with 50 ng L^{-1} showed values between 56% and 142% for estriol, cholesterol, triclosan, estradiol and estrone with RSD < 20%. Ibuprofen, octylphenol and nonylphenol showed recovery between 18% and 22% with RSD < 5%. For the study with seawater fortified with 100 ng L^{-1}, the recovery varied between 53% and 166% for cholesterol, estriol, triclosan, estradiol, estrone. The RSD was less than 31%. Ibuprofen and octylphenol would vary between 16% and 21%. In the analysis of the blanks with seawater, the concentrations of the analytes were lower than the LOD for all emerging contaminants.

Method application

The optimized protocol was applied to surface water samples to assess its applicability. Figure 6 presents the results for the quantification of EC in seawater samples.

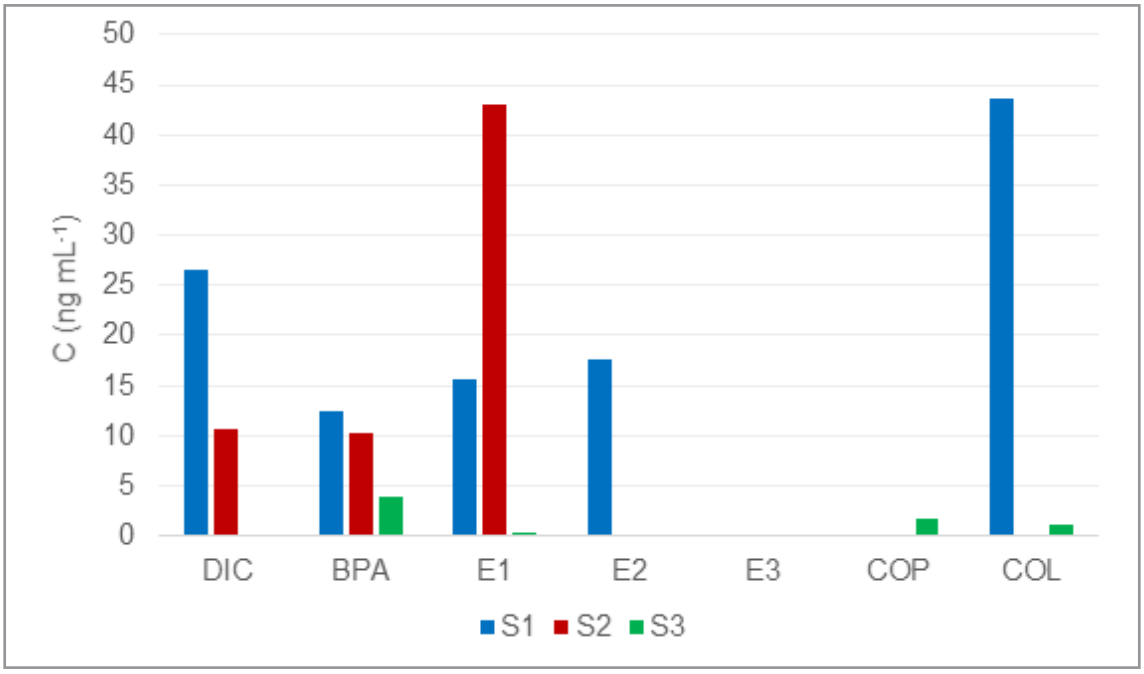


Figure 6. EC concentrations determined in seawater samples.

The chromatographic analytical method developed together with SPE enabled the determination of seven analytes in the sample: DIC (11-27 ng mL^{-1}), BPA (4-13 ng mL^{-1}), E1 (0.9-43 ng mL^{-1}), E2 (17 ng mL^{-1}), E3 (0.50 ng mL^{-1}), COP (2 ng mL^{-1}) and COL (2-43 ng mL^{-1}). Although the sampling site belongs to an environmental protection area, it is deficient in basic sanitation.

Approximately 80% of the region is served by a sewage network. Considering this fact, the presence of hormones and sterols, which are markers of sewage contamination, may be indicative of the region's sanitary deficiency [44]. Values are close to those reported by [43] in marine waters and with high salinity, determined by LC-MS/MS: BPA (0.19-0.49 ng mL^{-1}), DIC (<0.47–79.89 ng mL^{-1}), E1 (< 0.56–1.95 ng mL^{-1}), and E2 (<5.28–31.43 ng mL^{-1}), except BPA and E1. In this sense, complementary studies involving spatial and temporal sampling in this region can be performed using the procedure developed here for a detailed environmental assessment.

CONCLUSION

The derivatization step was improved and provided less reaction time, which favored an increase in the analytical frequency. Besides, this has been achieved by employing a low-cost and widely available household microwave device.

Also, the multivariate optimization of instrumental parameters of the GC-MS technique, generally neglected in the literature, provided a significant improvement in terms of analyte sensitivity. This was verified after the application of the method in the seawater samples, revealing the presence of the analytes in trace concentrations, compatible with those reported in the literature.

Because of these aspects, it is possible to conclude that the analytical strategies used in this work can be classified as simple, low cost, and obtained with a reduced number of experiments. This suggests that the steps reported here are not limited to EC but can be extended to the determination of other classes of compounds or other instrumental techniques, mainly, in the aspect that involves exploring the full potential of the equipment.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors are grateful to “Instituto Nacional de Ciências e Tecnologias Analíticas Avançadas” (INCTAA, proc. 573894/2008-6 and 465768/2014-8), “Financiadora de Estudos e Projetos” (FINEP, CT-HIDRO 01/2013) and “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq) for the financial support. This study was financed in part by the “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” – Brazil (CAPES) – Finance Code 001.

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FEATURE

BrJAC Celebrates 10 Years of Existence

Commemorative seal.
By Adriana Garcia.

The *Brazilian Journal of Analytical Chemistry* (BrJAC) completed 10 years of existence on June 18, 2020. To celebrate this significant milestone, an online event was held in which Prof. Dr. Marco Aurélio Zezzi Arruda, Editor-in-Chief of BrJAC, talked about the evolution and modernization that the journal has undergone in these 10 years, resulting in its high visibility worldwide. Then, Prof. Dr. Lauro Tatsuo Kubota, Editor-in-Chief of BrJAC since its creation until December 2018, spoke a little about how BrJAC was born and about the contribution of this journal to the field of analytical chemistry. Carlos Roberto Rodrigues, one of the idealizers of BrJAC and director of the first publisher of the journal, DKK Comunicação, spoke about how the BrJAC project was born.

BrJAC is the first Brazilian scientific journal dedicated to all branches of analytical chemistry. It was launched on June 18, 2010 to fulfill an idealistic purpose of a group of people to achieve actual academic–industrial integration towards innovation and technical–scientific development. This is reflected in the composition of BrJAC's Editorial Board, which brings together professionals of the field of analytical chemistry who work in academia, private companies, and public institutions. In its 10 years of existence, completed in 2020, BrJAC has increased in scientific quality and worldwide visibility.

The creation of BrJAC was conceived simultaneously with the creation of the Analitica Latin America (ALA) Congress. In 2008, Mr. Rodrigues coordinated several meetings for the organization of the 1st ALA Congress, and the Congress' Scientific Committee believed in the idea of creating an exclusive vehicle for communication of topics related to analytical chemistry. The intention was to create a new scientific journal to serve as a platform for disseminating research on analytical chemistry developed both in academic laboratories and in state and private research centers. In October 2009, with the journal's project ready, its idealizers participated in the 15th Brazilian Meeting on Analytical Chemistry (15º ENQA) in Salvador, BA, and conducted an opinion poll among the participants on suggested titles for the journal. *Brazilian Journal of Analytical Chemistry*, which was the title suggested by Luiz Bravo, one of the journal's idealizers, shot ahead in the poll. This is how BrJAC was born.

BrJAC's booth at one of the Analitica Latin America Congress editions.
Photo: Lilian Freitas.

BrJAC emerged during a very important moment in Brazil, when technological innovation seemed to assume an increasingly bigger role in public policies, planning, and business. In the beginning, the objective of BrJAC was to debate, to discuss, to show trends and needs with opinion editorials and interviews with renowned investigators, and to publish scientific papers.

From 2016 onwards, the Visão FOKKA agency became the publisher of BrJAC. From this, several changes were introduced, but always maintaining the main objective of the journal. Some improvements were implemented, such as the following: the introduction of a professional platform for online manuscript submission; renewal of the composition of the Editorial Board for BrJAC; definition of a period of 2 years for each member to remain on the Editorial Board; the inclusion of foreign advisers among the reviewers in order to increase the journal's internationalization; online publication of articles with their respective DOIs shortly after peer approval; and a totally new, intuitive, and responsive journal website for mobile, tablet, or personal-computer reading.

Over the past 10 years, BrJAC has counted on the collaboration of many professionals, both from academia and industry. In addition, the support of some sponsoring companies, such as Nova Analitica, Thermo Scientific, Gilson and Milestone, was also essential for its permanence and growth.

BrJAC "Young Talent in Analytical Chemistry" Award

In 2019, BrJAC created the "Young Talent in Analytical Chemistry" award to recognize outstanding researchers in analytical chemistry. This award will be offered annually at events related to analytical chemistry, such as the ENQA and the Analitica Latin America Congress, which take place in alternate years. The award will consist of a merit recognition diploma and a registration voucher for the national event on analytical chemistry (ENQA or Analitica Latin America Congress) subsequent to the award. The choice of the researcher to be awarded will be made by a committee designated by the Editor-in-Chief of BrJAC.

BrJAC's plush mascot usually raffled at meetings.
Photo: Lilian Freitas.

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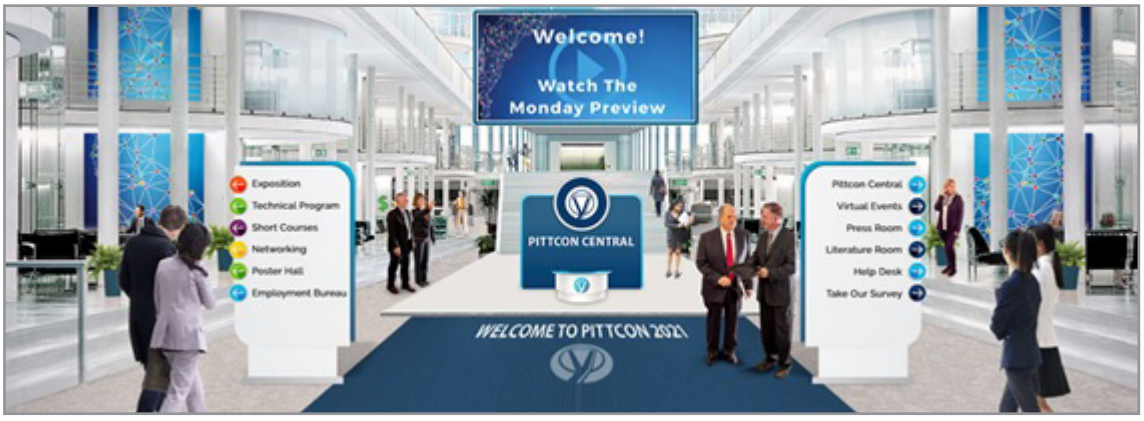
FEATURE

Pittcon 2021 was held as a Virtual Event

The Pittcon Conference & Expo, like the whole world, had to adapt due to the new coronavirus pandemic and held its 72nd edition as a virtual event, which took place March 8-12, 2021.



Pittcon 2021 Virtual Entrance - Pittcon image.

Pittcon is a conference and exposition on analytical chemistry that advances and enriches the scientific endeavor by connecting scientists worldwide, facilitating the exchange of research and ideas, showcasing the latest in laboratory innovation, and funding science education and diffusion.

The organizing committee of Pittcon 2021 wanted to ensure that the participants and exhibitors of the event, the people who make up the international scientific community, had access to the same dynamic exhibition and technical programming available in a Pittcon in person.

“Our decision process, our research, led us to attend a multitude of other virtual shows,” states Pittcon 2021 President Dr. Neal Dando. “We wanted to see what worked and what did not. We talked to our exhibitors, sent out surveys to our thousands of attendees, and most important of all, we listened.”

The mission of the Pittcon committee and staff was not only to present Pittcon 2021 as a different event from what Pittcon used to be, but also to present a virtual show different from other virtual shows.

Technical Program

The technical program consisted of 1,350 presentations, including symposia invited and organized sessions, contributed sessions, and oral and poster sessions. The conference was presented virtually throughout the week of the virtual event.

The Pittcon Technical Program hosted a transnational collection of chemists from the private, academic, and government sectors presenting their research, findings, insights and other advances to thousands of attendees. In addition, this year, for the first time in the conference’s over 71-year history, the Pittcon Technical Program was presented entirely via virtual platform.

“We are elated to have such a tremendous response to participate in our technical program this year,” states Neal Dando, Ph.D., Pittcon 2021 President. “The depth and modernity of our technical programming has always been one of our primary strengths. Witnessing this level of worldwide participation for our first virtual show is a testament to that.”

Virtual access platform for the Pittcon 2021 Content - Pittcon image.

The Pittcon conference is organized into ten tracks representative of contemporary analytical chemistry. These tracks are: Bioanalytical; Cannabis and Hemp; Energy; Environmental; Food Science and Agriculture; Forensics and Toxicology; Industry and Manufacturing; Life Sciences; Nanotechnology and Materials Science; and Pharmaceutical. Other areas of focus, such as Art and Historic Preservation are also presented at the conference, as well as topics that focus on subjects such as spectroscopy, chromatography, and even grant writing.

Pittcon also received Joseph B. Powell, Ph.D., Shell's Chief Scientist, Chemical Engineering, as the 2021 Wallace H. Coulter speaker. The Wallace H. Coulter Lecture is the annual keynote talk at Pittcon and an integral part of the Pittcon Technical Program. The lecture is part of a greater technical program that works to keep the scientific community informed on the latest research and developments, presented by leading scientists from around the world. This year's Coulter lecture was entitled 'The Industrial Laboratory for Energy and Chemicals: Past, Present and Future'.

Industrial laboratories have played an important role in the innovation and commercialization of new technologies. Dr. Powell's lecture presented case studies on technology development, including enhanced oil recovery, development of chemical processes with homogeneous and heterogeneous catalysts and advanced separations, and biofuels and new energies. The breadth of laboratory, pilot and demonstration plant studies required to understand underlying fundamentals and de-risk scale up were also described.

Awards

Each year, Pittcon provides a venue place where scientists who have made outstanding contributions to analytical chemistry and applied spectroscopy are honored. This year, Pittcon featured 15 such awards presented to 17 distinguished scientists from throughout the world.

The Pittsburgh Spectroscopy Award was presented to Dr. Isao Noda of the University of Delaware. Dr. Noda was chosen based on his outstanding contribution in the advanced understanding and applications in the field of spectroscopy, his leadership in the scientific community, and his guidance of young scientists. His research interest is in the broad area of polymer science and vibrational spectroscopy.

The Pittsburgh Analytical Chemistry Award was presented to Dr. Richard Yost of the University of Florida. Dr. Yost is widely recognized as a world leader in the field of mass spectrometry and analytical chemistry, most notably for his co-invention of the triple quadrupole mass spectrometer, which has revolutionized important measurements impacting human health and well-being in the fields drug metabolism, pharmacokinetics, environmental studies, and biological analyses.

The Pittsburgh Conference Achievement Award was presented to Dr. Robbyn Anand of Iowa State University. Dr. Anand and her research group have developed methods for single-cell analysis, electrokinetic separations in complex media, and voltammetry at bipolar electrodes. She is also the founder of the Midwest Retreat for Diversity in Chemistry.

The Pittcon Heritage Award was presented to Dr. Peter Hupe of Agilent Technologies (retired). Dr. Hupe founded Hupe-Busch, which eventually became the Agilent Technologies High Performance Liquid Chromatography (HPLC) Business. He is also being recognized for his technical and business contributions in the field of Preparative Gas Chromatography, his role in the founding of the respected HPLC Series of International Meetings, and his academic achievements as the Chair of Instrumental Analysis and Professor at the Free University of Amsterdam.

Pittcon 2021 also hosted sessions for three awards from the American Chemical Society (ACS) - the ACS Advances in Measurement Science Lectureship Awards, the ACS Analytical Division Satinder Ahuja Award for Young Investigators in Separation Science, and the ACS Award in Analytical Chemistry.

Pittcon 2021 also hosted two award sessions, each from the Society of Electroanalytical Chemistry (SEAC) and the scientific publisher LCGC. These awards are, respectively, the SEAC Royce W. Murray Award and the SEAC Charles N. Reilley Award, and the LCGC Lifetime Achievement in Chromatography Award and LCGC Emerging Leader in Chromatography Award.

Additional awards presented were the Coblentz Society Williams-Wright Award, the Chromatography Forum of the Delaware Valley's Dal Nogare Award, and the Ralph N. Adams Award. Of special note was the Chinese American Chromatography Association (CACA) Award, which celebrated its inaugural presentation at Pittcon 2021.

Exposition

At the exhibition the attendees had the opportunity to interact with the companies participating in the event through a virtual booth. The companies displayed their new products through images that had links to websites with product descriptions and videos. Attendees could also interact virtually with the exhibitors through an online service in real time by text or voice channel.

Exhibitors' virtual booths platform - Pittcon image.

Exhibiting companies included Analytik Jena US, Bruker Corporation, LECO Corporation, Metrohm USA, Milestone Inc., PerkinElmer, Shimadzu Scientific Instruments, Inc., Thermo Fisher Scientific, among many others.

Thermo Fisher's virtual booth - Pittcon image.

Virtual Pittcon will remain on-demand until June 12, 2021. Therefore, if you are registered, you can visit and see all the presentation you may have missed and review the presentation you want.

For more information on the Pittcon 2021 visit: https://pittcon.org/

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BrJAC

SPONSOR REPORT

PDF

This section is dedicated for sponsor responsibility articles.

A robust mass spectrometer for precision medicine – the Orbitrap Exploris 240 mass spectrometer for large-scale plasma protein profiling

Michelle L. Dubuke, Sitara Chauhan, Sarah Trusiak, and Emily I. Chen

Thermo Fisher Scientific, Precision Medicine Science Center, Cambridge, MA

This report was extracted from the Thermo Scientific Application Note 65952

Goal

To demonstrate the reliability and robustness of the Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer for large-scale, untargeted plasma protein profiling.

Keywords: Orbitrap Exploris 240 mass spectrometer, Orbitrap technology, mass spectrometry, Evosep One LC, protein biomarker, plasma workflow, serum workflow, Evosep, high throughput plasma protein profiling workflow

INTRODUCTION

Biomarkers are greatly needed for managing patients with diseases and improving efficacy of biotherapeutics. Blood is an ideal biological material for biomarker discovery because its collection can be done routinely and repeatedly in the clinic. With emerging efforts of establishing large biorepositories, research laboratories race to assemble robust and reproducible mass spectrometry-based workflows that can be scaled up to detect disease relevant plasma protein biomarkers, and can be implemented in the clinic to benefit the affected population.

Previously, we described an automated sample preparation solution that can be utilized to reduce analytical variability by using a liquid handling robotic platform (AN-65727). In this application note, we focus on describing a LC-MS/MS platform that can be easily implemented to conduct large-scale, human cohort plasma protein profiling studies. An easy to operate Evosep One LC was coupled to an Orbitrap Exploris 240 mass spectrometer using the Thermo Scientific™ EASY-Spray™ ionization source and a Thermo Scientific™ EASY-Spray™ HPLC column. We optimized the MS acquisition parameters for short LC gradients (60 SPD), and the resulting MS method is included as a pre-loaded method template (plasma profiling) in the Method Editor for the Orbitrap Exploris 240 MS. Using the preset method (LC and MS), we performed analyses of over 200 digested pooled human serum injections five weeks apart to examine reproducibility and robustness of the LC-MS/MS workflow.

EXPERIMENTAL

Source of chemicals and reagents

Fisher Chemical™ Optima™ LC/MS grade (A955-500 and W6500) acetonitrile and water were used in these experiments. The Thermo Scientific™ Pierce™ Peptide Retention Time Calibration Mixture (PRTC, P/N 99321), Thermo Scientific™ Pierce™ HeLa Protein Digest Standard (P/N 88328), Thermo Scientific™ EasyPep™ Mini MS Sample Preparation Kit (P/N A40006), and EASY-spray HPLC columns (ES903 and ES906) were obtained from Thermo Fisher Scientific. The Evosep One LC, EvoTips, and the Evosep column (EV-1064) used were from Evosep.

Sample preparation

Pooled human non-small cell lung cancer adenocarcinoma serum was purchased from BioIVT. Serum samples were depleted using the Thermo Scientific™ High Select™ Top 14 Abundant Protein Depletion Mini Spin Columns (A36369). Depleted serum samples were reduced, alkylated, trypsin digested, and purified using the EasyPep MS Sample Prep Kit. Concentrations of trypsin digested peptides purified from the EasyPep sample prep column were measured by a Thermo Scientific™ Pierce™ Quantitative Fluorometric Peptide Assay (P/N 23290). PRTC peptides were spiked in biological samples to monitor column stability, peptide retention time, peak width, and mass spectrometer mass accuracy.

Data analysis

A 3-stage data processing workflow in Thermo Scientific™ Proteome Discoverer™ 2.4 software was created to take advantage of the combination of spectral library searching, database searching, and additional modifications commonly found in biological samples all without impacting the false discovery rate (FDR) calculations. The human protein database (SwissProt, December 2018) was used for peptide identification. The search parameter settings for peptide identification are as follows: 10 parts per million (ppm) precursor mass accuracy, 0.02 Dalton (Da) fragment mass accuracy, static modification of carbamidomethyl +57.021 Da (C), and dynamic modification of oxidation +15.995 Da (M). 1% FDR was set as the filtering threshold for protein and peptide identification. In the first processing stage, the MSPep search engine was used to search the acquired data against a Prosit generated spectral library based on the Human SwissProt FASTA file with alkylated cysteine (+57.021 Da) as the only static modification. In the second processing stage, all medium and low confidence PSMs and all unidentified spectra were re-searched using SEQUEST HT against the Human SwissProt FASTA file with oxidation of methionine (+15.995 Da), protein N-terminal acetylation (+42.011 Da), protein N-terminal loss of methionine (-131.040 Da), and protein N-terminal loss of methionine with acetylation of the new N-terminus (-89.030 Da) as the dynamic modification, and alkylation of cysteine (+57.021 Da) as the only static modification. Finally, in the third processing stage, all medium and low confidence PSMs and all unidentified spectra were re-searched using SEQUEST against the Human SwissProt FASTA file with asparagine/glutamine deamidation (+0.984 Da), peptide N-terminal glutamine to pyro-glutamic acid (-17.027 Da), and peptide N-terminal carbamylation (+43.006 Da) as dynamic modifications, in addition to all of the protein N-terminal and static modifications listed above. A 1% peptide FDR was set for all search nodes.

Label-free quantification (LFQ) comparison of detected proteins was processed and visualized in Proteome Discoverer software, which applies normalization of the total abundance values for each run across all files. After aggregating all the normalized abundance values, Proteome Discoverer software scales the abundance values of each sample so that the average of all samples is 100.

Skyline software was used to monitor the retention time, peak area, full width half maximum (FWHM), and mass accuracy of the spiked PRTC peptides during the full acquisition run-time. Prism 8.4 software was used to perform statistical analysis.

RESULTS AND DISCUSSION

The Orbitrap Exploris 240 mass spectrometer showed excellent inter-laboratory reproducibility

With its small footprint, the Orbitrap Exploris 240 mass spectrometer introduces different hardware such as the S-lens interface to balance the robustness and sensitivity for translational research with the mass accuracy expected of Thermo Scientific™ Orbitrap™ mass spectrometers (Figure 1A). In addition to its small footprint, high instrument-to-instrument reproducibility allows researchers to increase throughput of data acquisition for large-scale studies. To examine the inter-laboratory reproducibility of the Orbitrap Exploris 240 MS, we ran the same system suitability method on four different Orbitrap Exploris 240 MS instruments at three different sites. As shown in the Figure 1B, we found similar performance of these four Orbitrap Exploris 240 mass spectrometers at both protein identification (2.58% CV) and peptide identification (5.89% CV).

Plasma profiling method template is pre-loaded on the Orbitrap Exploris 240 MS to harmonize data acquisition parameters and benchmark instrument performance

We optimized the MS parameters to acquire data from digested plasma/serum samples using Evosep LC gradients and the Orbitrap Exploris 240 MS. The optimized MS method is included as a pre-loaded method template (plasma profiling) in the Method Editor of the Orbitrap Exploris 240 MS (Figure 2).

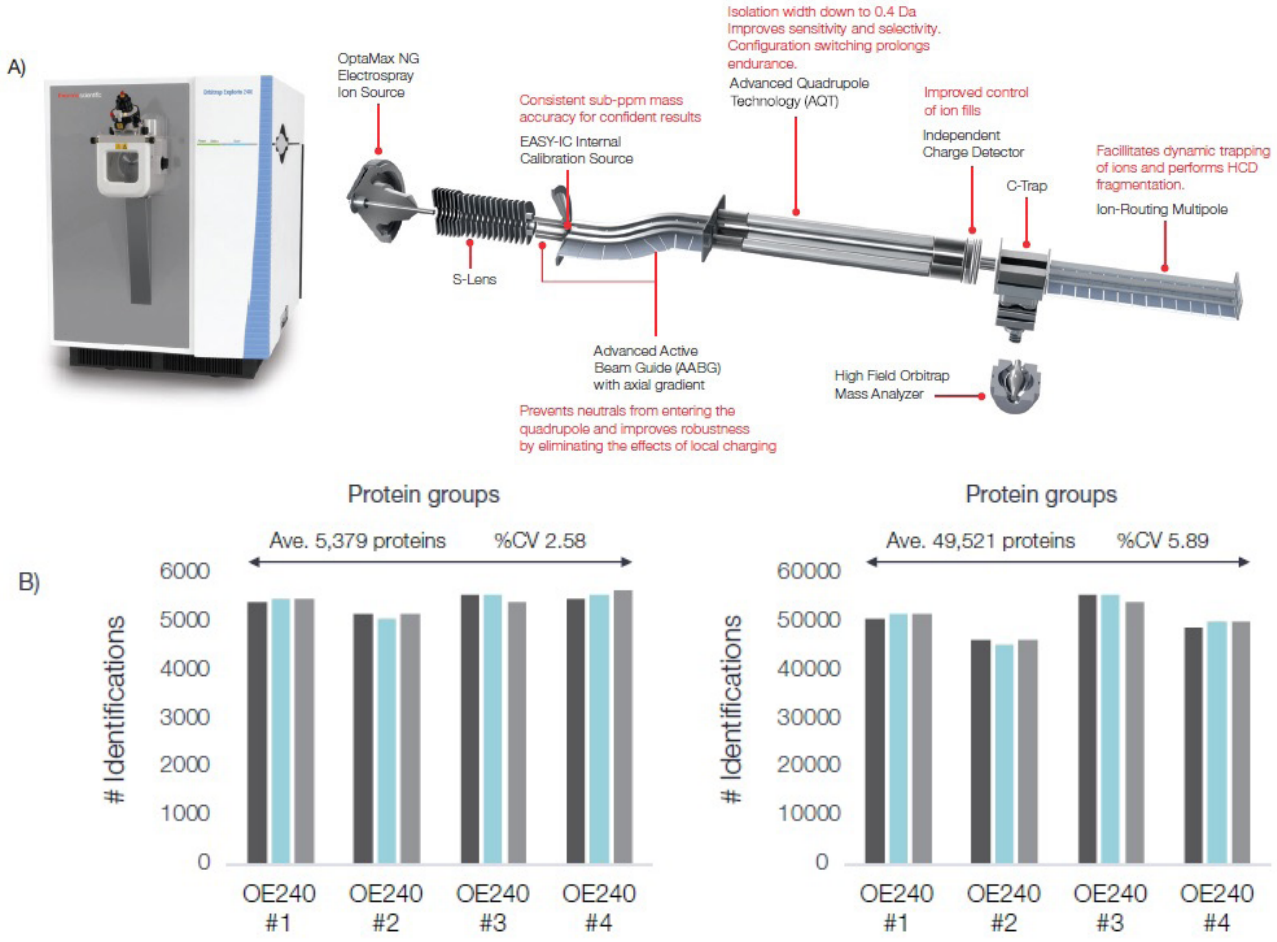
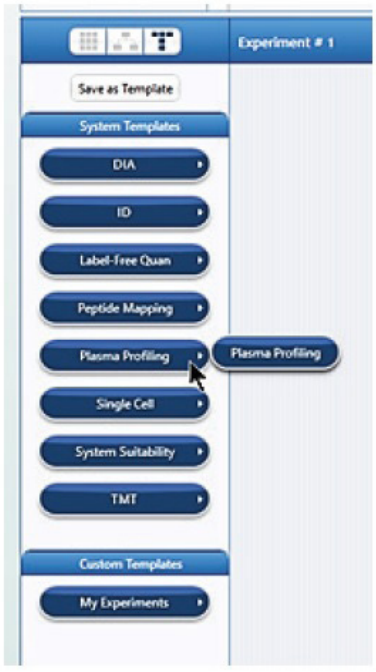


Figure 1. Instrument schematic and inter-lab performance of the Orbitrap Exploris 240 mass spectrometer. A) Schematic of the ion path of the Orbitrap Exploris 240 MS highlighting important features of the system for improved performance and reliability. B) Inter-laboratory study of the Orbitrap Exploris 240 MS – 1 µg of HeLa digest separated on an EASY-Spray HPLC column (ES903) (75 um x 50 cm) coupled with a Thermo Scientific™ UltiMate™ 3000 HPLC system over a 60 min LC gradient at 300 nL/min flow rate.

A)



B)

Global parmeters	
Source	EasySpray
Ion transfer tube temp	280 °C
Spray voltage	1.8 kV
MS parameters	
Orbitrap resolution	60,000 at *m/z* 200
RF lens	80%
AGC target	300%
Max inject time	Auto
MS/MS parameters	
Intensity threshold	1 x 10^5
Resolution	15,000 at *m/z* 200
AGC target	Standard (100%)
Maximum IT	Auto
Top N	20
Isolation window	1.6 *m/z*
Normalized collision energy	30%
Dynamic exclusion	25s

Figure 2. The plasma protein profiling MS method using short gradients (60 SPD and 30 SPD) from Evosep LC is pre-loaded as a method template in the method editor of the Orbitrap Exploris 240 MS. A) A screenshot of the plasma profiling method template in the method editor. B) Detailed MS parameters of the plasma profiling method template.

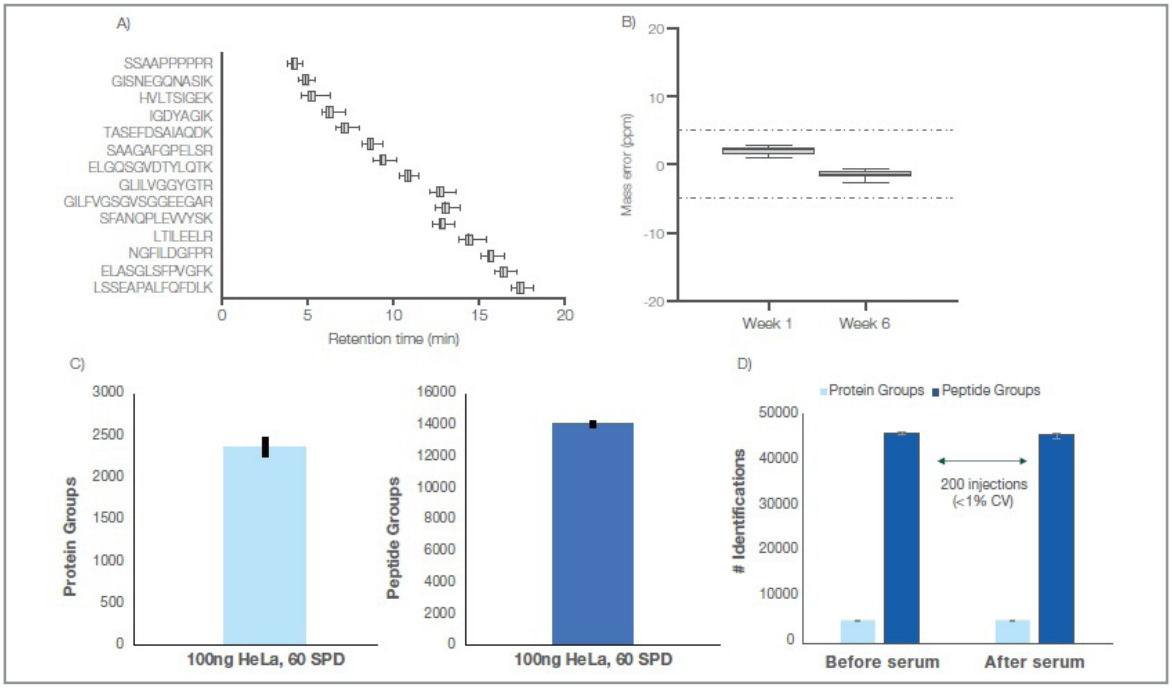


Figure 3. Analytical performance of the EvoSep One LC coupled with the Orbitrap Exploris 240 MS. A) Retention time stability of the PRTC peptides (50 fmol) over 200 runs. B) Mass stability of the PRTC peptides over 200 runs in two non-consecutive weeks (over 100 injections summarized in each time point). C) Protein and peptide identifications at 1 % FDR from 100 ng HeLa digest. D) Results from system suitability tests before and after 200 consecutive injection of human serum.

Using the pre-loaded method (LC and MS), we analyzed commercially available HeLa digest standard to validate the the performance of the Evosep LC and Orbitrap Exploris 240 MS (quality assurance or QA) and to quality control results from using the workflow over time (QC). We also spiked the PRTC peptide standard in human serum to quickly monitor the column integrity and MS stability during the experimental period. Using the 60 SPD LC method and EASY-Spray HPLC column (ES906), we observed minimal retention time shift (<5% CV) (Figure 3A) and mass accuracy of no more than +/- 5ppm over 200 injections that were five weeks apart (Figure 3B). Using the same LC-MS method, we detected close to 2,500 proteins and over 14,000 peptides from 100 ng of HeLa digest standard (Figure 3C). Lastly, to demonstrate the robustness of the Orbitrap Exploris 240 MS for plasma proteome biofluid analysis, we compared results from system suitability tests (method described in Figure 1) before and after 200 consecutive injections of human serum (200 ng per injection) and found good repeatibility (<1% CV difference) without recalibration of the mass spectrometer (Figure 3D).

The workflow solution of coupling the Evosep LC to the Orbitrap Exploris 240 MS showed robust and reproducible profiling of plasma proteins over hundreds of injections

In addition to robust analytical performance shown in Figure 3, we observed excellent reproducibility of protein and peptide identifications over 100 injections of human serum performed five weeks apart using this workflow at the 60 SPD throughput (Figure 4).

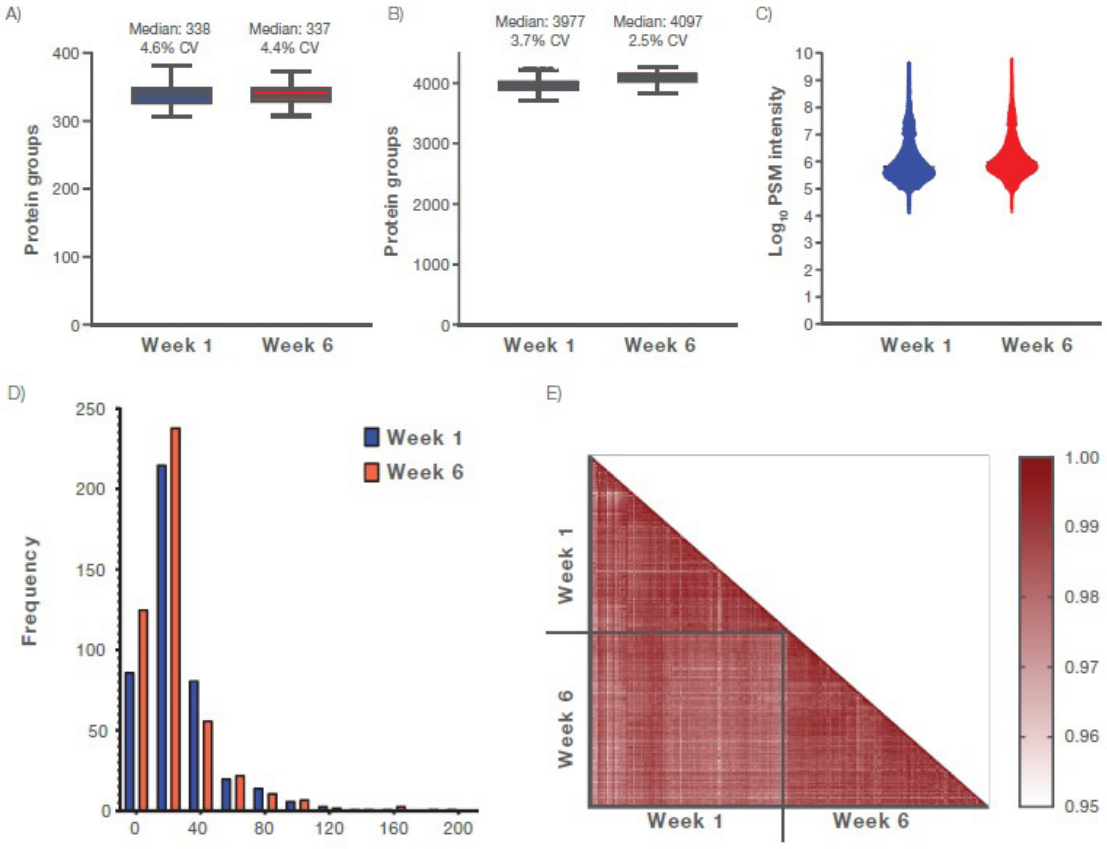


Figure 4. Robust and reproducible analytical performance of the plasma protein profiling workflow. A) Box-and-whisker plots show median and maximum 1.5 interquartile range (IQR) of protein (1% FDR) from 100 consecutive runs of 200 ng Top14 depleted serum at two time points (200 injections total). B) Peptide identification (1% FDR) from 100 consecutive runs of 200 ng Top14 depleted serum at two time points (200 injections total). C) Distribution of annotated PSMs intensities at each time point. D) A histogram of % CVs from LFQ of 100 Top14 depleted serum injections at each time point. E) Correlation of 200 runs from two different time points based on LFQ.

On average, 338 proteins (3977 peptides) were identified in week 1 from 100 injections of 200 ng Top14 depleted serum with less than 5% CV (Figure 4A & 4B). 337 proteins (4097 peptides) were identified in week 6 also with less than 5% CV from another batch of 100 injections of 200 ng Top14 depleted serum (Figure 4A & B). Moreover, over 5 orders of magnitude of dynamic range of PSMs was detected from over 200 injections of Top14 depleted human serum (Figure 4C).

While the depth of proteome coverage is important, robust quantification is equally important to identify actionable biomarkers. To evaluate the quantification consistency, we combined replicate runs from each time period using a feature mapping consensus workflow and looked at the distribution of the cumulative CV of LFQ. We found more than 70% of proteins were quantified with less than 20% CV from 100 replicate injections at each time point (Figure 4D) and LFQ values were highly correlative between week 1 and week 6 runs (Figure 4E). Our results showed good repeatibility (runs within the week) and reproducibility (runs between two weeks) of ths workflow for large-scale studies.

Varying loading amounts and gradient lengths allowed reproducible characterization of 500 plasma proteins

To determine if one can increase the depth of plasma proteome coverage by increasing the loading amount and gradient length, we tested two different loading amounts (200 ng and 500 ng) and two different gradient lengths (21 min and 44 min). We found that 500 plasma proteins could be detected by loading 500 ng of Top 14 depleted human serum with the 44-minute gradient (30 SPD) method and results showed consistent quantitative with a consistent quantitative performance. Among these 500 plasma proteins, many of them are known FDA approved biomarkers. We highlighted some of these FDA-approved biomarkers across different concentrations (Figure 5). Ultimately, our results demonstrated that the workflow solution can be scaled based on the user's need for analytical throughput, sample availability, and biological/ disease relevance for large-scale studies.

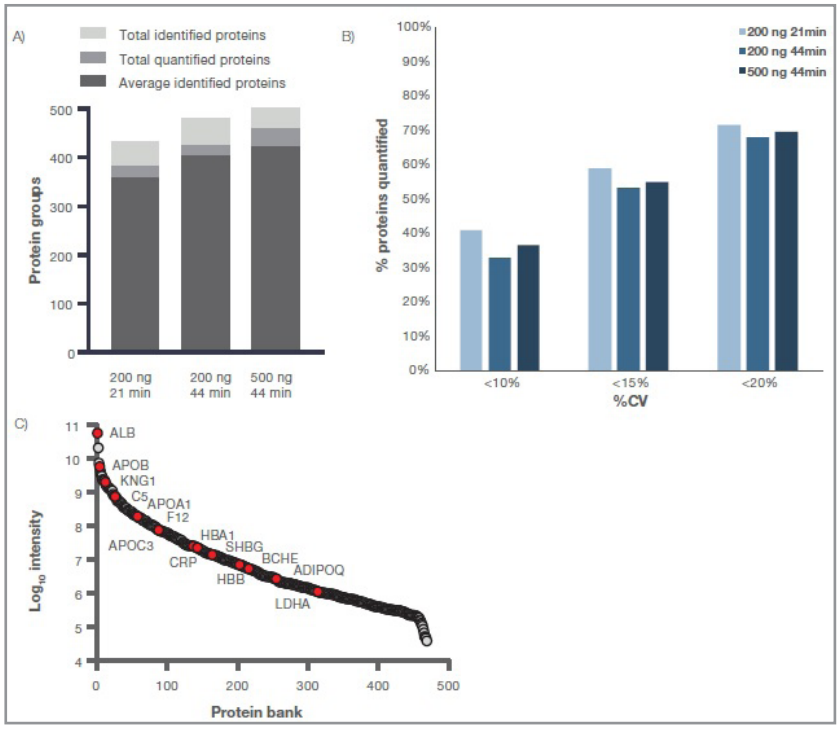


Figure 5. Reproducible detection of 500 plasma proteins from Top14 depleted human serum by varying loading amounts and gradient lengths. A) 200 or 500 ng of Top14 depleted human serum were loaded on Evotips for LC MS/MS analysis using the Orbitrap Exploris 240 MS and the 60 SPD (21 minute) or 30 SPD (44 minutes) Evosep LC methods. Three types of numbers were reported: averaged identified proteins (per run), total quantifiable proteins (n=5), and total identified proteins (n=5). Feature mapping was applied to repeated injections (n=5) to determine the total number of proteins identified and quantified. B) LFQ analysis of plasma proteins from replicate injections (n=5) of different loading amounts in combination with the gradient length. Percentages of protein quantified with less than 10, 15, and 20% CV were shown. C) Known FDA approved biomarkers across a range of concentrations were highlighted among 500 plasma proteins identified.

SUMMARY

In summary, we presented a robust, reproducible, and high-throughput workflow solution for plasma protein profiling, which can be integrated with our automated sample preparation solution to support biomarker discovery for large-scale human cohort studies. This analytical solution includes a robust and easy to use Orbitrap Exploris 240 mass spectrometer for qualitative and quantitative characterization of human plasma proteins from healthy and diseased individuals. Our results demonstrated:

- The Orbitrap Exploris 240 mass spectrometer showed excellent inter-laboratory reproducibility.
- The plasma profiling method template, which is pre-loaded on the Orbitrap Exploris 240 MS, could be used to harmonize data acquisition parameters and benchmark the instrument performance.
- The workflow solution of coupling the Evosep LC with the Orbitrap Exploris 240 MS showed robust and reproducible profiling of plasma proteins over hundreds of injections.
- The Orbitrap Exploris 240 MS is robust and easy to maintain, which is ideal for routine plasma protein profiling analysis.
- The workflow solution can be scaled based on the user's need for analytical throughput, sample availability, and biological/disease relevance for large-scale studies.

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Brazilian Journal of Analytical Chemistry
2021, Volume 8, Issue 31, pp 130–137

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Total elemental analysis in clinical research using the Thermo Scientific iCAP TQ ICP-MS

Tomoko Vincent

Applications SpecialistThermo Fisher Scientific,Bremen, Germany

This report was extracted from the Thermo Scientific Technical Note 43283

INTRODUCTION

Trace element analysis of biological samples provides significant information to support clinical research and forensic toxicology. An interesting example of trace elemental analysis for clinical research purposes is exploring the degradation of titanium based orthopedic and dental implants in humans. Following recent research on the possible carcinogenic effects of titanium dioxide the fate of titanium in the human body has become a growing area of clinical research focus. To support this there is a need for the development of robust analytical methods for the identification and quantification of titanium in a range of samples such as human body fluids and organs. However, the development of such a method is challenging due to the low concentration of titanium in these types of samples and the potential isobaric interferences which single quadrupole ICP-MS cannot remove.

Advancements in ICP-MS technology have led to the development of triple quadrupole (TQ) ICP-MS instruments, which have the required sensitivity as well as the capability to resolve isobaric interferences resulting from polyatomic and isotopic species.

This technical note focuses on the development of a robust method for the analysis of titanium and other trace elements in human serum reference materials using the Thermo Scientific™ iCAP™ TQ ICP-MS.

Keywords: Clinical research, isobaric interferences, serum, titanium, trace elemental analysis, urine

Sample preparation

The certified reference materials (Seronorm™ Trace Elements in Serum L-1 and L-2, SERO, Norway) and volunteered human urine were gravimetrically diluted by a factor of ten in pre-cleaned (72 hours in 2% nitric acid, washed in ultra-pure water) polypropylene bottles with nitric acid (0.5% m/m Fisher Scientific) and tetramethylammonium hydroxide (TMAH, 2% m/m SIGMA-ALDRICH) in ultra-pure water (18 MΩ cm). A calibration blank, a series of standards and a Quality Control (QC) were prepared using the same procedure, replacing the certified reference material with single element standards (SPEX CertiPrep). The elements and final concentrations are shown in Table 1. All samples and standards were spiked with an internal standard mix (10 µg L^{-1} Ge, Y, Rh, Te and Bi).

Instrumentation

The iCAP TQ ICP-MS consists of three quadrupoles to improve interference removal compared to single quadrupole (SQ) ICP-MS. The first quadrupole (Q1) rejects all unwanted ions such as precursor species that may recombine in the collision / reaction cell (CRC) and subsequently interfere with the target analyte.

The second quadrupole (Q2) is used to selectively shift the interference or target analyte with an appropriate reaction gas.

The third quadrupole (Q3) isolates the product ion and removes any remaining interferences through a second stage of mass filtration allowing for interference free analysis of the analyte.

In this analytical method, TQ mass shift mode was used for the target element titanium (Figure 1). Titanium was reacted with ammonia gas (NH_3) to create the cluster ($^{48}Ti(NH_3)_3NH$) at *m/z* 114 in Q2.

Table 1. Elements analyzed and concentration of calibration standards and the QC

	Major STD1	Major STD2	Major STD3	Major STD4	QC CCVs (mg L^{-1})
Ca	5	10	25	50	10
Fe	0.1	0.2	0.5	1	0.2
Mg	5	10	25	50	10
P	5	10	25	50	10
K	5	10	25	50	10
S	50	250	500	1000	100
Na	50	100	250	500	100
	Minor STD1	**Minor STD2**	**Minor STD3**	**Minor STD4**	**QC CCVs (µg L^{-1})**
Sb	0.5	1	2.5	5	1
As	0.1	0.2	0.5	1	0.2
Ba	5	10	25	50	10
Cd	0.1	0.2	0.5	1	0.2
B	5	10	25	50	10
I	5	10	25	50	10
Pb	0.1	0.2	0.5	1	0.2
Li	500	1000	2500	5000	1000
Mo	0.1	0.2	0.5	1	0.2
Rb	0.5	1	2.5	5	1
Sr	5	10	25	50	10
Ti	0.5	1	2.5	5	1
U	0.005	0.01	0.025	0.05	0.01
V	0.1	0.2	0.5	1	0.2
Zn	50	100	250	500	100
Se	0.1	0.2	0.5	1	0.2
Al	50	100	250	500	100
Cr	0.5	1	2.5	5	1
Mn	5	10	25	50	10
Ni	5	10	25	50	10
Co	0.1	0.2	0.5	1	0.2

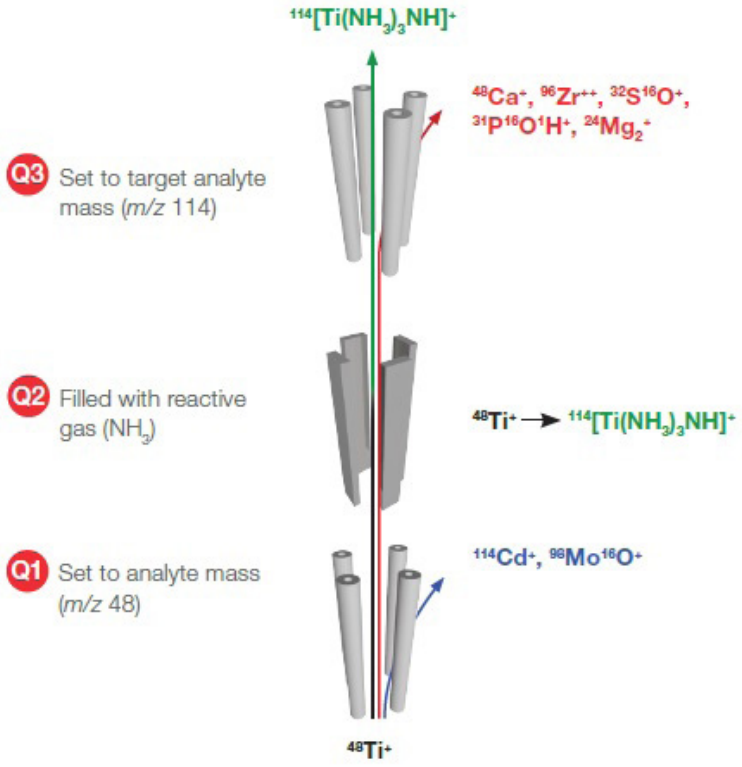

Figure 1. TQ mass shift mode for titanium.

The iCAP TQ ICP-MS also has the ability to operate in single quadrupole mode when advanced interference removal is not required. For many of the analytes in this analytical method, analysis using pure He as a collision gas and Kinetic Energy Discrimination (KED) mode is sufficient.

METHOD DEVELOPMENT AND ANALYSIS

The sample introduction system used is detailed in Table 2. The operating parameters were optimized by the default autotune procedure in the Thermo Scientific™ Qtegra™ Intelligent Scientific Data System™ (ISDS) software that controls the iCAP TQ ICP-MS.

Table 2. Instrument configuration and operating parameters

Parameter	Value	
Nebulizer	PFA nebulizer 0.2 mL min^{-1}, pumped at 40 rpm	
Spraychamber	Quartz cyclonic spraychamber cooled at 3 °C	
Injector	2.5 mm Quartz	
Interface	High matrix (3.5 mm), Ni cones	
RF power	1550 W	
Nebulizer gas flow	1.001 L min^{-1}	
QCell setting	**SQ-KED**	**TQ-NH_3**
Gas flow	4.5 mL min^{-1}	0.29 mL min^{-1}
CR Bias	-21 V	-7.9 V
Q3 Bias	-18 V	-11 V
Dwell time	0.2 seconds per analyte, 5 sweeps	

The optimum measurement mode for each analyte was automatically selected by the Reaction Finder method development assistant within Qtegra ISDS Software. Additional measurement modes were selected for Ti to compare the efficiency of the interference removal in TQ mass shift mode:

SQ-KED – single quadrupole mode with CRC pressurized with He, KED applied, no filter on Q1 and Q3 set to mass 48

SQ-NH_3 – single quadrupole mode with CRC pressurized with NH_3, no filter on Q1 and Q3 set to product ion mass of 114

TQ-NH_3 – triple quadrupole mode with CRC pressurized with NH_3, Q1 set to mass 48 and Q3 set to product ion mass of 114

An internal standard was also associated with each analyte on a mass basis. Internal standard association and measurement modes for the final analysis are shown in Table 3.

Table 3. Measurement modes and internal standards used for each element

	Measurement mode	Analyte/Product Ion mass	Internal standard
Na	SQ-KED	23	^{74}Ge
Mg	SQ-KED	24	^{74}Ge
P	SQ-KED	31	^{74}Ge
S	SQ-KED	34	^{74}Ge
K	SQ-KED	39	^{74}Ge
Ca	SQ-KED	44	^{74}Ge
Fe	SQ-KED	56	^{74}Ge
Li	SQ-KED	7	^{74}Ge
B	SQ-KED	11	^{74}Ge
Al	SQ-KED	27	^{74}Ge
V	SQ-KED	51	^{74}Ge
Cr	SQ-KED	52	^{74}Ge
Mn	SQ-KED	55	^{74}Ge
Co	SQ-KED	59	^{74}Ge
Ni	SQ-KED	60	^{89}Y
Zn	SQ-KED	66	^{74}Ge
As	SQ-KED	75	^{89}Y
Se	SQ-KED	78	^{74}Ge
Rb	SQ-KED	85	^{89}Y
Sr	SQ-KED	88	^{89}Y
Mo	SQ-KED	95	^{103}Rh
Cd	SQ-KED	111	^{103}Rh
Ti	TQ-NH_3	114	$^{74}Ge^{14}N^{1}H_2$
Sb	SQ-KED	121	^{125}Te
I	SQ-KED	127	^{125}Te
Ba	SQ-KED	138	^{103}Rh
Pb	SQ-KED	208	^{209}Bi
U	SQ-KED	238	^{209}Bi

The sample analysis consisted of an external calibration curve followed by replicate analyses of the urine and serum samples. Continuous calibration verication (CCV) samples were analyzed every 10 samples and a total of 124 samples were measured during the analysis. All samples were presented for analysis using a Teledyne CETAC Technologies ASX-560 Autosampler. The rinse solution used on the autosampler between samples was the same as the diluent (0.5% HNO_3/2% TMAH).

RESULTS

Titanium in biological samples is particularly challenging due to the isobaric overlap of ^{48}Ca and polyatomic interferences from SO^+ and POH^+. To evaluate the efficiency of interference removal, three different measurement modes (SQ-KED, SQ-NH_3 or TQ-NH_3) were used to measure a certified reference material (CRM). The results for titanium quantification in both Serum L-1 and L-2 for each of the measurement modes are shown in Table 4. The result from the TQ-NH_3 is the most accurate when compared to the reported values for these materials. The Reaction Finder method development assistant automatically selects this mode for analysis.

To demonstrate the improved interference removal, the effect of the presence of cadmium in the sample was investigated. A ten-fold diluted serum sample and a10 mg L^{-1} cadmium standard were analyzed with TQ-NH_3 mode and spectra recorded. The ten-fold diluted serum sample shows a typical spectral fingerprint associated with the creation of $Ti(NH_3)_3X^+$ clusters (Figure 2). The 10 mg L^{-1} cadmium standard (Figure 3) measured with the same conditions and measurement mode, shows no presence of Cd in the spectra (only residual counts from the analysis of the serum), the Cd having been eliminated by Q1. This prevents any trace Cd in the sample from interfering with the analysis of Ti at *m/z* 114.

Table 4. Comparison of titanium results in the serum CRMs with different measurement modes

	Ti SQ-KED, µg L^{-1}	Ti SQ-NH_3, µg L^{-1}	Ti TQ-NH_3, µg L^{-1}	Ti Reported Value, µg L^{-1}
Serum L-1	167	1800	6.64	6.8
Serum L-2	262	1850	6.38	6.8

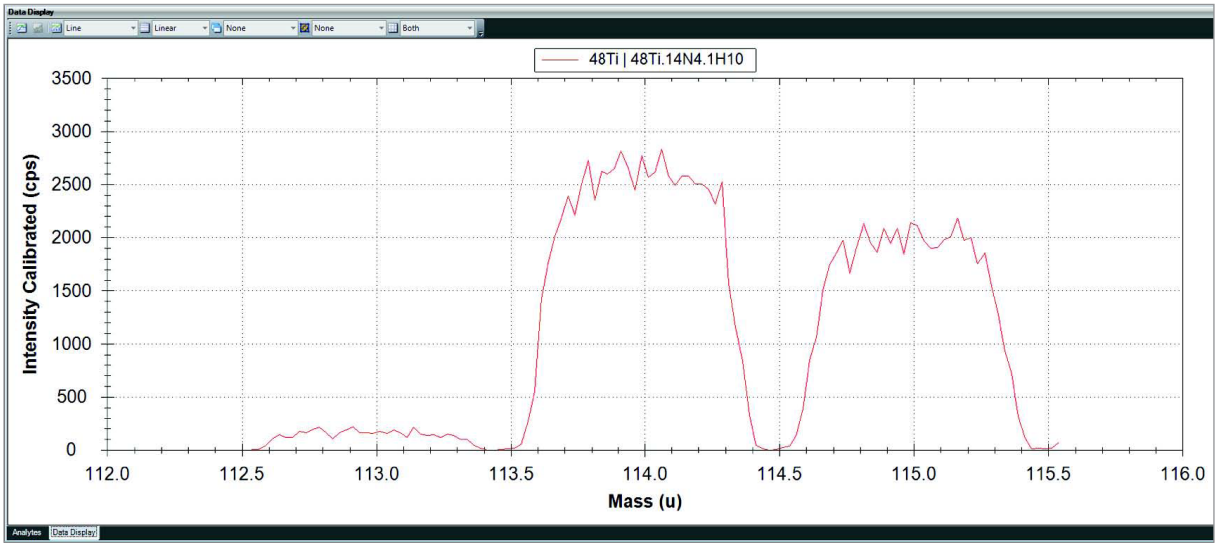

Figure 2. Spectra of serum sample (diluted 10-fold).

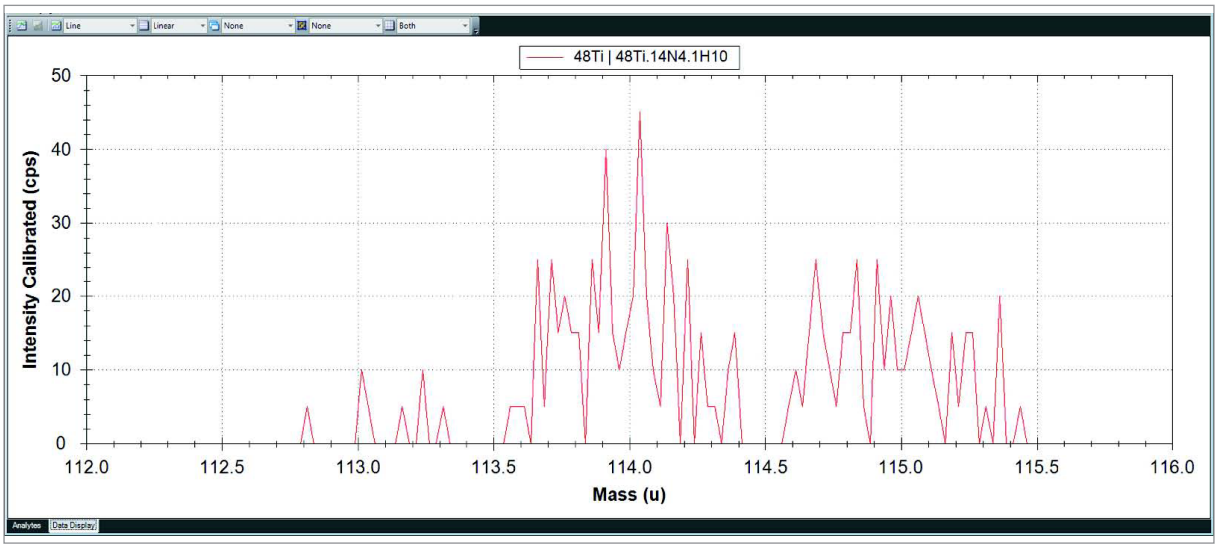

Figure 3. Spectra of 10 mg L^{-1} cadmium.

Selected calibrations for the multi-elemental analysis are shown in Figures 4 to 7. The calibration curve for the titanium with TQ mass shift mode (Figure 4) shows high sensitivity at 3903 cps/µg L^{-1} and excellent linearity with an R^2 value of 0.9998 for the calibration consisting of a blank and four standards (0, 0.5, 1, 2.5 and 5 µg L^{-1}).

All other elements analytes apart from Ti were analyzed using SQ-KED. When analyzing in this mode the first quadrupole simply acts as an ion guide. Calibration curves for arsenic and selenium using the SQ-KED mode are shown in Figures 5 and 6 respectively with the concentration range of 0.1 to 1 µg L^{-1}. The calibration curve for sulfur (Figure 7) is performed with the concentration range of 50 to 1000 mg L^{-1}. These are typical elements and typical concentration ranges expected in clinical research.

The results of the multi-elemental analysis of the serum CRMs are shown in Table 5. Measured values for the analytes in the reference materials are in good agreement with the reference or reported values. These values cover a wide concentration range from sub ppb to low % levels, demonstrating the importance of the dynamic range of the iCAP TQ ICP-MS. A urine sample, analyzed in the same analytical run, was found to contain typical elemental concentrations.

The detection limit (LOD) was determined based on three times the standard deviation of a ten-replicate measurement of the calibration blank. The method detection limits (MDL) for all of the elements analyzed were calculated by multiplying the LOD by the dilution factor (1:10) (Table 5). The LODs for all the elements of interest are well below the target levels required for clinical research sample analysis.

Table 5. Results for the serum CRMs and urine sample. The analyte labeled with a * are reported at mg L^{-1}, all other results are reported in µg L^{-1}.

	LOD	MDL	Serum L-1		Serum L-1		Urine
			Measured	Reference or reported value	Measured	Reference or reported value	Measured
Na*	0.0027	0.027	2743	2330-3504	3255	2820-4241	2977
Mg*	0.0001	0.0010	21.0	13.4-20.1	39.7	27.1-40.7	85.6
P*	0.0008	0.08	52.3	43.3-65.1	120	88-132	710
S*	0.145	1.3800	1100	1008	1495	1335	476
K*	0.0021	0.02	150	101-153	260	176-265	1946
Ca*	0.002	0.0200	90.1	69-104	124	95-143	99.8
Fe*	0.00002	0.00023	1.64	1.17-1.77	2.18	1.72-2.58	0.005

Table 5. Results for the serum CRMs and urine sample. The analyte labeled with a * are reported at mg L^{-1}, all other results are reported in µg L^{-1}. (Continuation)

	LOD	MDL	Serum L-1		Serum L-1		Urine
			Measured	Reference or reported value	Measured	Reference or reported value	Measured
Li	1.13	11.2920	5778	4202-6320	10806	7739-11639	22.4
B	0.67	6.746	70.1	79.4	87	82.1	1548
Al	0.20	1.9670	54.2	25.2-75.7	122	96-144	13.7
V	0.002	0.022	1.04	1.10	1.26	1.10	0.229
Cr	0.008	0.0800	1.70	1.30-3.05	5.20	4.00-7.50	0.838
Mn	0.008	0.084	10.7	7.9-11.9	14.2	11.6-17.4	0.914
Co	0.0001	0.0010	1.38	0.67-1.57	2.16	2.13-3.97	0.027
Ni	0.006	0.055	6.26	3.38-7.9	9.41	7.9-11.9	1.45
Zn	0.051	0.5130	1052	844-1269	1527	1404-1831	359
As	0.002	0.018	0.383	0.400	0.374	0.380	1.31
Se	0.010	0.1000	80.8	51-120	124	95-176	7.31
Rb	0.004	0.035	4.20	4.40	8.70	8.70	812
Sr	0.006	0.0570	95.7	95.0	106	110	89.2
Mo	0.005	0.048	0.710	0.760	1.20	1.21	7.62
Cd	0.001	0.0100	0.130	0.130	0.140	0.140	0.229
Ti	0.002	0.02	6.64	6.80	6.38	6.80	0.151
Sb	0.006	0.0600	11.6	10.4	16.1	15.0	0.040
I	0.022	0.219	75.5	71.8	69.9	60.9	82.8
Ba	0.003	0.0300	172	190	133	139	2.09
Pb	0.0007	0.007	0.370	0.400	0.666	0.660	0.446
U	0.0001	0.0010	0.288	0.302	0.357	0.359	0.020

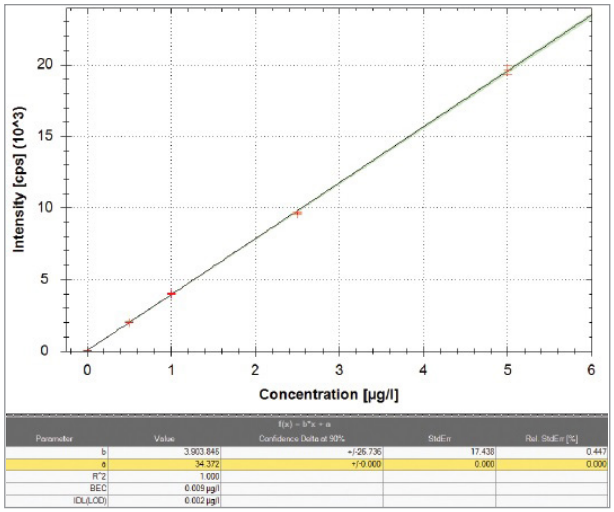

Figure 4. Calibration curve for titanium.

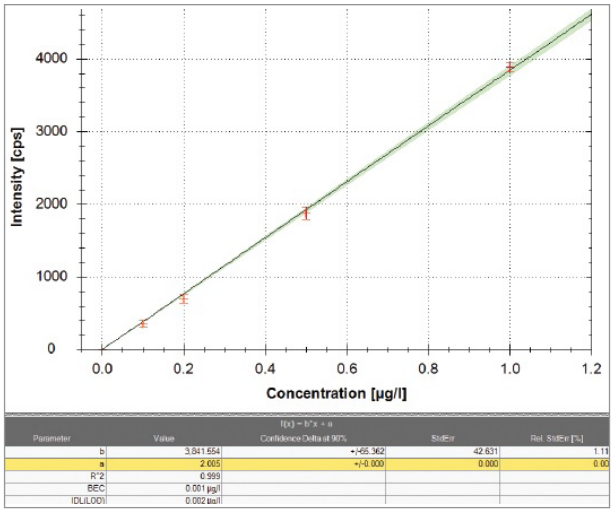

Figure 5. Calibration curve for arsenic.

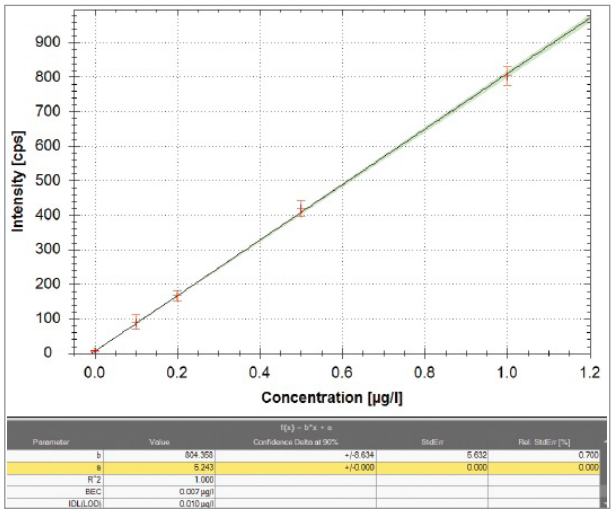

Figure 6. Calibration curve for selenium.

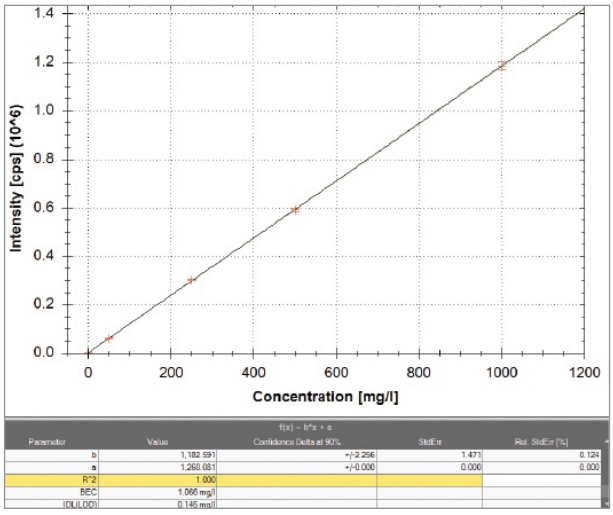

Figure 7. Calibration curve for sulfur.

The average results of the ongoing QC test over a period of eight hours (with a total of nine QC samples being measured) are shown in Figure 8. Average recoveries lie between 95 and 110% with standard deviations typically less than 2% (apart from B, As and Se where the SD was < 4% due to lower sensitivity). These results demonstrate the long term stability of the instrument when analyzing high matrix biological samples.

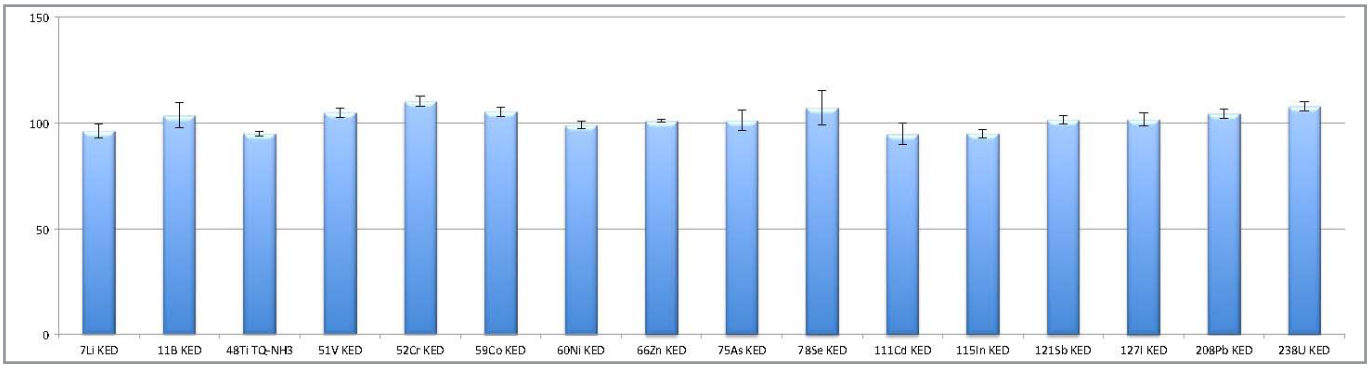

Figure 8. Calibration check verification standards (CCVs) measured during the analysis.

CONCLUSION

The Thermo Scientific iCAP TQ ICP-MS provides excellent performance for the determination of trace element analysis in biological samples making it ideal for clinical research. One key investigation is the degradation of metal-on-metal hip replacement implants, where Ti is often a component and where accurate analysis is problematic using SQ-ICP-MS.

With the iCAP TQ ICP-MS, powerful triple quadrupole technology provides the advanced performance required for the sensitive and accurate determination of Ti and other trace elements in complex samples, whilst the Reaction Finder tool allows for simple method setup by automatically selecting analytes of interest.

Find out more at thermofisher.com/TQ-ICP-MS

This Sponsor Report is the responsibility of Thermo Fisher Scientific.

Brazilian Journal of Analytical Chemistry
2021, Volume 8, Issue 31, pp 138–142

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SPONSOR REPORT

PDF

This section is dedicated for sponsor responsibility articles.

Preparation of Biological Samples for Trace Metal Analysis

Acid digestion of samples using ultrapure quartz inserts with low acid volumes ICP and AAS metal analysis

This report was extracted from the Milestone Industry Report: ETHOS UP / Biological

INTRODUCTION

Clinical laboratory testing plays a crucial role in the detection, diagnosis, and treatment of disease. Clinical laboratory personnel examine and analyze the chemical content of fluids. They test for drug levels in the blood that show how a patient is responding to treatment. They also prepare specimens for examination. With increasing automation and the use of computer technology, the work of technologists and technicians has become less hands-on and more analytical. The complexity of tests performed implicates the need of technological advanced instrumentation. Many laboratories often need to perform analysis of low-level trace metal samples. This is due to the small sample quantity they have available, since it often comes from a biopsy. Many laboratories specialized in analyzing biological materials are equipping their lab with instrumentation suitable for trace analysis, such as ICP-OES or ICP-MS.

In these conditions, sample preparation becomes a crucial operation before analysis. This is why Milestone has developed new micro-inserts for SK-15 rotor, capable to prepare samples employing small volume of acids.

Micro-inserts, based on so-called Vessel-Inside-Vessel Technology, are smaller secondary vials that can be placed inside the primary high-pressure microwave vessel of SK-15 rotor.

This configuration reduces the amount of acid required for digestion to near stoichiometric quantities, which reduces the dilution factor and decreases the final blank level.

EXPERIMENTAL

The objective is that of completing a digestion of biological sample (with an internal standard), with a maximum of 2 mL of nitric acid and to verify the recoveries. Moreover, we will test (with a different sample) that no cross contamination occurs

INSTRUMENT

The ETHOS UP matches the main requirements of many laboratories, thanks to its unique benefits, such as:

- High productivity
- Ease of use
- High safety
- High flexibility

The Milestone ETHOS UP is a very flexible and high-performing platform used to prepare samples for trace elements and routine analysis. The ETHOS UP is available with multiple configurations. The most suitable one for employing micro-inserts is the SK-15 high-pressure rotor.

The SK-15 rotor works with the Milestone “vent-and-reseal” technology for controlling and limiting the internal pressure of each vessel.

Figure 1. Milestone's ETHOS UP.

Figure 2. SK-15 easyTEMP High Pressure Rotor.

SK-15 HIGH PRESSURE ROTOR

The SK-15 perfectly matches the laboratories needs to determine trace elements, thanks to its capability to digest large sample amount and its high temperature/pressure capabilities.

The 15 positions high-pressure rotor is safely controlled via direct temperature sensor that constantly controls the digestion temperature during the run, ensuring perfect digestion of even the most difficult and reactive samples.

Micro-Inserts Technology (Vessel-Inside-Vessel Technology)

Below the inserts configuration used for the test.

Description	Picture	Working volume
TFM rack for 3 micro-inserts (PH00054) + Quartz vials (QB00039) or Teflon vials (MKE0006A) or Glass vial (70017)		4 mL ≤ Volume ≥ 1 mL

SAMPLE PREPARATION AND ICP-OES PARAMETERS

Test 1

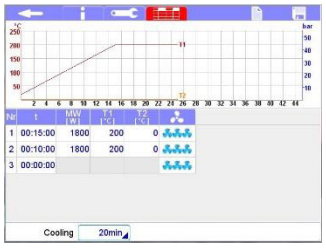
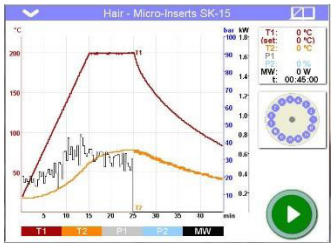
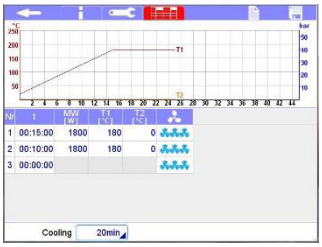
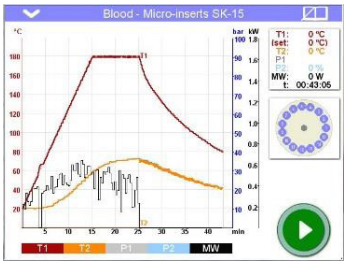
The micro-sampling inserts have been used to digest Hair samples, with 50 ppb of an internal standard containing the following elements: As, Cd, Cr, Pb, Se, Ni, Mn.

Test 2

We performed a second test with a sample of Animal Blood (non-certified), to check that any cross contaminations occur between each micro-insert vials.

Here is the list of samples that we have performed in micro-inserts configurations:

	Sample Name	Sample weight	Reagents into the vial	Reagents into the SK 15 vessel	Micro-sampling config.
Test 1	Human hair IAEA -085	100 mg	HNO_3 (2 mL)	H_2O dist. -10 mL	TFM rack for 3 micro-inserts. Quartz vials
Test 2	Animal Blood sample	100 mg	HNO_3-(2 mL)	H_2O dist. -10 mL	TFM rack for 3 micro-inserts. Quartz vials

	Sample	Method	Temperature profile
Test 1	Human hair IAEA-085		
Test 2	Animal blood		

ICP-OES PARAMETERS, AGILENT ICP-OES (710 SERIES)

Power	1.30 kW
Plasma Flow	15.0 L/min
Auxiliary Flow	1.50 L/min
Nebulizer Flow	0.75 L/min
Replicate read time	10 s
Instrument stabilization delay	15 s
Sample Uptake Delay	30 s
Pump Rate	15 rpm
Rinse Time	10 s
Replicates	3

ANALYTICAL RESULTS

Recovery Tests with Human Hair (spike recovery results of 50 ppb As, Cd, Cr, Pb, Se, Ni and Mn)
(Results expressed in ppb)

Replicate	As	Cd	Cr	Pb	Se	Ni	Mn
1	49.2	48.1	54.9	47.9	51.9	50.7	51.0
2	39.0	47.5	53.0	49.4	49.4	49.9	51.4
3	49.2	48.3	53.2	48.3	51.9	50.4	52.3
Average	**45.8 ± 5.9**	**48.0 ± 0.4**	**53.7 ±1.0**	**48.5 ± 0.8**	**51.1 ± 1.4**	**50.3 ± 0.4**	**51.6 ± 0.7**
% Recovery	**91.6**	**95.9**	**107.4**	**97.1**	**102.1**	**100.7**	**103.1**

CROSS CONTAMINATION TESTS WITH ANIMAL BLOOD

(Results expressed in ppb)

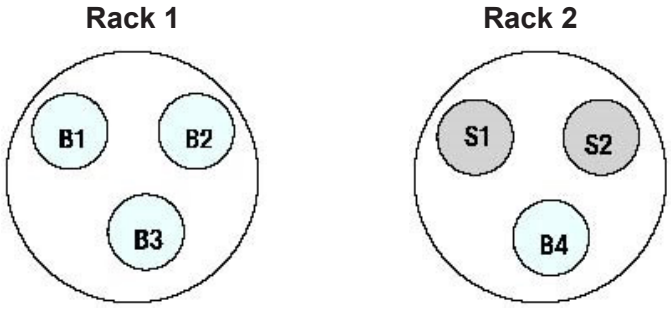


Figure 1. Scheme of sample positions in SK-15 vessels. B = Blank; S = Sample

Sample	Cr (µg/L)	Cu (µg/L)	Mn (µg/L)	Ni (µg/L)	Pb (µg/L)	Zn (µg/L)
B1	< 2	< 2	< 2	< 2	< 2	< 10
B2	< 2	< 2	< 2	< 2	< 2	< 10
B3	< 2	< 2	< 2	< 2	< 2	< 10
S1	54.79	39.56	14.38	31.61	11.00	148.5
S2	56.08	39.52	14.30	32.59	10.80	148.2
B4	< 2	< 2	< 2	< 2	< 2	< 10
Average	**55.4**	**39.5**	**14.3**	**32.1**	**10.9**	**148.4**
Std. Dev.	**0.9**	**0.0**	**0.1**	**0.7**	**0.1**	**0.2**

CONCLUSION

Human Hair and Animal Blood samples were prepared for elemental analysis using quartz micro-inserts configuration (vessel-inside-vessel technology).

Micro-inserts demonstrate to provide a robust and reproducible way to prepare biological samples for trace metal analysis with low acid volumes and small quantity of sample. The data reported in this industry report shows that the “Vessel-Inside-Vessel Technology” is a great solution for clinical laboratories that need to examine and analyze biological material employing small volume of acids and small quantity of sample.

ABOUT MILESTONE

At Milestone we help chemists by providing the most innovative technology for metals analysis, direct mercury analysis and the application of microwave technology to extraction, ashing and synthesis. Since 1988 Milestone has helped chemists in their work to enhance food, pharmaceutical and consumer product safety, and to improve our world by controlling pollutants in the environment.

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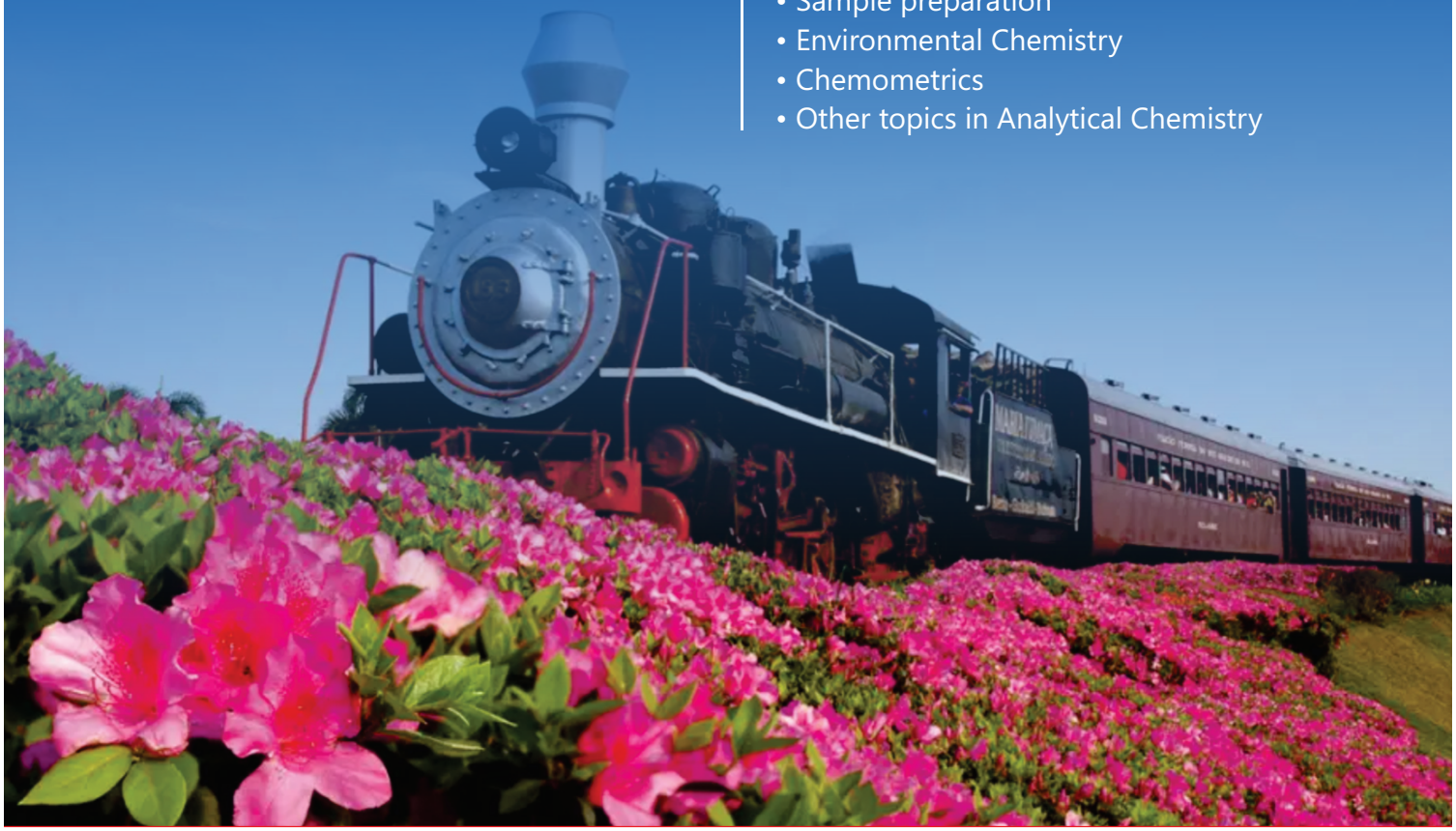

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A Milestone in Analytical Chemistry

Milestone vision is to help chemists by providing the most innovative technology for metal analysis, direct mercury analysis and applying microwave technology to extraction, ashing and synthesis.

Milestone has been active since 1988 in the field of advanced microwave sample preparation. Franco Visinoni, the founder and CEO of Milestone Corp. (Italy), is the “chief dreamer” of the society, which is based on continuous innovation and technological improvement. The key of the success of Franco Visinoni and, therefore, of Milestone lies in bringing together individuals from diverse scientific, listening to them and engineering disciplines to solve real-world problems with innovative microwave instrumentation.

Franco Visinoni became a chemist with a strong analytical background and in 1975 started by working in a big international company, but his expectations and carrier prospect was too much small for him at that time. So, he began doing carrier in the marketing field.

“I was good. I understood that I was good at surveying and anticipating the needs of the market. I founded the unsatisfied requests. The company that I work for asked me to write all the technical specifications of one of these “missing products.” I did it and they manufactured what I said. It was a success! Therefore, I became the marketing manager for Europe.”

However, since he was a child, he had the dream of founding his own company. With the help of his brother and his wife, he founded Milestone.

“When I came to Milan to register the new society, I read on the International Herald Tribune a declaration of Tom Watson, the founder of IBM, that I interpreted as a wish for me: ‘When you have a small company, a lot of fear, and a great opportunity you can do amazing things’.”

In 1989, Franco Visinoni conceived the first microwave unit voted to excellence: MLS-1200 Standard. It could extremely reduce the sample preparation time, thus, offering quality and standardization of results. This is only the beginning step of a long and fruitful growing path.

1989 – MLS-1200 first microwave system.

At Miconex 1990, China.

Milestone now has more than 50 patents and more than 20,000 instruments installed worldwide covering government, academic, contract, and manufacturing industries. It is the acknowledged industry leader in microwave instrumentation technology. Milestone commitment is to consistently provide the scientific and industrial communities with the most effective, safest, and highest quality instrumentation for chemical applications.

The Milestone today's product line includes the most effective, safe and productive instruments for Microwave Digestion for trace and ultra-trace analysis, Microwave Extraction, Direct Mercury Analysis, Microwave-Assisted Synthesis and Microwave Ashing.

Milestone's passion and commitment to being a trusted partner to their users start at the first touch, and is solidly supported by the entire team.

Milestone's team.

The excellent support of the skilled Milestone Application Team through all these years has highly contributed to the success of the Milestone instruments and of the company. All systems are produced, assembled and checked by Milestone Production Department according to ISO procedures, to ensure they fully satisfy the needs of our customers. Milestone today is still guided by its president, Franco Visinoni together with his daughter, Vanessa Visinoni, while managed by the CEO, Diego Cortesi, responsible for each decision and actions of the company.

Milestone is operative worldwide through a network of very reliable partners; USA headquarters are located in Shelton, Connecticut. The presence of Milestone in Japan is assured by Milestone General KK, near Tokyo, and in Korea by Milestone Korea, located in Seoul. These branches have sales and technical assistance departments as well as application laboratories supporting our customers. Besides this, Milestone is present through more than 70 exclusive distributors, who guarantee everywhere high standards of quality and assistance.

Building before Milestone's foundation.

Milestone's headquarters in 2021.

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Learn more at **www.milestonesrl.com** VIDEO

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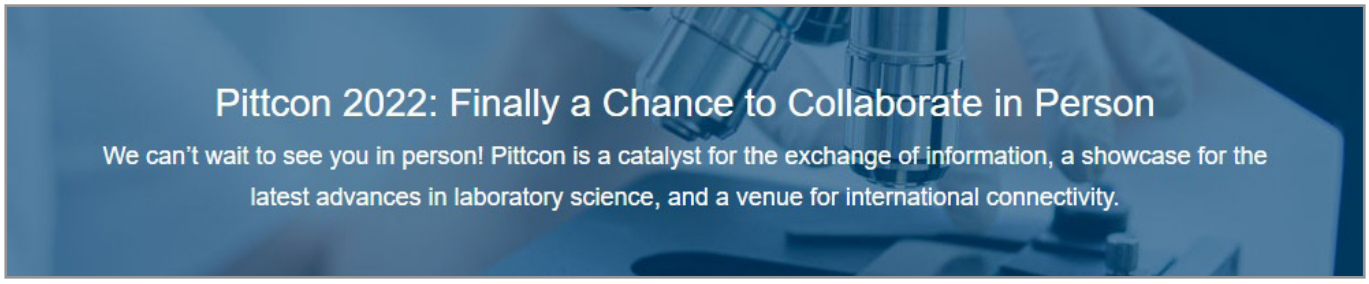

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Learn how the beverage industry performs tests to safeguard consumer health, beer taste, and quality. In this article, Margareth Krauter, the head microbiologist and Quality Assurance specialist at Grupo Petrópolis, one of Brazil's largest beverage manufacturers, speaks with SelectScience® about the many considerations of beer quality control. Read this Artcle **here**

Interview: ***The importance of maintaining sample integrity throughout the COVID-19 pandemic***

As the pandemic continues to surge, so does the global demand for viral transport media to help ensure sample integrity. Sample collection, storage, and transportation safety have never been more important than in the COVID-19 era. Amanda Kulwicki, Global Brand Manager at Thermo Fisher Scientific, discusses how her team ensures sample integrity with confidence. Read this Interview **here**

Webinar: ***Bioanalytical method development for oligonucleotides: Overcoming the analytical challenges***

In recent years, the development of next-generation oligonucleotide therapies with high target specificity and stability has greatly increased, yet the precise and productive analytical testing of these molecules has remained a significant challenge.

In this webinar, Mary Trudeau, principal applications scientist at Waters Corporation, outlines the key challenges in method development for oligonucleotides and solutions to address them. Access this webinar **here**

Video: ***Choosing an automate sample prep technique to match your analytes and matrix***

When choosing a sample preparation method, it is important to consider the chemistry of your compounds, including properties such as volatility or polarity. In this video presentation, Dr. Diane Turner, Founder and Director of Anthias Consulting Ltd., discusses how to utilize information about your compounds to choose the most suitable automated sample prep technique. Access this video **here**

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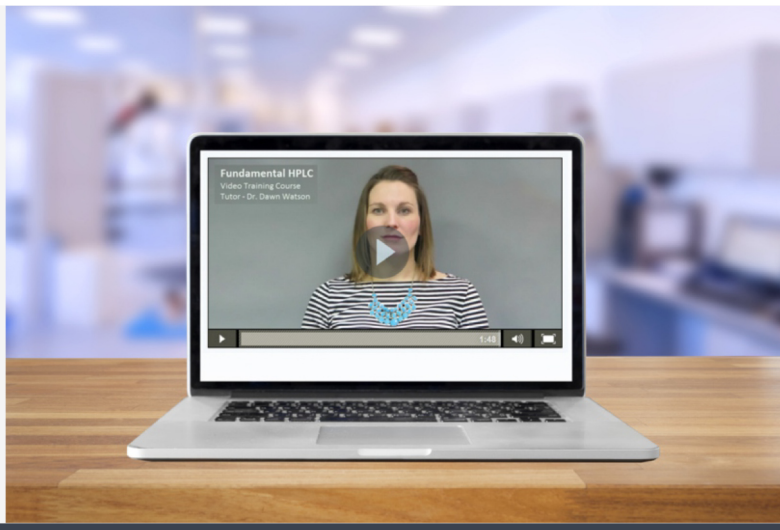

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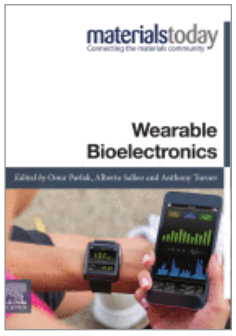
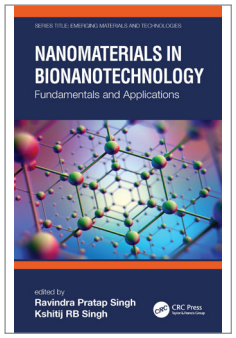
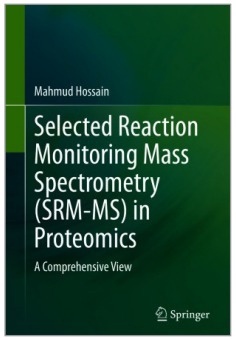


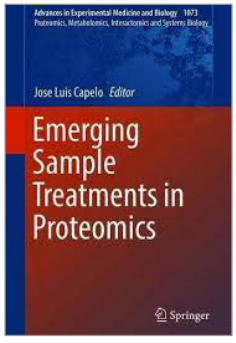
Wearable Bioelectronics – A volume in Materials Today
Onur Parlak, Alberto Salleo and Anthony Turner, Editors
2020 Publisher: Elsevier
This book presents the latest on physical and (bio)chemical sensing for wearable electronics. It covers the miniaturization of bioelectrodes and high-throughput biosensing platforms while also presenting a systemic approach for the development of electrochemical biosensors and bioelectronics for biomedical applications. Topics covered include self-powering wearable bioelectronics, electrochemical transducers, textile-based biosensors, epidermal electronics and other exciting applications. Read more …



Nanomaterials in Bionanotechnology – Fundamentals and Applications
Ravindra Pratap Singh, Kshitij RB Singh, Editors
August, 2021. Publisher: CRC Press
This book offers a comprehensive treatment of nanomaterials in biotechnology, from fundamentals to applications along with their prospects. It explains the basics of nanomaterial properties, synthesis, biological synthesis, and chemistry and demonstrates how to use nanomaterials to overcome problems in agricultural, environmental, and biomedical applications. Covers nanomaterials for environmental analysis and monitoring for heavy metals, chemical toxins, and water pollutant detection. Read more …



Selected Reaction Monitoring Mass Spectrometry (SRM-MS) in Proteomics – A Comprehensive View
Hossain, Mahmud, Authors
2020. Publisher: Springer
Covering a wide-ranging facet of a “gold-standard” targeted mass spectrometry (MS) method for the consistent detection and accurate quantification of preselected proteins in complex biological matrices, this book provides knowledge-based planning and optimized design of SRM-MS experiments and data analysis; Describes SRM-MS applications in proteomics; Covers various software and databases for SRM-MS analyses. Read more …



Emerging Sample Treatments in Proteomics
José Luis Capelo Martínez, Editor
June 2019. Publisher: Springer
Proteomics is a well-established area of Science; yet with a strong area in constant evolution, namely sample treatment. There few books that currently cover the field of emerging sample treatments in proteomics, this new volume will be the first to cover all emerging and existing studies. This unique book presents the latest advances in the field focusing on emerging trends linked to high-resolution mass spectrometry, technology addressed to treat samples faster and to attempts to simplify the proteome for the reader. Read more …

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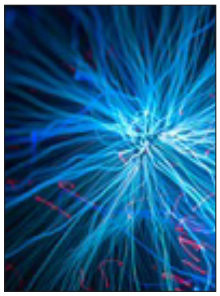

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Featured Article: ***New Transmission Electron Microscope Dramatically Expands Cryo-EM Access for Academia and Industry.*** By Anke Mulder. Over the last several years, cryo-EM has revolutionized structural biology by enabling scientists to obtain high-resolution models of molecules that could not be uncovered using other approaches. Learn more on how cryo-EM is expanding access to academia and industry. Read more

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Chromatographyonline delivers practical, nuts-and-bolts information to help scientists and lab managers become more proficient in the use of chromatographic techniques and instrumentation, thereby making laboratories more productive and businesses around the world more successful.

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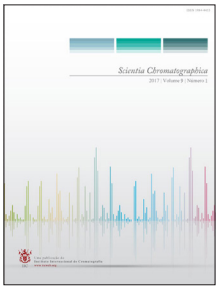

Scientia Chromatographica

Scientia Chromatographica is the first and to date the only Latin American scientific journal dedicated exclusively to Chromatographic and Related Techniques (Mass Spectrometry, Sample Preparation, Electrophoresis, etc.). With a highly qualified and internationally recognized Editorial Board, it covers all chromatography topics (HPLC, GC, SFC) in all their formats, in addition to discussing related topics such as “The Pillars of Chromatography”, Quality Management, Troubleshooting, Hyphenation (GC-MS, LC-MS, SPE-LC-MS/MS) and others. It also provides columns containing general information, such as: calendar, meeting report, bookstore, etc. Read more

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Interview: ***Fundamental Research and Applications of ICP-OES and ICP-MS Toward Improved Understanding of Elemental Chemical Analysis*** With John W. Olesik, who directs the Trace Element Research Laboratory in the School of Earth Sciences at The Ohio State University. Read more

EVENTS 2021 – It is suggested to consult the event's official website for updates.

June 14 – 18, 2021
XVII Italian-Hungarian Symposium on Spectrochemistry (IHSS) – Current Approaches in Health and Environmental Protection, On-Line
https://www.ihss2020.unito.it

June 22 – 25, 2021
17th National Meeting on Mycotoxins (ENM 2021) – Hybrid event in person and online
Rio de Janeiro, RJ, Brazil |
https://www.enmicotoxinas.com.br/

August 30 – September 3, 2021
International Conference on Electronic Materials (2021 IUMRS-ICEM) and the XIX Brazilian Materials Research Society Meeting (XIX B-MRS) – ONLINE
https://www.sbpmat.org.br/19encontro/

October 4 – 8, 2021
20th IUPAB Congress, 45th Annual SBBf Meeting, and 49th Annual SBBq Meeting – Virtual Format
http://iupab2020.sbbq.org.br/interna-278/home

October 11 – 15, 2021
34th Latin American Congress of Chemistry – CLAQ 2020; 18th Latin American Congress of Chromatography – COLACRO; 10th Colombian Congress of Chromatography – COCOCRO; 4th Colombian Congress of Biochemistry and Molecular Biology - C2B2
Convention Center, Cartagena de Indias, Colombia
https://claq2020.com/en/bienvenida/

October 18 – 21, 2021
Metrology 2021 – Online
https://metrologia2021.org.br/

November 30 – December 2, 2021
FCE Pharma
São Paulo, SP, Brazil
https://www.fcepharma.com.br/o-evento

November 30 – December 2, 2021
Analitica Latin America Conference & Expo
São Paulo, SP, Brazil
https://www.analiticanet.com.br/

November 16 – 19, 2021
60th Brazilian Chemistry Congress – Virtual
http://www.abq.org.br/cbq/

December 12 – 16, 2021
XXIII International Mass Spectrometry Conference (IMSC 2021)
Sheraton Grand Rio Hotel & Resort, Rio de Janeiro, RJ, Brazil
https://www.imsc2020.com/

EVENTS 2022 – It is suggested to consult the event's official website for updates.

May 25 – 28, 2022
XXII Brazilian Congress of Toxicology (CBTox 2022)
Balneário Camboriú, SC, Brazil
https://www.cbtox2021.com.br/

June 4 – 8, 2022
18th International Conference on Electroanalysis (ESEAC 2022)
Vilnius, Lithuania
http://www.eseac2020.com/

September 25 – 28, 2022
20th National Meeting on Analytical Chemistry (20th ENQA) & 8th Ibero-American Congress of Analytical Chemistry (8th CIAQA)
Bento Gonçalves, RS, Brazil
https://enqa.com.br/

Date to be defined

44th Annual Meeting of the Brazilian Chemical Society (RASBQ)
Maceió, AL, Brazil
http://www.sbq.org.br/reunioes-anuais

XVIII Chemometrics in Analytical Chemistry (CAC)
Courmayeur, Italy / Chamonix, France
http://cac2020.sciencesconf.org

GUIDELINES FOR AUTHORS

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Journals

1. Orlando, R. M.; Nascentes, C. C.; Botelho, B. G.; Moreira, J. S.; Costa, K. A.; Boratto, V. H. M. *Anal. Chem*., **2019**, *91* (10), pp 6471-6478 (https://doi.org/10.1021/acs.analchem.8b04943).
   - *Publications with more than 10 authors, list the first 10 authors followed by a semicolon and et al.*
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Books

2. Burgot, J.-L. *Ionic Equilibria in Analytical Chemistry*. Springer Science & Business Media, New York, **2012**, Chapter 11, p 181.
3. Griffiths, W. J.; Ogundare, M.; Meljon, A.; Wang, Y. Mass Spectrometry for Steroid Analysis. In: Mike, S. L. (Ed.). *Mass Spectrometry Handbook*, v. 7 of Wiley Series on Pharmaceutical Science and Biotechnology: Practices, Applications and Methods. John Wiley & Sons, Hoboken, N.J., **2012**, pp 297-338.

Standard methods

4. International Organization for Standardization. ISO 26603. Plastics — *Aromatic isocyanates for use in the production of polyurethanes — Determination of total chlorine*. Geneva, CH: ISO, **2017**.

Master's and doctoral theses or other academic literature

5. Dantas, W. F. C. *Application of multivariate curve resolution methods and optical spectroscopy in forensic and photochemical analysis*. Doctoral thesis, **2019**, Institute of Chemistry, University of Campinas, Campinas, SP, Brazil.

Patents

6. Trygve, R.; Perelman, G. US 9053915 B2, June 9, **2015**, Agilent Technologies Inc., Santa Clara, CA, US.

Web pages

7. http://www.chromedia.org/chromedia [Accessed 10 January 2019].

Unpublished source

8. Viner, R.; Horn, D. M.; Damoc, E.; Konijnenberg, A. *Integrative Structural Proteomics Analysis of the 20S Proteasome Complex* (WP-25). Poster presented at the XXII International Mass Spectrometry Conference (IMSC 2018) / August 26-31, **2018**, Florence, IT.
9. Author, A. A. *J. Braz. Chem. Soc*., in press.
10. Author, B. B., **2019**, submitted for publication.
11. Author, C. C., **2019**, unpublished manuscript.

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