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Edited by

Sibel A. Ozkan

Faculty of Pharmacy Department of Analytical Chemistry Ankara University Ankara, Turkey

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PREFACE

This 6th volume of *Recent Advances in Analytical Techniques* contains five comprehensive chapters. The concepts described in this volume reflect the important recent advances in analytical chemistry, including modern quality management aspects of these methods that can find wide use in industry. In addition, the chapters cover important recent trends in analytical methods, including the use of Analytical Techniques for Analysis of Metals and Minerals in Water; Lipidomics Techniques and their Application for Food Nutrition and Health; Recent Advances in the Analysis of Herbicides and Their Transformation Products in Environmental Samples; Nano Porous Anodic Aluminum Oxide: An Overview on Its Fabrication and Potential Applications; PIXE/PIGE Measurements of Archaeological Glass, its Conceptualization and Interpretation: A Case Study. I hope that the readers will greatly enjoy reading the excellent chapters contributed by eminent scientists in their respective fields. I would like to thank all the authors contributing to this volume for their superb contributions.

Also, I would like to thank the Bentham staff, including Ms. Mariam Mehdi (Assistant Manager of Publications), and Mr. Mahmood Alam (Director of Publications) at Bentham Science Publishers for their untiring efforts and efficient interactions with the authors in the publication process.

Sibel A. Ozkan Faculty of Pharmacy Department of Analytical Chemistry Ankara University Ankara, Turkey

List of Contributors

Harshdeep Kaur	Department of Chemistry, Punjab Agricultural University, Ludhiana, Punjab, India
Jiamin Xu	National R&D Branch Center for Freshwater Aquatic Products Processing Technology (Shanghai), Integrated Scientific Research Base on Comprehensive Utilization Technology for By- Products of Aquatic Product Processing, Ministry of Agriculture and Rural Affairs of the People's Republic of China, Shanghai Engineering Research Center of Aquatic-Product Processing & Preservation, College of Food Science & Technology, Shanghai Ocean University, Shanghai 201306, China
Jiahui Chen	National R&D Branch Center for Freshwater Aquatic Products Processing Technology (Shanghai), Integrated Scientific Research Base on Comprehensive Utilization Technology for By- Products of Aquatic Product Processing, Ministry of Agriculture and Rural Affairs of the People's Republic of China, Shanghai Engineering Research Center of Aquatic-Product Processing & Preservation, College of Food Science & Technology, Shanghai Ocean University, Shanghai 201306, China
Jing Su	Xinhua Hospital, Shanghai Institute for Pediatric Research, Shanghai Key Laboratory of Pediatric Gastroenterology and Nutrition, Shanghai Jiao Tong University, School of Medicine, Shanghai 200092, China
Jian Zhong	National R&D Branch Center for Freshwater Aquatic Products Processing Technology (Shanghai), Integrated Scientific Research Base on Comprehensive Utilization Technology for By- Products of Aquatic Product Processing, Ministry of Agriculture and Rural Affairs of the People's Republic of China, Shanghai Engineering Research Center of Aquatic-Product Processing & Preservation, College of Food Science & Technology, Shanghai Ocean University, Shanghai 201306, China
Makhan Singh Bhullar	Department of Agronomy, Punjab Agricultural University, Ludhiana, Punjab, India
Pervinder Kaur	Department of Agronomy, Punjab Agricultural University, Ludhiana, Punjab, India
Roman Balvanović	Vinča Institute of Nuclear Sciences, National Institute of Serbia, University of Belgrade, Belgrade, Serbia
Saša Đurović	Laboratory of Chromatography, Institute of General and Physical Chemistry, Studetski trg 12, 11158 Belgrade, Serbia
Saša Šorgić	Oenological Laboratory, Heroja Pinkija 49, 26300 Vršac, Serbia
Saša Popov	Oenological Laboratory, Heroja Pinkija 49, 26300 Vršac, Serbia MS Enviro, Njegoševa 22, 26300 Vršac, Serbia
Snežana Filip	University of Novi Sad, Technical Faculty "Mihajlo Pupin" Zrenjanin, Djure Djakovica b.b., 23000 Zrenjanin, Serbia
Shudan Huang	National R&D Branch Center for Freshwater Aquatic Products Processing Technology (Shanghai), Integrated Scientific Research Base on Comprehensive Utilization Technology for By- Products of Aquatic Product Processing, Ministry of Agriculture and Rural Affairs of the People's Republic of China, Shanghai Engineering Research Center of Aquatic-Product Processing & Preservation, College of Food Science & Technology, Shanghai Ocean University, Shanghai 201306, China

- Ting ZhangNational R&D Branch Center for Freshwater Aquatic Products Processing Technology
(Shanghai), Integrated Scientific Research Base on Comprehensive Utilization
Technology for By- Products of Aquatic Product Processing, Ministry of Agriculture
and Rural Affairs of the People's Republic of China, Shanghai Engineering Research
Center of Aquatic-Product Processing & Preservation, College of Food Science &
Technology, Shanghai Ocean University, Shanghai 201306, ChinaUjjal KumarData to College of Clinate Data to Clinate Data t
- Sur Department of Chemistry, Behala College, University of Calcutta, Kolkata, India
- Xichang Wang National R&D Branch Center for Freshwater Aquatic Products Processing Technology (Shanghai), Integrated Scientific Research Base on Comprehensive Utilization Technology for By- Products of Aquatic Product Processing, Ministry of Agriculture and Rural Affairs of the People's Republic of China, Shanghai Engineering Research Center of Aquatic-Product Processing & Preservation, College of Food Science & Technology, Shanghai Ocean University, Shanghai 201306, China
- Žiga Šmit Faculty of Mathematics and Physics, Jožef Stefan Institute, University of Ljubljana, Ljubljana, Slovenia

Analytical Techniques for Analysis of Metals and Minerals in Water

Saša Đurović^{1,*}, Saša Šorgić², Saša Popov^{2,3} and Snežana Filip⁴

¹ Laboratory of Chromatography, Institute of General and Physical Chemistry, Studetski trg 12, 11158 Belgrade, Serbia

² Oenological Laboratory, Heroja Pinkija 49, 26300 Vršac, Serbia

³ MS Enviro, Njegoševa 22, 26300 Vršac, Serbia

⁴ University of Novi Sad, Technical Faculty "Mihajlo Pupin" Zrenjanin, Djure Djakovica b.b., 23000 Zrenjanin, Serbia

Abstract: Investigation of the water samples for content of bulk, trace and heavy metals is of great importance for the humanity. For this purpose, a large number of analytical techniques have been developed. Beside analytical techniques, there are systems and methods for pretreatment and preparation of the samples for analysis. There are also procedures for sampling and sample preservation which are essential for the final result. There are several available instrumental techniques for the analysis of metals in water samples (AAS, GFAAS, ICP-OES, ICP-MS, *etc.*), which can be divided into several groups such as volumetric, spectrophotometric, electrochemical, chromatographic, *etc.* All these techniques may be coupled among themselves and with techniques for sample preparation such as preconcentration techniques. This improves the performance of the applied techniques and decrease the possibility of the contamination of samples. This chapter provides an insight into all these processes and issues from sampling, sample conservation, pretreatment and preparation to the application of different analytical techniques for analysis of water samples.

Keywords: Analysis, Classical methods, Instrumental methods, Metals, Sampling, Sample conservation, Sample pretreatment and preparation, Water.

INTRODUCTION

Water is one of the most precious resources in the world. Contamination of this resource is an important issue to deal with. Presence of the toxic pollutants shows the negative effect on the environment, human health, as well as negative economic effects. Heavy metals in water may originate from natural sources, *i.e.*, eroded sediments, volcanos, *etc.*, or from anthropogenic sources such as waste

* Address correspondence to Saša Đurović: Laboratory of Chromatography, Institute of General and Physical Chemistry, Studetski trg 12, 11158 Belgrade, Serbia; Tel: +381659577200; Email: sasatfns@uns.ac.rs

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disposal, industrial effluents, *etc.* These metals may negatively influence the organic life. Their action is connected with their properties, availability, and concentration. Availability depends on the form of these elements, which may be dissolved (dangerous form) and particulated (bounded form in sediments, organic compounds, *etc.*). Balance between these two forms is regulated by pH value and redox potential [1].

It has been reported that the concentration of heavy metals is significantly higher in the populated areas with industry, comparing to their concentration in the wild [2 - 4]. For such reasons, there is a high possibility of contamination of drinking water in these areas followed by an expression of negative effect on human health [5 - 7]. Thus, it is important to develop analytical techniques for monitoring water samples originated from both urban and wild areas. It should be taken into account that these techniques should be able to detect and quantify very low levels of analyzed elements because some elements are present in rather a trace or even ultra-trace levels (μ g/L or even ng/L levels). However, it has been mentioned that these levels may be higher in urban areas due to the presence of the industry [8, 9].

Another group of elements is major or bulk elements. Their concentration in the environment is much higher (in mg/L levels). Although they are essential for human health, presence in excessive amounts may lead to different disorders and illnesses [1].

Due to the significance of knowing the levels of all these elements in the water, this chapter's aim is to summarize available methods for sampling, storage, pretreatment, and preparation of water samples for the analysis. Besides, an important task is to present all available analytical techniques for analyzing the metals in both major and trace levels.

SAMPLING, STORAGE, AND PRESERVATION OF THE SAMPLES

Sampling is probably the most important and critical step in all analytical procedures because even a tiny mistake may cause a huge error in obtained result, making the analysis useless. For such reason, sampling procedures need to be followed strictly. Taking the diversity in the nature of the sample itself and concentration of the metals into an account, different sampling methods have been developed [1]. Therefore, the main aspects of the water samples' collection have been defined. It is essential that the collected sample is a representative sample of water which is to be analyzed. To accomplish this, large volumes of water are usually required. Representative samples must be homogenized for preparation of samples for the analysis. It should be also bear in mind that the shorter time between the sampling and analysis is in strong correlation with reliability of the

Analysis of Metals and Minerals

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obtained results [10, 11]. It needs to be pointed out that occurrence of the turbidity and/or suspended matter, and application of the methods for their elimination are very important factors in the analytical process [11 - 13]. Chemical profile of the water also has significant influence on the choice of the sampling method. Therefore, a discrete sample should be taken when composition of water is unchanged over the time. However, obtained results showed the composition of the analyzed water at the certain moment. On the other hand, when it comes to the average composition of desired component(s) during the certain period of time, a composite sample should be taken (mix of different samples taken at different period) [14 - 16].

Contamination of the sample during the manipulation is an important factor, which contributes to the final result of the analysis. Magnitude of the concentration of the analyzed element is also a significant contributor. Lower order of magnitude usually means a higher error in the final result. Usual reasons for losing the heavy metals are adsorption on the surface of the storage vessel and/or contamination. Significance of this issue is proven by the available publications on this subject, reporting the necessary steps and precautions to avoid contamination [17 - 19]. Factor, which should be also taken into account, is chemical and biological inertness of the sampling equipment, *i.e.*, used equipment must not change the composition of the water sample. Selected containers must be made of such material that prevents any possible undesirable processes such as adsorption and desorption. Material of the sampling container should be chosen according to the objective of the analysis. If the heavy metals are analyzed, container must not be made of metal in order to prevent contamination of the sample due to the metal leaching. Considering all relevant factors, e.g., sampling efficiency and cost, samples may be kept in a plastic container made of polyethylene or polyvinyl chloride. It is essential that plastic containers are cleaned prior to sample's collection. This could be accomplished by rinsing with diluted hydrochloric acid, distilled and distilled water [20]. Extreme caution is needed when chemical separation is required because chemical reaction may occur, causing the changes in chemical composition of the sample. Such an event happens due to the variations in certain parameters (pH, redox potential, oxygen, etc.). In such cases, samples have to be stored at low temperatures (dark place and/or frozen) [1].

When it comes to the bulk elements, such as sodium (Na) and potassium (K), the analyst should be aware that those elements may leach from the glass bottle. Therefore, when bulk elements are to be analyzed, borosilicate or polyethylene vessels should be used. It is also recommended to lower the pH down with nitric acid (pH \approx 2) in order to prevent the adsorption of these elements on the vessel's wall [21]. Besides, zinc (Zn), manganese (Mn), iron (Fe), and copper (Cu) could

Lipidomics Techniques and their Application for Food Nutrition and Health

Shudan Huang¹, Jiamin Xu¹, Jiahui Chen¹, Ting Zhang¹, Jing Su², Xichang Wang¹ and Jian Zhong^{1,2,*}

¹ National R&D Branch Center for Freshwater Aquatic Products Processing Technology (Shanghai), Integrated Scientific Research Base on Comprehensive Utilization Technology for By-Products of Aquatic Product Processing, Ministry of Agriculture and Rural Affairs of the People's Republic of China, Shanghai Engineering Research Center of Aquatic-Product Processing & Preservation, College of Food Science & Technology, Shanghai Ocean University, Shanghai 201306, China

² Xinhua Hospital, Shanghai Institute for Pediatric Research, Shanghai Key Laboratory of Pediatric Gastroenterology and Nutrition, Shanghai Jiao Tong University, School of Medicine, Shanghai 200092, China

Abstract: Due to the chemical complexity and wide concentration range of lipids in biological samples, it is necessary to apply different analytical strategies to identify and quantify lipid species and amounts. In this book chapter, we mainly introduced the techniques, workflow, and applications of lipidomics in food nutrition and health. First, we mainly introduced the common lipidomics techniques, such as direct infusion mass spectrometry-based techniques, chromatographic separation mass spectrometry-based techniques, mass spectrometry imaging, and nuclear magnetic resonance. Second, we described the common lipidomics workflow, including sample preparation, MS data acquisition, and data processing. Third, we mainly discussed the application of lipidomics in food nutrition and health. Finally, we briefly summarized and discussed the future perspectives of lipidomics. All these discussions suggested that lipidomics could ensure food quality, examine dietary lipid nutrition, and prevent and detect diseases.

Keywords: Chromatography, Cancer, Data processing, Lipidomics, Mass spectrometry imaging, Metabolic syndrome, MS data acquisition, Nuclear magnetic resonance, Nutrition, Neurological disorders, Sample preparation, Shotgun lipidomics.

^{*} **Corresponding author Jian Zhong:** National R&D Branch Center for Freshwater Aquatic Products Processing Technology (Shanghai), Integrated Scientific Research Base on Comprehensive Utilization Technology for By-Products of Aquatic Product Processing, Ministry of Agriculture and Rural Affairs of the People's Republic of China, Shanghai Engineering Research Center of Aquatic-Product Processing & Preservation, College of Food Science & Technology, Shanghai Ocean University, Shanghai 201306, China; E-mail: jzhong@shou.edu.cn

INTRODUCTION

In recent years, with the development of the economy, the living standards of people have been significantly improved, and people pay much attention to food nutrition and health. Various nutrients play different roles in the body for people. However, food safety and nutrition problems have frequently aroused the panic of people, which might have a bad impact on the lives and health of people. Numerous news has reported the safety and quality of food, such as melamine adulterated milk and [1] gutter oils [2], which improved the concern of the public. Besides, with the globalization of the food market, people can easily get more foods from different legislation and regulations to improve food safety and quality. Although with the persistence of legislation and regulations, there are still amounts of unscrupulous producers and traders who want to produce or sell adulterated foods to earn a big profit, ignoring the health of the consumers [4]. Therefore, we need novel techniques to help to dissolve these problems.

There are lots of nutritional substances in foods, like protein, sugars, vitamins, lipids, inorganic salt, and water [5]. As shown in Fig. (1), there are 9 representative lipids and their reaction products in the food system. Among the nutritional substances, lipids have several key functions, which can be found almost in every cell membrane. The lipid is hardly soluble in water, and very easy to be dissolved inorganic, and has the characteristics of structural diversity and variety [6]. First, it can protect the body from injury and act as a cushion to the body. Second, according to the need, the lipid can insulate the body and keep it warm. Third, it can make the skin and hair lubricated. Moreover, it can help transport the essential fat-soluble vitamins A, D, E, and K and essential fatty acids. Last but not the least, lipid provides energy for people. Each gram of lipids provides 9 calories, which is more than twice that of carbohydrates or protein [7, 8]. In terms of the disease, the lipid has been found to have relations with Alzheimer's disease [9], diabetes [10], tumors [11], and other diseases.

In the 1960s and 70s, lipid was one of the most intensely studied areas of biology. Most of the bioinformatics resources of Kyoto Encyclopedia of Genes and Genomes (KEGG) have relied on the messages, which obtained from that time [13]. In 2003, lipidomics was firstly proposed as one of the main branches of metabolism [14]. It is generally believed that lipidomics can analyze the characteristics of all lipid molecules in organisms and their roles in protein expression and gene regulation. Therefore, the research of lipidomics belongs to the category of life sciences and is closely related to human health, so its importance in the research of diseases has also attracted widespread attention [15]. At present, the contents of lipidomics research mainly include three major

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aspects: lipid and its metabolite analysis and identification, lipid function and metabolic regulation (including key genes/proteins/enzymes), and lipid metabolism pathways and networks [16, 17].

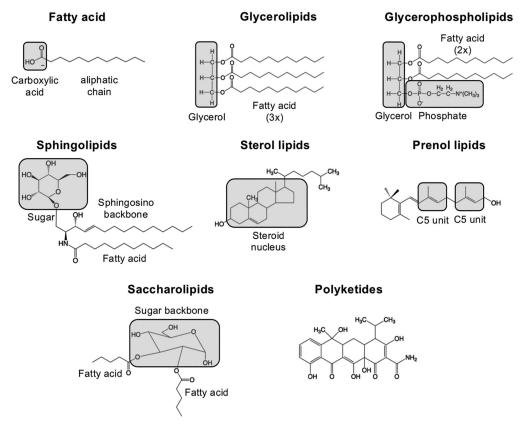


Fig. (1). Categorization of lipids. Reprinted from Elsevier Publisher (2020) [12].

There are several aims of lipidomics, such as to study the lipids in biological fluids, tissues, and cells through various methods, and to explore the body lipids changes in quality metabolism under different diseases or drug interference states, to study the possible mechanism of disease and the mechanism of action of drugs from the perspective of lipid metabolism network, and to search for key lipid biomarkers that can characterize a disease or drug intervention [12, 18, 19].

In this book chapter, we mainly introduce the techniques, workflow, and applications of lipidomics in the fields of food nutrition and health (Fig. 2). The development of lipidomics techniques can attribute to the applications, such as identifying food adulteration more correctly and detecting diseases quickly.

CHAPTER 3

Recent Advances in the Analysis of Herbicides and their Transformation Products in Environmental Samples

Pervinder Kaur^{1,*}, Harshdeep Kaur² and Makhan Singh Bhullar¹

¹ Department of Agronomy, Punjab Agricultural University, Ludhiana, Punjab, India ² Department of Chemistry, Punjab Agricultural University, Ludhiana, Punjab, India

Abstract: Herbicide residues in crop, soil and contamination of groundwater have become a worldwide concern in recent decades as their presence at low concentrations entails an unacceptable risk to human health and non-target organism. The magnitude of exposure and concentration at a particular time may trigger bioaccumulation and bio magnifications of herbicide residues and their degraded products, causing mutagenic, carcinogenic and teratogenic effects on humans, flora and fauna and microbiological living system. These atrocious circumstances have raised concern about their presence in environmental compartments and necessitate the continuous monitoring of herbicide residues in various matrices. However, determining the herbicide residues in the soil and crop is challenging because of the very low concentration of analyte, complicated sample matrices and low maximum residue limit (MRL) imposed by the regulatory agencies. The detection limits imposed by environment quality legislation can only be achieved by using appropriate sample preparation techniques, which comprise isolation and concentration of the analytes with nominal matrix interference, thus allowing its facile detection and quantification through instrumental analysis. In recent years, the requirements for separation and pre-concentration procedures have undergone numerous changes, and various sample preparation methods have been used. The final step in the analytical process involves the identification and quantification of the herbicide residues using suitable instrumentation, and over the years, herbicides have been determined by spectrophotometric, chromatographic, electrochemical, electrophoretic, hyphenated and biosensors. This book chapter provides a comprehensive overview of the novelties and the advantages of different techniques employed for the detection of herbicides and their transformation products in environmental samples.

Keywords: Biosensors, Chromatographic Methods, Electrochemical, Herbicides, Hyphenated, Maximum Residue Limit, Microextraction, Pretreatment, Quantification, Transformation Products.

^{*} Corresponding author Pervinder Kaur: Department of Agronomy, Punjab Agricultural University, Ludhiana, Punjab, India; E-mail: pervi_7@yahoo.co.in

INTRODUCTION

Weed management is essential for agricultural production and will play an important role to meet future food production requirements. Over the years, herbicides have emerged as an effective method for controlling weeds. Herbicides use has been increasing throughout the globe due to increasing labour cost, choice of application of herbicides, quick weed control in crop and non-crop areas, *etc.* Significant amounts are also used in the lawns, parks, golf courses, forestry, industry and for maintenance of rights-of-way for pipelines, power-lines and highways. Herbicides are among the largest growing segments, accounting for 60% (Fig. 1a) of total crop protection globally, and their use is projected to increase by 15-20% per year. Of the total herbicide consumption, rice and wheat account for a major share of 25.0 and 20.0% in the world market. In maize, total herbicide consumption is 15%, and vegetables and oilseed pulses account for 10-15% of the herbicide consumption. Fruits, cotton, sugarcane and other crops involve 4-6% herbicide consumption (Fig. 1b). Table 1 enlists some of the widely used herbicide classes for control of weeds in different crops.

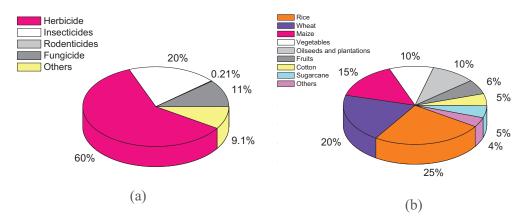


Fig. (1). (a) Total pesticide consumption in the world (b) Total herbicide consumption in different crops [1].

Though herbicides help in the protection of crops from weeds and are vital for higher productivity at a lower cost but their indiscriminate and non-judicious usage can ultimately cause health hazards because of bioaccumulation and biomagnifications of herbicide residues. Not only herbicides but their transformation products (Table 1) can also pose a residual problem and are thus required to be considered while taking the environmental risk estimation (Table 1).

Increased concern about the presence of herbicides in environmental compartments necessitates the continuous monitoring of herbicide residues and

Analysis of Herbicides

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their transformation products in various matrices. International agencies, such as European Union (EU), CODEX and USEPA have decided on maximum residue limits (MRLs) (Table 2) in order to keep a check on herbicide residues in various food commodities and keep them within safe limits. It is mandatory for herbicides to meet the criteria for food safety regulation to consider them safe for application.

Herbicide	Transformation Product/Metabolites	Matrix	References
Penoxsulam	2-amino-TP, 5-OH-XDE-638, BSTCA, BSTCA-methyl and BST	Soil	[2]
Bispyribac sodium	Sodium 2-(4,6-dimethoxy-2- pyrimidinyl)oxy)-6-((4-hydro- y-6-methoxy-2-pyrimidinyl)oxy benzoate and sodium 2-((4,- -dimethoxy-2-pyrimidinyl)oxy) 6- hydroxy benzoate	Soil	[3]
Linuron	-N(OCH)CH ₂ OH, -NHCH ₃ , and -NHOCH ₃	Plant	[4]
2, 4-D	2,4-dichlorophenol (2,4-DCP), 2-chlorophenol (2-CP), pbenzoquinone (PBQ), 2-chlorohydroquinone (2-CHQ) and 4-chloro1,3-benzenediol (4- Chlororesorcinol, 4-CR)	Water	[5]
Pendimethalin	N-(1-ethyl1-propyl)-3,4 dicarboxy-2,6-dinitrobenzenamine-N-oxide, N- (1-ethylpropyl)-3,4 dimethoxy-2,6-dinitrobenzenamine and benzimadazole-7-carboxyaldehyde	Soil	[6]
Atrazine	Desethylatrazine (DEA) and Deisopropylatrazine (DIA)	Water	[7]
Simazine	2-hydroxysimazine	Water	[8]
Butachlor	N-(butoxymethyl)-N-(2-chloroethyl)-2,6-diethylaniline, (N- (butoxymethyl)- 2-chloro-N-(2-ethylphenyl) acetamide, N- (butoxymethyl)-2,6-diethyl-N-propylaniline, 2-chloro-N-(- ,6-diethylphenyl) acetamide and 2,6-diethylaniline	Soil	[9]
Pretilachlor	N-ethyl-2-chloro-2',6'-diethylacetanilide, 2-hydroxy2',6- -diethylacetanilide, 2,6-diethyl-N- (propyloxyethyl)acetanilide and 2,6- diethyl-N-(propyloxyethyl)aniline	Soil	[10]
Quizalofop	6-chloroquinoxalin-2-ol), (<i>R</i>)-2-(4-hydroxyphenoxy)propionic acid and 2,3-dihydroxyquinoxaline	Soils	[11]
Cyhalofop- butyl	Cyhalofop acid	Soil	[12]
Clodinafop - propargyl	Acid derivative-clodinafop	Wheat	[13]
Imazamox	5-(methoxymethyl)-2,3-pyridinecarboxylic acid and 2-carbamoyl- 5-(methoxymethyl)-3-pyridinecarboxylic acid	Water	[14]
Imazethapyr	2,3-pyridinecarboxylic acid and 7- hydroxy-furo[3,4-b]pyridine-5(- H)-one	Water	[14]
Triflusulfuron	2-amino-4-(dimethylamino)-6-(2,2,2-trifluoroethoxy)-1,3,5-triazine (2) and 6-methyl-2- methylcarboxylate benzene sulfonamide	Soil	[15]
Prosulfuron	phenyl sulfonamide, desmethyl prosulfuron and amino triazine	Soil	[16]

CHAPTER 4

Nano Porous Anodic Aluminum Oxide: An Overview on its Fabrication and Potential Applications

Ujjal Kumar Sur^{1,*}

¹ Department of Chemistry, Behala College, University of Calcutta, Kolkata, India

Abstract: The quick development in nanotechnology has raised the status of this modern technology owing to the decrease in the sizes of structures and devices. There has been considerable dispute concerning the future consequences of nanotechnology. Nanotechnology has the probable ability to design many new and novel materials and devices in smaller dimensions with broad-range applications in medicine, electronics and sustainable energy production. Nano porous anodic aluminum oxide (AAO) films consisting of self-organized hexagonal arrays of invariable parallel nanochannels have been widely applied as the building block to fabricate various functional nanostructures of different morphologies such as nanoparticles, nanowires and nanotubes. These functional nanostructures can be potentially utilized in various applications like magnetic storage media, optoelectronics, bio/chemical sensors, photonics and plasmonics. This chapter describes the different fabrication processes of AAO films in detail along with citation of a few interesting applications.

Keywords: Anodic aluminum oxide, Aluminum, Anodization, Electrochemical replication, Hard anodization, Hexagonal, Nanotechnology, Nanostructures, Nano porous, Nanochannels, Nanoimprint lithography, Plasmonics, Self-organization, Surface-enhanced Raman scattering, SERS substrates.

INTRODUCTION TO NANOTECHNOLOGY

Study of structures of dimensions of 100 nanometers or less $(1 \text{ nm} = 10^{-9} \text{ m})$ and development of new materials or devices with dimensions on this scale is known as nanotechnology. The idea of "nanotechnology" was first proposed by famous American physicist Richard Feynman in his famous lecture titled 'There is plenty of room at the bottom' delivered at the American Physical Society meeting at Caltech in 1959.

^{*} Corresponding author Ujjal Kumar Sur: Department of Chemistry, Behala College, University of Calcutta, Kolkata, India; E-mail: uksur99@yahoo.co.in

Anodic Aluminum Oxide

Feynman first introduced the concept of nanotechnology and predicted a procedure by which manipulation of individual atoms and molecules can be carried out by means of accurate tools. Nanotechnology started its venture in the early 1980's with two topmost advances; the study of cluster science and discovery of Scanning tunneling microscope by Binnig and Rohrer.

Nanotechnology got a major boost when integrated circuits (ICs) were invented in 1958. According to Moore's law [1], the number of transistors in the computing hardware which can be located economically on an integrated circuit has augmented exponentially, becoming doubled roughly every two years. Dr. Gordon E. Moore, the co-founder of Intel, first observed this in 1965 (Fig. 1).

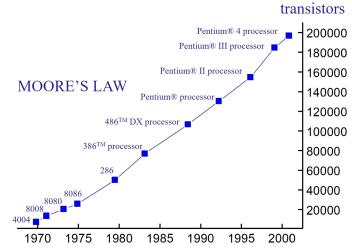


Fig. (1). Schematic diagram showing Moore's law.

As the present computing-hardware technology has fizzled out in 2012 regarding the fabrication procedure and device operation. Consequently, alternate patterning techniques and computation outlines are required. Introduction of quantum, molecular and optical computers, carbon-nanotubes based devices can provide a new dimension of computation. Carbon based nanomaterials such as fullerenes, carbon nanotubes and graphene had transformed the computing-hardware technology and one can expect that these carbon nanomaterials based devices will substitute the prevailing silicon (Si)-technology based devices in the near future by providing an overall upgradation of computation speed and efficiency.

Different physical properties like mechanical, optical, electrical, *etc.* can be modified meaningfully by reducing the dimension of the system and size-related intensive properties, such as quantum confinement in semiconductor nanomaterials, surface plasmon resonance in metal nanoparticles will be exhibited

by nanostructured materials. The mechanical, thermal and catalytic properties of these nanomaterials will change in comparison to the bulk materials due to the increase in surface area to volume ratio.

WHY DO WE WANT TO FABRICATE NANOSTRUCTURES ?

In addition to fundamental physical interest in the nanometer size regime, the different physical properties of nanosized structures compared to their bulk and molecular counterparts enable unique potential technological applications in medicine, electronics and optical devices.

INTRODUCTION TO POROUS ANODIC ALUMINUM OXIDE

Anodic aluminum oxide (AAO) films can be grown employing the electrochemical oxidation of an aluminum (Al) substrate in an electrolytic cell (Fig. 2). AAO films have been studied and applied in different industrial applications, including the construction of electrically insulating layers (dielectrics), anti-corrosive coatings and decorative coloration of metal surfaces [2, 3]. This fabrication procedure of AAO films is commonly known as anodization. Nano porous AAO films with hexagonal arrangement of monodisperse nanopores are common template systems for the fabrication of various functional nanostructures (*e.g.* nanofabrication of quantum dots) [4 - 6]. In 1995, self-organized AAO film was discovered by Japanese scientists Masuda and Fukuda using the right anodization conditions [7].

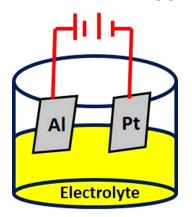


Fig. (2). Typical electrolytic cell used for anodization of Al.

Conventionally, a two-step anodization process is employed to produce such selforganized arrays of AAO nanochannels. Some of the applications of porous AAO films are mentioned as follows:

CHAPTER 5

PIXE/PIGE Measurements of Archaeological Glass, its Conceptualization and Interpretation: A Case Study

Roman Balvanović^{1,*} and Žiga Šmit²

¹ Vinča Institute of Nuclear Sciences, National Institute of Serbia, University of Belgrade, Belgrade, Serbia

² Faculty of Mathematics and Physics, Jožef Stefan Institute, University of Ljubljana, Ljubljana, Slovenia

Abstract: Reliable scientific answers to questions posed by social sciences, like archeology, to exact sciences, like physics or chemistry, depend not only on meaningfully posed questions, well-selected and pretreated samples and accurate and precise measurements, but also on an area of interpretation that exists between the two fields. This interdisciplinary area consists of many representations of measurement data, notions, and concepts that evolve through solving particular problems. However, this set of concepts is not always determinate, clear and consistent, obscuring the problem and obstructing the interpretation of results. The chapter explains this starting with a concrete example of measurements of archaeological glass using simultaneous PIXE/PIGE measurements, explaining general and technical details of measurements, and proceeds to show how the measurements are treated, processed, and displayed in ways to comply with the concepts, interpret the results, and provide adequate answers to the questions posed by archeology. The chapter also offers some possible improvements through a few novel concepts and ways of interpretation.

Keywords: Compositional group, Conceptualization, Geological class, Kernel density estimate, PIGE, PIXE, Principal component analysis, Rare earth patterns, Roman glass.

INTRODUCTION

The chapter focuses on the most important issues and approaches regarding the measurement and interpretation of analytical data in Archaeometry, illustrated by PIXE/PIGE measurements of ancient glass. It describes the most important con-

^{*} **Corresponding author Roman Balvanović:** Vinča Institute of Nuclear Sciences, National Institute of Serbia, University of Belgrade, Belgrade, Serbia; E-mail: broman@vinca.rs

Measurements of Archaeological Glass

cepts encountered in efforts how to understand and interpret the measurements that are meaningful for both the analyst and the archaeologist.

The chapter introduces several novelties. The equations for how to analyze glass with PIXE-PIGE without measuring the proton number have not been published before. There is also further improvement in the correction for geometrical effects. New ways to use principal component analysis (PCA) in data interpretation are suggested, enabling the depiction and comparison of large amounts of data in multivariate space. A step toward the identification of entities and relations in the interdisciplinary space between archaeological concepts and scientific measurements is made with the desire to lay a foundation for a formal interdisciplinary theory. All of this will be illustrated in the case study of the collection of eighty pieces of glass fragments (window panes, glass vessels, lamps, glass adornments) from the sixth century AD Byzantine settlement of Jelica, Serbia.

SIMULTANEOUS PIXE AND PIGE MEASUREMENTS OF ARCHAEOLOGICAL GLASS

How to analyze the chemical composition of glass? Glass is composed of light and heavy elements that, in principle, require different techniques for their determination. However, the metals in glass are in an oxide form with a known stoichiometric ratio, so it is then sufficient to determine the glass metal content. Though specific glasses may contain boron and fluorine, it is normally assumed that the analysis shall extend from (including) sodium onwards. The elements of Z>10 emit measurable X-rays, so X-ray fluorescence-based techniques may provide several advantages: they cover a large range of elements and are nondestructive, which is important when dealing with the objects of cultural heritage. For excitation of X-rays, irradiation with photons (X-ray fluorescence, XRF), electrons in the electron microscope (energy-dispersive spectroscopy, EDS) or charged particles (proton-induced X-ray emission, PIXE) can be used. The X-rays of sodium have an energy of 1.04 keV, so they absorb already in a few micrometers of material, including the irradiated sample itself. Corrosion processes that leach sodium out of the surface layer, causing sodium depletion, further afflict these shallow regions. The measurements are then preferably made in vacuum (EDS, PIXE) using thin-window X-ray detectors with no other absorbing material. For measurements at ambient pressure, helium flux may be used (XRF, in-air PIXE). For controlling the X-ray self-absorption, the glass object is usually sampled, and the sample is mirror-polished with abrasives and diamond paste. This is especially required for the measurements in an electron microscope, as the range of electrons of a few 10 keV energy is typically a few micrometers. Protons in the MeV energy range are much more penetrative and

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can reach several 10 μ m deep into the object; however, self-absorption of X-rays defines the mean X-ray production range of about 10 μ m for medium Z elements. It is sufficient that an unprepared glass surface can be used in PIXE measurement if the iridescent layer is peeled off. Another advantage of the proton beam is the small bremsstrahlung background. Bremsstrahlung X-rays are produced by accelerated charges: these are primary and displaced electrons in the case of the electron beam, and the resulting radiation can completely screen the characteristic X-rays of the elements heavier than iron. A complete analysis of a glass sample can be done by combining EDS with XRF. In vacuum, PIXE measurement is done in the same way as EDS in the electron microscope; the sample needs to be polished because of the self-absorption of sodium X-rays, though the sensitivity levels are significantly lower due to the smaller background, so heavier elements can be detected as well.

PIXE can also be applied in a way that the proton beam is extracted into the air through a thin window made of thin metal of plastic foils; the current material is silicon nitride (Si₃ N_4) of sub-micrometer thickness. The objects are then analyzed in situ without limitation of their size. The measurements are almost nondestructive, as the radiation damage is minimal, and preparation of the measuring spots can be avoided, as some clean plane spots are easily found on the whole object. Several facilities apply helium flush in the irradiated area, which reduces proton energy loss between the exit window and target and, at the same time, reduces the absorption of soft X-rays [1]. For the apparatus in Ljubliana, we decided on ambient aerial atmosphere with the purpose to save helium, as helium supplies in the world are diminishing. This implied limitation for soft X-rays, which are extensively attenuated in air, shifting the lightest element to be detected to silicon. Important glass elements of sodium, magnesium and aluminum thus became invisible. Their detection is nevertheless possible by another spectroscopic method, detection of gamma rays induced by inelastic proton scattering (proton-induced gamma emission, PIGE). Gamma rays are more penetrative than X-rays of low-Z elements, so sodium is analyzed below the depths afflicted by corrosion processes. Detection of sodium by PIGE is then practiced also in systems using vacuum or He atmosphere [2]. For measurements in air, PIGE can also be used for the analysis of magnesium and aluminum. The side effect of measurement in the air is the detection of X-rays from atmospheric argon, which can be exploited as well as an indicator of the impact of proton number or for monitoring the influence of experimental geometry on selfattenuation of X-rays.

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Sibel A. Ozkan



Sibel A. Ozkan

Sibel A. Ozkan is currently working as a Full Professor of Analytical Chemistry at Ankara University, Faculty of Pharmacy since 1986. She is an active member of the European Chemical Society-DAC member on behalf of the Turkish Chemical Society. She is a member of the European Pharmacopoeia-EDQM- Chromatography Section. She is a member of PortASAP: - European network for the promotion of portable, affordable and simple analytical platform: Core group of Cost Action CA 16215. Working Group 4.

She has been involved in several analytical chemistry projects related to LC methods, separation techniques, chiral separation, drug analysis in dosage forms and biological samples, electrochemical biosensors, nanosensors, DNA biosensors, enzyme sensors, biomarkers, environmental sensors, method development, and validation of drug assay.

She has published about 400 original and review papers and Editor of 10 scientific books from HNB Publishing (2012), Springer (2015), Bentham (5 Volumes, 2018-2020), Elsevier (2019, 2021), CRC (2022) and more than 50 book chapters in different years (from Elsevier and Springer).

She received the Ankara University Encouragement Award (2003), Turkish Pharmacists Association-Scientific Award (2008), Supervisor of The Best Ph.D. Thesis Award (Health Sciences) in Turkey from the High Council of Education of Turkey in 2017 and Ton Duc Thang University "Woman in Science 2019 Award" (Vietnam), Ankara University Science Award (2020).

She is the Editor of the Journal of Pharmaceutical and Biomedical Analysis (SCI) and Regional Editor (Europa) of Current Pharmaceutical Analysis (SCI). Besides, she is an Editorial Board member of Talanta (SCI), Chromatographia (SCI), Biosensors&Bioelectronics X, Critical Reviews in Analytical Chemistry, Analytical Bioanalytical Chemistry, Turkish Journal of Chemistry, and other journals.