APPLICATIONS OF MODERN
MASS SPECTROMETRY

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Applications of Modern Mass Spectrometry

(Volume 1)

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PREFACE

Mass spectrometry, combined with the modern tools of chemo- and bioinformatics, has emerged as one of the most powerful tools in research with numerous applications in industry. The analytical capacity of mass spectrometry has exceeded beyond its conventional role of determination molecular mass and structural determination. Recent developments in ionization, detection techniques and data analysis tools, as well as applications of mass spectrometry in various fields continue to open up new vistas in this rapidly evolving field. The present volume of ***"Applications of Modern Mass Spectrometry"*** provides a useful insight into some of these developments. The present 1st volume of this book series comprises of 5 comprehensive reviews, written by the leading practitioners of mass spectrometry. These articles present diverse applications of mass spectrometry in fields such as animal nutrition, food and environmental analysis, biomedical and forensic sciences, as well as toxicology and explosives. Qualitative and quantitative analysis and identification of proteins and peptides are the cross cutting themes in many of these articles.

The review by Gonzalez-Ronquillo *et al.* focuses on the accurate quantification of microbial protein synthesis in ruminants by employing ^{15}N isotopic mass spectrometry. Zhu *et al.* review the recent developments in qualitative and quantitative analysis of proteins and peptides in food matrices through LC-MS techniques. Izadmanesh and Ghasemi have provided an extensive review of the use of chemometric tools for the analysis of complex mass spectrometric data. Heavy metal toxicity is a global health challenge, and reliable methods are required for the detection of heavy metals in water and other samples. Developments in this area employing ICP-MS methods are reviewed by Boruah and Biswas. Finally Anilanmert and Cengiz have reviewed the relevant literature on the direct and on-site detection of various explosives and their residues by modern mass spectrometry. Each article presents case studies of the use of various innovative mass spectrometric tools, along with their strengths and limitations.

We are grateful to all the authors for their excellent scholarly contributions, and for the timely submissions of their review article. We would also like to express our gratitude to Ms. Fariya Zulfiqar (Manager Publications) and Mr. Mahmood Alam (Director Publications) of Bentham Science Publishers for the timely completion of the volume in hand. We sincerely hope that the efforts of authors and production team will help readers in better understanding and appreciating the versatility and robustness of mass spectrometry, and motivate them to conduct good quality research and development work in this exciting area.

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CHAPTER 1

Applications of Mass Spectrometry for the Determination of Microbial Crude Protein Synthesis in Ruminants

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Abstract: The importance of quantifying ruminal microbial crude protein synthesis has promoted the development and comparison of several different methods for precise determination of both the amount and rate of synthesis. One major challenge is in estimating and differentiating protein in the rumen between microbial, dietary, and endogenous fractions, and to correctly isolate the solid and liquid microbial fraction of the rumen contents. This is further complicated by the goal of using non-invasive methods as much as is feasible, such as avoiding the use of fistulated animals; the selection of an appropriate microbial marker, specifically one that behaves similarly in the solid-associated and liquid-associated microbial fractions. It is also vital to be able to accurately estimate the contribution of microbial protein to overall nitrogen used by the animal, which can be accomplished by the use of ^{15}N labeled, as assimilated by ruminal bacteria, and by the quantification of labeled nitrogen *via* mass spectrometry ($^{15}N/^{14}N$). This review focuses on challenges regarding accurate quantification of microbial crude protein synthesis in the rumen, as well as providing the methodology for quantification using the ^{15}N marker. This review is based on the collection of scientific papers from the main research groups in feed and animal nutrition in ruminants.

Keywords: Endogenous excretion, ^{15}N, Microbial protein, Purine derivatives, Ruminants.

INTRODUCTION

Ruminants are inefficient utilizing dietary nitrogen, so they have to use microbial protein (MP) to meet their metabolizable protein requirements [1, 2]. In order to

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know the crude protein (CP) requirements of ruminants and improve their efficiency, microbial crude protein (MCP) synthesis is used. Menezes *et al*. [3] mentions that if more CP is given than required by the ruminant, the excreted Nitrogen (N) increases instead of improving their performance.

The importance of quantifying MCP in the rumen has spurred the development and comparison of several different methods of analysis. However, these methods do not always satisfy the required scientific needs for specificity, efficiency, or cost, nor do they always address concerns regarding quantitative inconsistency in replicating this technique for estimation. Some of the major challenges of these methods focus includes 1) how to estimate and differentiate protein of microbial, dietary, and endogenous origins; 2) proper isolation of the microbial fraction in the particulate and fluid fractions of the rumen; 3) how to accurately quantify microbial protein in a minimally invasive manner, such as without the use of fistulated animals (ruminally and duodenally); and 4) the choice of an appropriate microbial marker that behaves similarly in both solid and liquid fractions [4, 5]. Regarding this final challenge, a currently used microbial marker is the labeled nitrogen ^{15}N, which is one of the most reliable and recommended methods, unlike others than often overestimate or underestimate MCP [6 - 10]. This review focuses on discussion of these challenges regarding accurate quantification of MCP synthesis in the rumen, as well as providing the methodology for quantification using the ^{15}N marker. This review based on the collection of scientific papers from the main research groups in feed and animal nutrition in ruminants.

MICROBIAL CRUDE PROTEIN SYNTHESIS

Nutritional studies in ruminants are aimed at the selection of feeds based on high efficiency of MCP synthesis in the rumen along with the available N sources and energy support. A key strategy for improving production has, therefore, been designed to maximize the efficiency utilization of available feed resources in the rumen by providing optimum conditions for microbial growth and thereby, supplementing dietary nutrients to complement and balance the products of rumen digestion to the animal's requirement. Supplementation with rumen-protected lysine and methionine can improve N use efficiency, maximizing the CP requirement from 18 to 15% in dairy cattle, without affecting milk yield production or animal performance [11].

Feed consumed by the ruminant, such as forage and cereals, enters the rumen and is available for degradation by rumen microbes. One fraction is rapidly degraded (Fraction A), while another is degraded slowly (Fraction B). Both fractions are then used for microbial growth and synthesis of MCP (Fig. **1a**) [12]). This process

depends on several factors, such as type of feed, ratio of forage: concentrate in the diet, fractional rate of degradation in the rumen, physiological stage of the animal, presence of secondary compounds (saponins, tannins, polyphenols, *etc*.), as well as the use of additives, such as enzymes or ionophores, all of which can affect digestion and microbial kinetics.

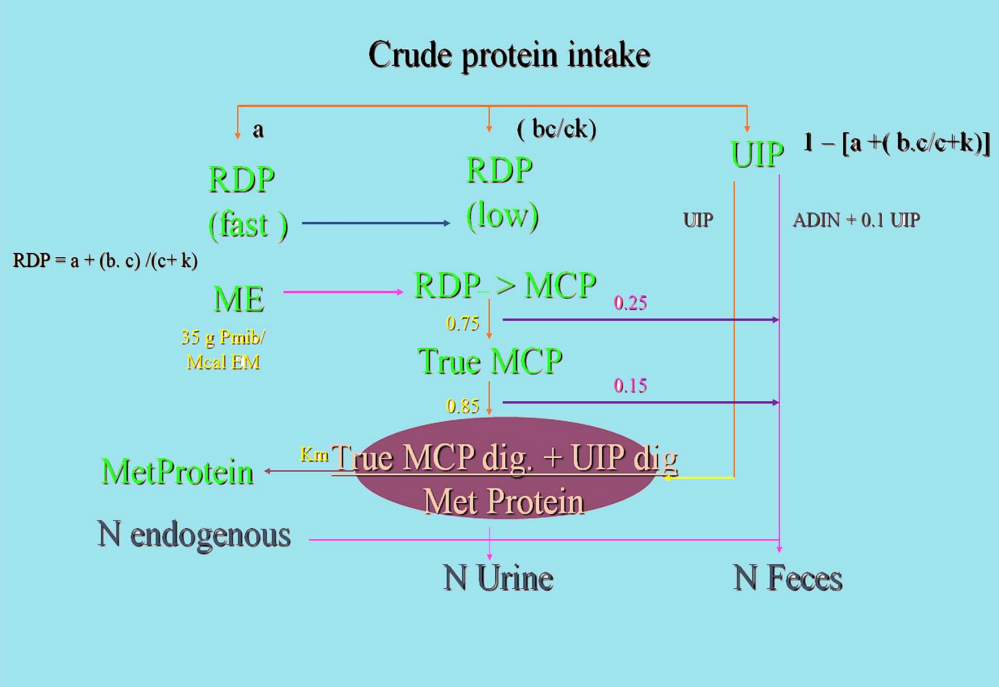

Fig. (1a). Ruminal degradable fractions, and microbial crude protein synthesis into the rumen (adapted from AFRC [12]).

In addition to the factors mentioned above, the synthesis of MCP is dependent on the energy supplied, which averages 35 g MCP/Mcal of metabolizable energy (ME) intake across particulate-associated bacteria (PAB) and liquid-associated bacteria (LAB). If the diet is energy deficient, there will be a corresponding reduction in MCP synthesis and ammonia from breakdown of amino acids and NPN will be absorbed into the bloodstream rather than being used for formation of MCP [13]. If the diet is too high in protein, excess protein will also be converted to ammonia and absorbed across the rumen wall into the bloodstream [14].

Once this MCP has been synthesized in the rumen, depending on the method of estimation [15], 75% is true microbial protein, the rest (25%) are nucleic acids, 85% is true digestible microbial protein (15% are undegradable amino acids)

CHAPTER 2

Qualitative and Quantitative LC-MS Analysis in Food Proteins and Peptides

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Abstract: LC-MS combines high separation ability of liquid chromatography with strong mass spectrometric structure identification. The advantages of LC-MS include high sensitivity and selectivity, minimal sample throughput, fast analysis speed and extensive structural information. It has been widely used in many fields, such as natural product analysis, pharmaceutical and food analysis, and environmental analysis.

In recent years, a great deal of researches have been conducted on the qualitative and quantitative aspects of food proteins and peptides. A variety of qualitative analyses of food proteins and peptides have been performed by LC-MS, such as accurate analysis of relative molecular weight, primary structural sequence, disulfide bond position, post-translational modifications (PTMs), *etc*. The quantitative analysis of proteins and peptides by LC-MS has been mainly achieved by two methods, *i.e*., label-free methods (peak intensities approach and spectral counting approach) and labeled methods (chemical labeling, metabolic labeling and enzymatic labeling methods). This chapter focuses on the application of qualitative and quantitative analysis of proteins and peptides in food sources.

Keywords: Food proteins and peptides, Mass spectrometry, Qualitative analysis, Quantitative analysis.

INTRODUCTION

The rapid growth of proteomics research initiatives depends on the use of mass spectrometry (MS). With the development of soft ionization technology, the ability of MS to analyze proteins and peptides has been greatly improved. In 1988, Tanaka *et al*. [1] invented matrix-assisted laser desorption ionization (MALDI), where samples were dissolved in a suitable solvent and mixed with appropriate matrix, and ionized by laser beams. It produces singly charged species

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for biomacromolecules, which is very important for identifying molecular ions of proteins. In 1989, Fenn [2] proposed electrospray ionization (ESI) technology, where samples were dissolved in a buffer or polar solvent, and then introduced into the mass spectrometer in the form of a spray. It produces multiply charged species, which make the mass range of ESI theoretically unrestricted. At present, ESI-MS is usually used for qualitative and quantitative studies of various non-volatile and thermally labile samples [3]. These ionization technologies have greatly facilitated the widespread use of MS in proteomics. Fenn and Tanaka shared the 2002 Nobel Prize in Chemistry for their contribution on ESI and MALDI, respectively. Since then, MS developed rapidly as one of the most popular methods in analysis.

Various liquid chromatography (LC) and MS platforms have been organically integrated and are playing an increasingly important role in the field of proteins and peptides analysis. This chapter focuses on the researches and applications of LC-MS in the qualitative and quantitative aspects of food proteins and peptides.

QUALITATIVE MEHTODS OF LC-MS ON FOOD PROTEINS AND PEPTIDES

Induced by light, humidity or temperature, protein and peptide components in food may cause spatial changes due to subtle changes in the primary structure, post-translational modifications (PTMs) and disulfide bonds, leading to losses of nutrients. Qualitative characterization is particularly important for discovering the changes, which is the first step to avoid these losses.

Due to its high sensitivity and accuracy, LC-MS has been developed as a routine method for characterizing complex food protein mixtures, such as the analysis of precise relative molecular mass, primary structural sequences, disulfide bond positions, and PTMs, *etc*.

The first step is to digest sample proteins into peptides using proteolytic enzymes such as trypsin, chymotrypsin. After separation using one- or multidimensional LC, the digested peptides are ionized and the selected ions are sequenced to produce signature tandem mass spectrometry (MS/MS) spectra. Digested peptides are identified by using automated database search programs, which correlate the experimental MS/MS spectra with theoretical spectra predicted for each peptide contained in protein sequence database [4]. Several MS/MS database search tools are currently available, including SEQUEST [4a] and Mascot [4b] as widely used commercial applications, X!Tandem [4e], OMSSA [4f], and ProbID [5] as open source database search tools, and SpectrumMill [5] and Phenyx [6] as integrated programs (that provide other functionalities in addition to database search). Then peptide assignments are statistically validated and incorrect identifications are

filtered out. Sequences of identified peptides are used to infer which proteins are present in the digested samples. Some peptides are presented in more than one protein, which complicates the protein inference process.

The Primary Structure and Relative Molecular Mass of Proteins and Peptides

Kou *et al*. [7] isolated and purified an antioxidant peptide CPe-III from the hydrolysates of chickpea albumin. Chickpea albumin isolates (CAI) were extracted from chickpea [8]. Chickpea albumin hydrolysates (CAH) were prepared from CAI by alcalase and flavorzyme proteases [9]. CAH was purified by gel filtration of Sephadex G-25, and lyophilized. The results of DPPH radical scavenging activity [10] showed that three peptide fractions obtained from gel filtration of CAH, Fraction I, Fraction II and Fraction III, were 23.15%, 34.02% and 41.3%, respectively. The results of hydroxyl radical scavenging activity [11] showed that at concentrations of 0.5 mg/mL, Fraction I, Fraction II and Fraction III inhibited hydroxyl radicals as 40.64%, 56.54% and 74.56%, respectively. The results of ABTS radical cation ($ABTS^{*+}$) decoloration assay [12] showed that Fraction III exhibited the highest antioxidative activity (0.967 ± 0.018 mmol/L). The reducing power [13] of Fraction III was the highest and exhibited a concentration dependence, as reported before [14].

Antioxidative peptides from Fraction III of CAH were purified and identified by RP-HPLC-ESI-MS/MS (LCQ Advantage MAX). From full scan LC-MS data, the highest peak was identified as a decapeptide (RQSHFANAQP, mw 1155 Da), corresponding to CPe-III, by Xcalibur software and BioWorks 3.3. CPe-III could be a suitable natural antioxidant in chickpea-related food.

Zhang's team [15] isolated and identified antioxidative peptides from rice endosperm protein (REP) enzymatic hydrolysate. First, REP was defatted and freeze-dried. Then, REP was hydrolysed with alcalase, chymotrypsin, papain, flavorase and neutrase, respectively, followed by measuring the antioxidant activities. The results of radical scavenging activities showed that these five enzymatic hydrolysates had the ability to scavenge DPPH radicals. At a concentration of 1.50 mg/ml, the values were 71.26 ± 1.06%, 35.26 ± 1.34%, 44.31 ± 2.12%, 24.31 ± 1.66%, and 85.86 ± 1.33% corresponding to alcalase, chymotrypsin, papain, flavorase and neutrase, respectively. Moreover, the results of superoxide radical (O_2^-) and hydroxyl radical ($^{\cdot}OH$) scavenging capacities suggested that at 2.00 mg/ml, the neutrase hydrolysate had the highest values, 75.69±1.23% and 82.93±1.21%, respectively. Fraction 3 of the neutrase hydrolysate had the highest antioxidant activity in four ion-exchange isolated fractions. The subfractions F3b and F3c further separated by RP-HPLC from

CHAPTER 3

Chemometrics as a Powerful and Complementary Tool for Mass Spectrometry Applications in Life Sciences

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Abstract: Because of its unique capabilities, mass spectrometry is an indispensable part of life science research. In this chapter, a review is made on aids of chemometrics in life sciences applications of mass spectrometry. Because of the increasing complexity of biological samples and ongoing technological enhancements of mass spectrometers, huge sum of data are provided for each biological sample. If the routine exploratory tools are used for data exploration, much of the information is not extractable and hence it gets lost. However, chemometrics helps to explore data thoroughly and extract maximum amount of information. The most common aids of chemometrics in bio-based mass spectrometry data is for experimental design, noise reduction, classification, library search, identification of biomolecules, finding the biomarkers, data compression and data mining.

This chapter is focused on the different aspects of using chemometrics for the analysis of mass spectrometry data in omics and biomedical images. In the first part, chemometrics applications for mass data in omics sciences (metabolomics and proteomics) are revealed. The mass data in omics are mainly provided by hyphenation of mass spectrometry with chromatographic techniques, i.e., gas chromatography (GC), liquid chromatography (LC) and electrophoretic techniques. In the second part of the chapter, the benefits of using chemometrics for mass spectrometry images are revealed. The data of these images are gathered by mass spectrometer itself or hyphenation with chromatographic techniques. Since, hyphenated methods are used for both omics and biomedical imaging, some of the chemometrics methodologies used in these two disciplines may be the same.

Keywords: Biomolecules, Biomarker detection, Biomedical imaging, Chemometrics, Data analysis, Data binning, Data compression, Data mining, Experimental design, Genomics, Life science, Mass spectrometry, Metabolomics, Multivariate curve resolution-alternating least squares (MCR-LAS), Multivariate

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methods, OMICS, Proteomics, Regions of Interest (ROI), Statistics, Variable selection, XCMS software.

INTRODUCTION

Mass spectrometry (MS) records the mass-to-charge ratio (*m/z*), of the sample components. In a simplified scheme, mass spectrometer consists of three parts: the ionization source, the mass analyzer, and detector. Therefore, the components must get ionized in the ionization source before *m/z* measurement. The ionized components are then transferred to the mass analyzer that separates the components according to *m/z* of the ions. Then, the detector detects the separated components, and mass spectrum is recorded. A mass spectrum is depicted as a diagram which *m/z* in x-axis and the intensity of the signal for is in y-axis [1]. In recent mass spectrometers carrying Orbitrap or FT-ICR analyzers, the mass analyzer itself performs the mass analysis and detection. There is not a separate detector where the ions will hit. Nowadays, most of life science studies use high resolution mass spectrometry in Orbitrap equipment [2].

For many years, mass spectrometry has played an important role in the health and life sciences researches. Investigation of complex biomolecules by mass spectrometry approaches is crucial in molecular life science research. In order to monitor qualitative and quantitative changes within hundreds or thousands of biologically active components, including proteins/peptides, lipids and metabolites mass spectrometry is an indispensable part. The mass spectrometry aided studies can help to understand pathophysiology of disease development at a molecular level and to monitor the effect of pharmacological treatment [3].

Often, the scientific measurements can be collected in a data matrix, where each row constitutes an observation and the columns represent the measured variables or factors (e.g., wavelength, mass number, chemical shift). This type of data collection generates huge data tables, which are hard to extract relevant information without appropriate tools. The power of chemometrics becomes relevant when working with these data which leads to the extraction of plentiful of useful information. However, in biology, chemometrics has been largely neglected in favor of traditional statistical methods. The overwhelming size and complexity of the data has forced biologists toward the use of multivariate statistical methods such as robust modeling [4 - 7].

Chemometrics is an interdisciplinary science that uses mathematics, statistics and computer sciences to overcome the huge data challenges. The interpretation of chemical data by chemometric methods helps to design, select and optimize experiments, and extract relevant information. Chemometrics can be defined as computer applications in chemistry, including data acquisition and processing,

optimization, intelligent laboratory systems, robotics, statistics, pattern recognition, cluster analysis, library search, structure property relationship, modeling information theory, artificial intelligence and expert systems [8, 9]. The computer hardware and software have widespread use in mass spectrometry [10, 11]. Conversely, the challenging problems from mass spectrometry has stimulated development of chemometrics. The chemometrics is a powerful tool for data acquisition, data handling, instrument control, and data interpretation in mass spectrometry [12].

In this chapter, we are going to have a brief survey of adaptation of different chemometric methods into the mass spectrometry and mass data collected from biological samples. This chapter will provide an overview on how chemometrics has evolved as an invaluable and indispensable part of mass spectrometry in life sciences. However, it should be reminded that this chapter may not cover all data analysis and computing approaches used in the life sciences mass spectrometry data.

MASS SPECTROMETRY OMICS DATA

OMICS can be defined as the study of the abundance and (or) structural characterization of biomolecules in living organisms (see Fig. **1**). The high-throughput omic technologies are useful for clinical characterization of diseases in organisms and evaluation of efficacy of existing or under-development therapies [13].

OMICS include DNA studies (genomics and epigenomics), RNA studies (transcriptomics), proteins study (proteomics), and metabolites studies (metabolomics). Recently, another omic subdiscipline called fluxomics, is developed to study the total set of fluxes in the metabolic network (fluxome) of the bio organisms. Other omic platforms include lipidomics, glycomics, foodomics, interactomics, and metallomics [14].

The technologically improved analytical techniques produce large omic data sets with complex structures. In mass spectrometry based analytical methods (GC-MS, LC-MS, GC×GC-MS, LC×LC-MS and electrophoresis-MS), highly complex OMIC data, lead to exploratory and interpretative challenges. To get useful information on major events taking place in an investigated system, data need to be processed and analyzed. The multivariate data are highly complex, and data analytical techniques must be used to cope with data challenges, including noise, collinearities, and missing data [15].

As indicated in Fig. (**1**) mass spectrometry is mostly used in metabolomics and proteomics. Hence, in this chapter, the data analysis strategies will be mostly

CHAPTER 4

Recent Developments of Allied Techniques of Qualitative Analysis of Heavy Metal Ions in Aqueous Solutions with Special Reference to Modern Mass Spectrometry

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Abstract: Heavy metal ions are basic elements of earth crust. These metal ions are non-biodegradable in nature and tend to accumulate in our ecosystem in due course of time. Some of the most toxic heavy metal ions include arsenic, mercury, cadmium, lead, nickel *etc*. The toxicity level depends on density for any biological system. Due to increasing applications of heavy metal ion compounds in industrial, agricultural and medical fields, water pollution induced by excess levels of heavy metal ion becomes a big crisis for us. As such, detection of heavy metal ions in water is an important issue for us. Mass spectroscopy methods are the most conventionally applied methods for the detection of heavy metal ions in water. Some of the mass spectroscopic methods are atomic absorption spectroscopy, inductively coupled plasma mass spectroscopy, graphite furnace atomic absorption spectroscopy *etc*. These methods have well detection capability of heavy metal ions in water with good selectivity and sensitivity. Along with mass spectroscopic methods, the use of optical fiber technology for heavy metal ions detection is remarkable. Optical fiber based sensors system for the detection of heavy metal ions basically works by changing the effective refractive index of its surroundings. For selective binding of heavy metal ions, sensitive layers are coated on optical fiber probe. Laser or light emitting diode is used as a light source in an optical fiber sensor for signal purpose. Accordingly, output response for various heavy metal ions is recorded on an optical spectrometer. From their output response, we can determine the concentration of metal ions present in water. It is noticed that optical fiber sensor can also have good sensitivity and selectivity towards the detection of heavy metal ions as mass spectroscopy methods.

Keywords: Arsenic, Cadmium, Colorimetric, Detection, Electrochemical, Heavy metal ion, Lead, Mass spectrometry, Mercury, Optical fiber sensor, Sources of heavy metal ions.

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INTRODUCTION

Heavy metals are the natural elements of the earth crust. The term "heavy" refers to relatively high density and atomic mass. This means that heavy metals possess high mass to volume ratio. The metals having atomic weights in between 63.5 and 200.6 g/mol with density 5 g /cm^3 can be labeled as heavy metals [1, 2]. But it is worthy to mention that any metal can be called heavy metal if its' presence in any system crosses the limit of its permissible limit irrespective of atomic mass or density. When the presence of any metal in any system exceeds its permissible limit, then it becomes toxic. Hence, it can be implicitly stated that heavy metals are toxic for any biological system for longer endurance [3, 4].

Metalloid such as arsenic is also called a heavy metal because it emerges to be highly toxic at a very low level of concentration [5, 6]. There are other metals such as iron, copper, manganese, zinc, cobalt *etc*. They are regarded as micro-nutrients of biological system. These micro-nutrients facilitate lots of activities in human body and also in plant. In case, the concentration of these metals increases beyond the permissible limit; they become poisonous and may cause various health issues [7, 8]. The most commonly known heavy metal ions are mercury (Hg), lead (Pb), cadmium (Cd), arsenic (As), chromium (Cr) and nickel (Ni). Even at very low concentration such as parts per billion (ppb) *i.e.* μg/L, these metal ions are harmful and intake or inhalation may cause different types of ailments [9].

In general, heavy metals are ubiquitously found. Nowadays, anthropogenic sources have become a major concern for us. Heavy metal compounds are used in different industrial sectors, and in agricultural field as pesticides, fertilizers, *etc*. Similarly, in semiconductor industry, arsenic is used as a doping element. After utilization of these heavy metal compounds, they finally enter environment [10, 11]. Being non-biodegradable in nature, these metal ions accumulate in our ecosystem and contaminate aquatic bodies eventually. As it is known that water is a primary essential element of the food cycle, hence utilization of heavy metal polluted water leads to contamination of food cycle. Through drinking of contaminated water or food, these heavy metals enter our body. These heavy metal ions have a tendency to bind with the thiol group of proteins [12]. In this way, these heavy metal ions are bio accumulated in different organisms of human body. Due to this bio accumulation, various types of diseases such as cancer, nervous system damage, kidney, liver, lung problem, high blood pressure *etc*. may occur [13].

There are several organizations such as the World Health Organization (WHO), the Environment Protection Agency (EPA), and the Centre for Disease Control

(CDC) for monitoring the effect of these metal ions to our environment. In Fig. (**1a**) and Fig. (**1b**), we have shown the most commonly found heavy metal ions and their various sources, respectively. These heavy metal ions are called "environment health hazards" depending on their toxicity and contamination in air, water and soil [9, 14]. In Table **1**, the most toxic heavy metal ions along with WHO permissible limits and their effect on human body are enlisted.

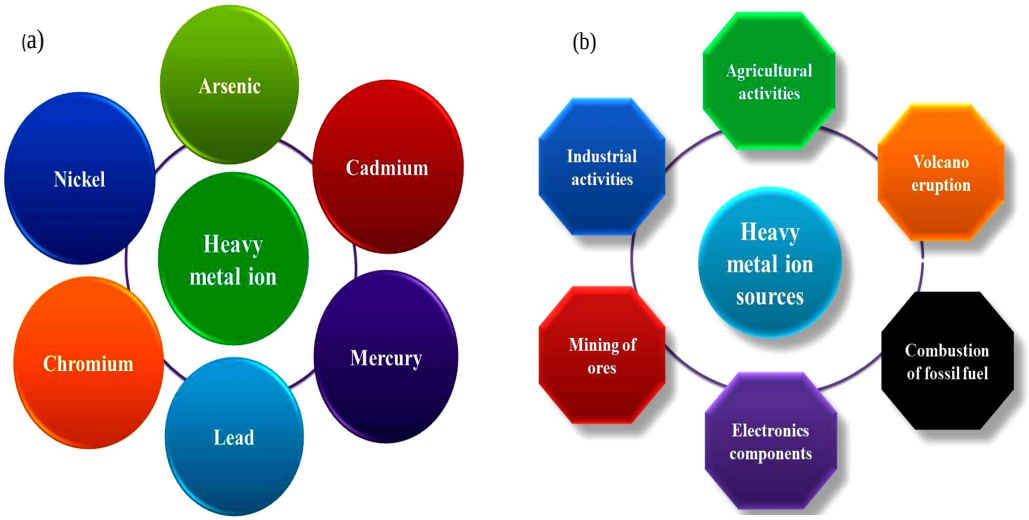

Fig. (1). (a) The most toxic heavy metal ions and (b) various sources of heavy metal ions.

Table 1. Toxic heavy metal ions, their sources, WHO permissible limit and their various health effects.

Heavy Metal	Sources	WHO Limits (ppb)	Health Effects	References
Mercury (Hg)	Volcanic eruptions, gold mining, burning of fossil fuels	2	Damage of kidney, heart, muscle, respiratory system, liver.	[15]
Arsenic (As)	Electronics industry, agricultural use of pesticides, fertilizer	10	Skin cancer, neurological damage, high blood pressure, kidney, reproductive system damage.	[16]
Cadmium (Cd)	Plastic materials, paints, pigments, photovoltaic cells	5	Cell damage, weight loss, metabolic disorder, renal cancer.	[17]
Lead (Pb)	Plastic pipe, batteries, paints pigments.	10	Anemia, kidney damage, memory loss, hepatopathy, neurological problem.	[18]

CHAPTER 5

New Techniques and Methods in Explosive Analysis

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Abstract: In forensic analytical chemistry, chemical investigation of the liquid/gas/solid evidences from the crime scene after the explosion (soil, water, concrete/glass/ wood pieces, metal, clothes taken from suspects, *etc*.) and reliable identification of explosive residues on such evidences remain an active area of research due to increased demand for homeland security against terrorist and warfare threats, as well as environmental monitoring. GC-MS, LC-MS, and LC-MS/MS offer distinct advantages for laboratory analysis of explosives in post-blast samples, including soil/ water/plant matrices, *etc*. Time-of-Flight, Ion-Trap, and Orbitrap technologies provide high resolution, better analyte identification, and accurate mass information at sub-ppm levels. Direct analysis techniques, such as ambient MS has a wider range of applications and offer high sensitivity/selectivity and direct analysis from the surface of interest. Techniques like Direct Analysis In Real Time (DART) and Desorption electrospray ionization (DESI), which can ionize substances directly on surfaces, offer new opportunities for security screening of explosives. Orbitrap MS was also used together with Raman microscopy for detailed molecular-level characterization of explosives and the chemical analysis of latent fingerprints. Electro-flow focusing ionization with in-source collision-induced dissociation can be used for MS detection and chemical imaging for speciation of the signatures of explosive devices and to detect proper spatial discrimination of explosive traces. Miniaturization to be used in-field analysis with low cost is a technique work on currently. Multi-analyte detection with high selectivity/sensitivity to be able to use in as many different matrices and to be able to analyze without or with a very short and simple sample preparation methods are targeted for future analyses. Despite other reviews focussing on a certain group of techniques, this chapter summarizes some important developments in the standard MS techniques and ambient MS techniques used in laboratory, on-site, and miniaturized mass spectrometric analysis of high energetic materials in the last two decades, mostly conducted in the last decade.

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INTRODUCTION

In recent years, various terrorist attacks have taken place, especially in the urban cities, and unfortunately, a number of these attacks have resulted in the deaths of many innocent people [1].

Analyzing the post-blast debris for explosives in terroristic attacks helps in tracing the origin of the explosives used and the possible suspects in order to prevent further threats [1]. In forensic analytical chemistry, analysis of the liquid, gas or solid evidences (soil, water, concrete/glass/ wood pieces, metal, clothes of the suspects, *etc*.) found in the crime scene after the blast and reliable identification explosive residues on these, are very important for solving the crimes, terrorist attacks, warfares and for finding the type of the explosives and their sources. Explosive detection methods have been developed for humanitarian demining, environmental issues (since explosive residues in the environment are a threat to the human health) as groundwater and soil remediation, security screening, intelligence activities, criminal forensics, as rapid sample screening and/or quantification [2]. The results of these analyses are frequently used as evidences in courts, from the point of identification of explosives used in terrorist attacks (to identify the type of bomb), to find its country of origin or manufacturer, and to aid in connecting a suspect with the crime scene [3].

Various screening and/or quantitation methods have been developed in recent years using ion mobility spectrometry, colorimetric method, cyclic voltammetry, optical sensors, UV-Raman spectroscopy, GC-MS, LC-MS, *etc*. and new techniques as Accu-Time of Flight (TOF) Direct Analysis in Real-Time **(**DART)/MS and HPLC-photodiode array (PDA)-APCI negative ionization-Linear Trap Quadrupole **(**LTQ) MS2/Orbitrap Fourier Transform Mass Spectrometer (FTMS), also exists in literature [4 - 12]. Various sample preparation methods, such as; solid-phase extraction (SPE), solid-phase microextraction (SPME), supercritical fluid extraction (SFE), solid-liquid extraction (SLE) have been used [13 - 22]. Standard techniques for detection and quantification of explosives include gas and liquid chromatography and spectroscopy (especially mass spectroscopy) [23]. Multi-explosive identifications are achieved using these techniques. Especially in combat with terrorism, field-portable techniques gain much interest. Portable detectors are mostly based on ion mobility spectrometry and surface acoustic wave techniques. Other techniques as

Raman, terahertz spectroscopy *etc*. are also developed for remote analyses. In techniques as multiarray colorimetric detection, multiple types of sensors may be required regarding a given environment, according to a required limit of false positives, confidence needed for identification, and the molecules which are needed to be detected. Unlike the other reviews which are focused on specific MS techniques as ambient MS techniques, this chapter reviews the modern applications of MS techniques in explosive analysis from a broad scope regarding the last two decades, focusing mainly on the studies and techniques pertaining to the last decade. You can see a photo from the attack in Vodafone Arena Stadium in Istanbul in the website of European Press Agency [24].

A CONCISE INFORMATION ON EXPLOSIVES

Explosives are chemicals, having great potential energy, which is transformed into stable compounds through speedy decomposition after a sudden impact, electricity, or spark, releasing a huge sound, heat, blast, and gases [25]. Explosive materials are usually prepared from a hydrocarbon-based fuel component, and a nitrogen- or oxygen-based trigger (as nitrate or a peroxide) [26]. Explosives have

enough oxygen in their molecules to initiate and continue the very rapid progressive combustion [25]. The amount of discharged energy changes with the properties of the material, such as composition, structure, density, heat of formation and decomposition, *etc* [27]. There are two classes for military explosives as “low” or “high” explosives, depending upon the speed of propagation of the combustion reaction [28]. The rate of decomposition of low explosives is adequately slow to be used safely as a propellant in a gun. Deflagration or burning can be started with these. For high explosives, the decomposition reaction propagates faster than the speed of sound, so rapidly that can be called as “instantaneous”, creating a shockwave [26, 28]. High energy materials are named as explosives, propellants and fireworks according to their properties and uses [27]. Mercury fulminate, Lead azide, octahydro-1,3,5--tetranitro-1,3,5,7-tetrazocine (HMX), Pentaerythritol tetranitrate (PETN), Amatol, Tetryl, 2,4,6-trinitrotoluene (TNT), 2,6-dinitrotoluene (2,6-DNT), Tetrytol, ethylcentralite (EC), Ethylene glycol dinitrate (EGDN), nitroglycerine (NG), 2,3- dimethyl-2,3-dinitrobutane (DMNB), Cyclonite, Tritonal, Pentolite, Ednatol, Torpex, Haleite, 1,3,5-trinitroperhydro-1,3,5-triazine (RDX), triacetone triperoxide (TATP), hexamethylene triperoxide diamine (HMTD), hexanitrostilbene (HNS), Ednatol, Pentolite, Torpex, Cyclonite, nitrate/fuel oil (AN/FO=ANFO), *etc*. are among some important high explosives [27, 28]. It is estimated there are at least 150 separate materials in use today [26].

The most common explosives used by the terrorists are high explosives such as

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ATTA-UR-RAHMAN, *FRS*

Prof. Atta-ur-Rahman, Ph.D. in Organic Chemistry from Cambridge University (1968) has 1,150 international publications (43 international patents and 254 books). He received the following awards: Fellow Royal Society (FRS) London (2006), UNESCO Science Prize (1999), Honorary Life Fellow Kings College, Cambridge University, (2007) Academician (Foreign Member) Chinese Academy of Sciences (2015), Highest Civil Award for Foreigners of China (Friendship Award, 2014), High Civil Award Austria ("Grosse Goldene Ehrenzeischen am Bande") (2007), Foreign Fellow Chinese Chemical Society (2013), Sc.D. Cambridge University (UK) (1987), TWAS (Italy) Prize (2009) He was the President of Network of Academies of Sciences of Islamic Countries (NASIC), Vice President TWAS (Italy), Foreign Fellow Korean Academy of Science & Technology, President Pakistan Academy of Sciences (2003-2006) and (2011 – 2014). He was the Federal Minister for Science and Technology of Pakistan (2000 – 2002), Federal Minister of Education (2002) and Chairman Higher Education Commission/Federal Minister (2002-2008), Coordinator General of COMSTECH (OIC Ministerial Committee) (1996-2012), and the Editor-in-Chief of Current Medicinal Chemistry.

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SYED GHULAM MUSHARRAF

Prof. Dr. Syed Ghulam Musharraf is amongst the most notable young scientists based on his seminal contributions to mass spectrometry and its applications. He obtained post-doctoral training from Austria and USA. On his return, he established a world-class mass spectrometry research laboratory in Pakistan. He has effectively used several mass spectrometric tools for the high-throughput analyses of bioactive compounds and the standardization of botanicals. He pioneered in biomarker-based metabolomics research in Pakistan for early diagnosis of lung, breast, oral and blood cancers, as well as thalassemia. He is the author of over 150 research publications (impact factor around 400, h-index = 25, Google Scholar). Based on his scientific contributions, he has received several international/ national awards and honors.