DEVELOPMENT OF A MINIATURE MASS SPECTROMETER AND IONIZATION METHODS FOR DIRECT SMALL BIOMOLECULE ANALYSIS

by

Xiao Wang

A Dissertation

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Doctor of Philosophy

Weldon School of Biomedical Engineering

West Lafayette, Indiana

December 2017

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Zheng Ouyang, Co-Chair
Weldon School of Biomedical Engineering
Dr. Fang Huang, Co-Chair
Weldon School of Biomedical Engineering
Dr. Riyi Shi
Weldon School of Biomedical Engineering
Dr. Yu Xia
Department of Chemistry

Approved by:
Dr. George R. Wodicka
Head of the Graduate Program

For the time to be memorized

ACKNOWLEDGMENTS

I still remember the first night that I arrived at Purdue five years ago. It was a new start and a new world for me. Time flies and it is time to start again. I really appreciate all the people I met and accompanied during the past five years.

Firstly, I would like to thank Dr. Zheng Ouyang who is my advisor for the last five years. He is the person who brought me to the world of mass spectrometry, ambient ionization and portable instrumentation. His persistence inspired and encouraged me to pursue my own goals. He instructed me how to present research works and how to communicate with others. Also, he shared his life experiences with us when we met difficulties and helped me to get through any troubles.

I would also thanks Dr. Fang Huang as my co-chair of the committee and Dr. Riyi Shi and Dr. Yu Xia as my committees. They offered a lot to help my research and career development. I would also thanks Professor Graham R. Cooks for his kind and supportive help.

Special acknowledgements to Dr.Yue Ren, Dr. Linfan Li and all the people who make significant contributions to the development of Mini β mass spectrometer. I would also thank Purspec technologies and thank the people there, including but not limited to: Ang Li, Xinwei Liu, Kai Liu, who made the first Mini β work possible.

I would also like to give special thanks to all the group members in Purdue Ouyang's lab, Purdue Aston lab, Purdue Xia's lab, and the MS lab in Tsinghua University. It is really my pleasure to work with all of the talented people there. Dr. Tsung-Chi Chen helped me to know how to use miniature mass spectrometer, Dr. Ziqing Li taught me the chemistry in MS world, Dr. Anyin Li helps me with nano-electrospray ion source and Dr. Pu Wei about how to prepare the solution for analysis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

HGP: Human Genome Project

LOD: Limit-of-detection

LC: Liquid chromatography

GC: Gas chromatograph

MS: Mass spectrometry

MALDI: Matrix assisted laser desorption ionization

ESI: Electrospray Ionization

FDA: Food and Drug Administration

CT: Computed tomography

MRI: Magnetic Resonance Imaging

UV: Ultraviolet

DESI: Desorption electrospray ionization

RF: Radio Frequency

AC: Alternative Current

DC: Direct Current

DAPI: Discontinues Atmospheric Interface

ID: Inner Diameter

OD: Outer Diameter

SDI: Synchronized Discharge Ionization

ABSTRACT

Author: Wang, Xiao. PhD
Institution: Purdue University
Degree Received: December 2017
Title: Development of a Miniature Mass Spectrometer and Ionization Methods for Direct Small Biomolecule Analysis
Major Professor: Zheng Ouyang

Abstract

Precision medicine is one of the most popular topics in the world. The successful analysis of biomarkers and biomolecules in an accurate and precise way is key for precision medicine. Currently, most developed methods are focused on gene sequencing, proteomics or targeted molecular therapy. Qualitative and quantitative tests of small biomolecules are still lacking in attention. The rapid, direct analysis of small biomolecules with an accessible instrument is highly demanded. This study focuses on mass spectrometry based technologies that seek an easy-to-use and high throughput solution for direct biomolecule analysis.

The development of a portable mass spectrometer at Purdue has been underway for more than a decade. The miniature ion trap mass spectrometer can provide in-field analysis capability for a large variety of samples, such as drug molecules, lipids or peptides. Ionization methods such as paper spray, extractive spray and paper-capillary spray have been developed to couple with the miniature mass spectrometer. The advancement of ambient ionization facilitates real-time analysis using Miniature mass spectrometry. Taking advantage from the solid fundamental research done in Purdue, this study focuses on the development of a new miniature mass spectrometer system and new ionization methods for direct quantification of biomolecules. Firstly, a new vacuum design for the miniature mass spectrometer was constructed and tested. Using this new design, a

new miniature mass spectrometer, Mini β, was evaluated in detail. Lastly, a new ionization method was developed for the direct analysis of chemicals on surfaces.

The vacuum system is one of the most important systems in a mass spectrometer. For the miniature ion trap mass spectrometer developed in Purdue, a discontinuous atmospheric pressure interface (DAPI) is used with a Hipace-10 turbo pump. In this study, a new turbo pump, Hipace-30, is used to improve the pumping speed and analytical performance of the instrument. A dual-linear ion trap system is constructed using the new vacuum design. The pressure variation curve, isolation performance, collision induced dissociation efficiency and the mass selective axial transfer efficiency are studied in detail. The duty cycle of the new system is increased by a factor of three. Using the new turbo pump, a supplementary DAPI can be used to actively control the pressure in the vacuum chamber to a desired value which increases the ion manipulation efficiency.

A new miniature mass spectrometer, Mini β, has been designed and constructed based on the new pumping configuration with a dual quadrupole linear ion trap system. The analytical performance of the new system is evaluated in this study. As a linear ion trap mass spectrometer, the Mini β can achieve sub-ppb level of detection limit using nano-ESI and ppb level using paper capillary spray cartridge. Mini β has a resolving power higher than 700 with a scan speed of 1000Da/s and is capable to analyze both positive and negative ions with MS^n capability. The new system also demonstrates the possibility of analyzing biomolecules, including lipids, peptides and small proteins. A paper-capillary spray cartridge ion source has been designed to couple the new Mini β instrument. Using the new Mini β mass spectrometer with sample cartridge, the successful detection of illicit drug in urine and illegal additive in medicine has been demonstrated using real samples.

For the ionization and sampling method, a sampling probe is designed based on the DAPI system and an internal discharge ionization method has been evaluated. The ionization process is synchronized with the DAPI operation. Non-volatile chemicals with a vapor pressure around 10^{-4}torr on a surface can be directly analyzed. Sample with lower vapor pressure can still be analyzed by using a 1-watt heater to heat the sample gas. Methanol and water vapor were found to be effective to improve the ionization efficiency for analyte that generates protonated ions.

With the advanced vacuum and sample introduction technology, the new miniature mass spectrometer, Mini β, and the new ionization and sampling technology, portable mass spectrometer system can be further implemented into the precision medicine area for clinical applications.

1. INTRODUCTION

1.1 Precision Medicine and Biomarker Analysis

Every patient has unique characteristics. The living environment, medical history and genetic basis are pivotal factors that influence the diagnostic and treatment results of a patient[1]. The concept of precision medicine dates back to the 19th century, where blood transfusions are based on the blood type of each patient. Since the first draft sequence of the human genome in 2001, precision medicine has become increasingly popular and effective, benefiting from scientific and technical advances. Precision medicine takes advantage of statistical results and divides patients into subpopulations based on their characteristics. This ensures the delivery of a tailored treatment at the right time to a specific patient[2-3]. Additionally, tailored medication treatment reduces treatment-related toxicity as well as expenses. Another aspect of precision medicine differentiates patients based on the susceptibility to a disease and provide advanced targeted prevention therapies. Disease can be controlled and treated in early stages to avoid further damage, increasing the patient's quality of life.

Biomarker tests[4], as well as the biomolecule tests[5], are key factors for the implementation of precision medicine. These biological analyses provide information from molecular to organ level which can guide medication actions. The advance of precision medicine requires these biological analyses to be more accurate and reliable. The collection of information and generation of a database for analysis results will improve method validation and clinical practice implementation. Precision medicine requires biomolecular tests to be precise and accurate to optimize treatment strategies for optimal outcomes. Furthermore, test results can provide researchers with valuable treatment efficacy data and highlight any potential health risks. These biomolecule tests also offer insight into the correlation between pivotal factors such as the environment, genetic and disease in

the long term. However, an inaccurate test or an improperly handled test will do more damage than before and can potentially be as problematic as a bad drug.

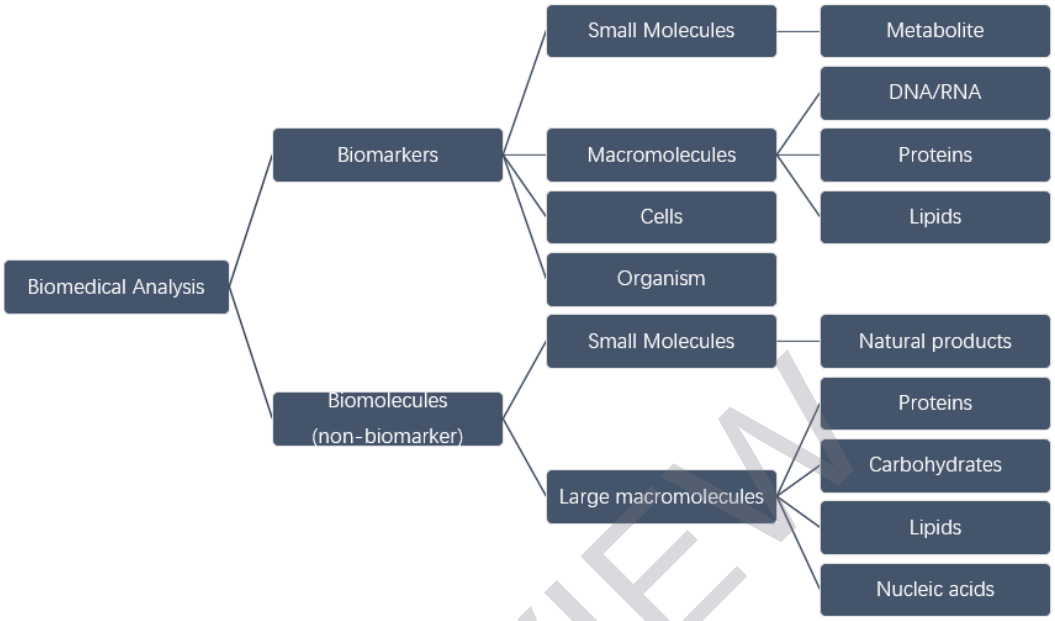

Figure 1. The definition and classification of biological analysis.

The concept of biomolecules and biomarkers share some similarity but also have their own focuses. As shown in Figure 1, biomarker tests emphasize more on the examination of biological indicators generated from human body, such as molecules, cells and even organs, which represent the status of various human diseases. Including molecules, cells and even organs. Biomolecule tests focus more on the analysis of various molecules in the human body, including endogenous molecules and exogenous molecules, such as pharmaceutical drugs, heavy metals and semisynthetic molecules. Exogenous molecular tests and metabolized exogenous molecular tests play a significant role in precision medicine which also works as a complement for biomarker tests.

Technologies have been developed to provide biomarker analysis in different scopes. Medical imaging including MRI, CT, PET and ultrasound are used for imaging organs *in vivo*, which provide guiding information for treatment. After the accomplishment of Human Genome Project (HGP)[6] in 2003, high-throughput genomic sequencing technologies or called next-generation

sequencing[7], was industrialized which provides production of thousands or millions of sequences simultaneously. Electrophoresis, protein chips, and liquid chromatography (LC) coupled with high resolution mass spectrometry, like time-of-flight mass spectrometry or orbitrap mass spectrometry help humans comprehend the importance of protein expression, structure and conformation. The advancement of LC-MS also benefits the analysis of lipid and small molecules in humans with high quantification accuracies and good limit-of-detections (LOD). Additionally, the use of matrix assisted laser desorption ionization with mass spectrometry (MALDI-MS)[8] to analyze microorganisms has been approved by the US Food and Drug Administration (FDA) [9-10].

Biomarker tests have several different uses in clinical practice (summarized in table 1). One biomarker test is used to understand a patient's molecular level and justify the status of the patient in a different domain. This information can be used as a patient's screening, diagnosis and classification evidence. Numerous biomarkers have been revealed to be highly correlated to some diseases including cardiovascular disease[11], Alzheimer Disease[12], cancer[13] and glomerular disease[14]. Another purpose for biomarker tests is to lead treatment-related actions, such as drug selection, adjustment of drug dosing and timing, and the forecast of possible adverse effects. Posttreatment biomarker tests can provide information through treatment evaluation and potential disease related risks.

The analysis of biomolecules without biomarker identity has undoubted significance. One typical application is therapeutic drug monitoring (TDM)[15-16]. Drug blood level is not only determined by the gene expression but also by the environment. The patient's age, body weight, mental condition, or even food and water intake can influence one's short term drug blood concentration significantly. However, the therapeutic window of drugs may not be wide. A rapid, accurate and sensitive drug level measurement can help to improve the optimization of drug dosing and the treatment efficacy,

and at the same time, minimize side effects due to the overdosing. Other typical applications are drug compliance monitoring[17] (increase health care delivery), drug metabolism detection (reduce exceptional adverse effect) and restricted drug control (control the drug of abuse).

Table 1. The classification of clinical biomarker uses and objectives (reprint from reference)[18]

Clinical Biomarker Use	Clinical Objective
Screening	Detect and treat early stage disease in the asymptomatic population.
Diagnosis/differential Diagnosis	Definitively establish the presence and precise description of disease.
Classification	Classify patients by disease subset.
Prognosis	Estimate the risk of or the time to clinical outcomes.
Prediction/treatment stratification	Predict response to particular therapies and choose the drug that is most likely to yield a favorable response in a given patient.
Therapy-related risk management	Identify patients with a high probability of adverse effects of a treatment.
Therapy monitoring	Determine whether a therapy is having the intended effect on a disease and whether adverse effects arise.
Posttreatment monitoring	Provide early detection and treatment of advancing disease or complications

In summary, precision medicine has huge advantages over traditional clinical practice and will lead the advancement of future medicine. Precision medicine highly relies on the accuracy and precision of the detection and analysis of biomarkers and biomolecules. The tests of small biomolecules and small molecule biomarkers play an unneglectable role in precision medicine. Many clinical application protocols have been established based on the precision medicine for better prediction, diagnosis, treatment and treatment evaluation.

1.2 Technologies for Small Biomolecule Analysis and Clinical Applications

Different kinds of technologies have been established for the analysis of biomarkers and have their own focus. Computed tomography (CT), magnetic resonance imaging (MRI) and ultrasound are representative imaging methods for organ level imaging. Cytology uses immunostaining, cell biopsy and cell culture followed by microscopic observation to study biomarkers in cellular level.

High-throughput DNA sequencing, *in situ* hybridization and computational genomics are technologies for DNA and RNA level biomarkers has attracted the world's attention since the beginning of the 21th century. High resolution mass spectrometry like matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF)[19], orbitrap[20], Fourier transform ion cyclotron resonance (FT-ICR)[21] were widely used in the 'omics' study such as proteomics, lipidomic and metabolomics[22].

The development of technologies for the tests of the small biomolecules are mainly based on immunoassay methods followed by optical observation[23] or chromatographic methods that use UV or mass spectrometry detection.

Immunoassays are based on the binding of antibodies or antigens to analytes in patient samples such as serum, blood or urine. Immunoassays run in two different formats, one is in multiple step mode and the other is a simple one step mode. The multi-step mode separates the analyte by adding reagents and washing it away to achieve separation. Single step immunoassay mixes the analyte and the reagent together and use other physical methods to quantify the result, like optical or colorimetric measurements. Immunoassays also can employ calibrators which contain known concentration of analyte. The comparison of the response of the calibrator and the real sample can provide enough information for a semi-quantitative result.

Liquid chromatograph (LC) and gas chromatograph (GC) followed by UV detector or mass spectrometer are the gold standard of quantitative analysis of small molecules in the past century. As the name implies, the physical state of chromatographic technologies is in either liquid phase or in gas phase. A fluid (either liquid or gas phase) containing dissolved mixture sample is called mobile phase. A structure that fluid passes through but holds another material is called stationary phase. When the mobile phase washes through the stationary phase, molecules travel in different

speeds based upon the partition coefficient between the molecule and stationary phase. Separation is achieved based on the divergent speeds and travel time of different molecules.

However, both immunoassay and chromatography have their drawbacks. Although immunoassay is fast, simple and straight forward, it can only detect one or a specific group of analytes by using one type of assay, and the analysis result can only be semi-quantitative. The test accuracy heavily depends on the PH level and the test time. The sensitivity of immunoassay is mainly determined by the binding energy of antibodies. Chromatography can help to analyze multiple target analytes in a single time with high sensitivity and selectivity; however, it usually has a high purity requirement and needs massive sample preparation. In most cases, sample preparation and instrumentation setup requires a professional operator with a relatively long time to obtain a result. Although chromatography can work as a gold standard in analytical chemistry, it cannot fulfill the requirements needed for precision medicine.

In precision medicine, quantitative results need to be in real time instead of taking hours, to ensure that the treatment or the surgical action can be guided on time. Therefore, immunoassay and chromatography methods are not optimal choices in most cases. The lack of technology and innovation for the real-time quantitative analysis of small biomolecules hinders the progress of precision medicine. An instrument with potential for direct biomolecular quantitative analysis would be beneficial for the implementation of precision medicine.

1.3 Ambient Ionization and Miniature Mass Spectrometer

Ambient ionization mass spectrometry is a technology which minimizes the sample preparation and ionization before sample analysis by mass spectrometry. Analytical conclusions can be obtained in minutes with sufficient specificity and sensitivity. By optimizing the pumping, mass analyzer, ion source and ion transfer components of a portable mass spectrometer, mass