



QUALITATIVE AND QUANTITATIVE DETECTION FOR BACTERIA AND BACTERIAL ENDOTOXIN USING LASER AND MICROWAVE SPECTROSCOPY

By

Muhammad Mahmoud Sayed Elsayeh

A Thesis Submitted to the
Faculty of Engineering at Cairo University
in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY
in
Biomedical Engineering and Systems

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Title of Thesis:

Qualitative and Quantitative Detection for Bacteria and Bacterial Endotoxin using
LASER and Microwave Spectroscopy

Key Words:

Rapid Microbial detection; Rapid Pyrogen Detection; Microwave Spectroscopy; Dielectric
Spectroscopy; Ultra Wide Band; Cepstrum Analysis; Raman Spectroscopy;

Summary:

This work presents two novel methods, the first method uses electromagnetic waves in the Ultra Wide Band (UWB) region of the microwave spectrum to detect and identify bacteria and bacterial endotoxins. The developed method is based on the properties of interaction between organic materials and electromagnetic waves. The interaction is measured quantitatively and qualitatively. The scattered parameters of sample network are measured and cepstrum coefficients are estimated for the analysis of the scattered parameters signals energies.

The second method based on Raman spectroscopy to detect and identify bacteria and bacterial endotoxin. It uses the frequency properties of Raman scattering through the interaction between organic materials and electromagnetic waves. The scattered intensities are measured and wave number converted into frequency, then the cepstral coefficients are extracted for both the detection and identification. The methodology depends on normalization of Fourier transformed cepstral signal to extract their classification features.

Numerical simulation and practical experiments' results proved effective identification and detection of bacteria bacterial endotoxin even with concentrations as low as 0.0003 Endotoxin unit (EU)/ml and 1 Colony Forming Unit (CFU)/ml using signal processing based enhancement techniques for both methods.

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Dedication

This thesis is dedicated to my mother.

Table of Contents

ACKNOWLEDGMENT	V
DEDICATION.....	VI
LIST OF TABLES:.....	IX
LIST OF FIGURES:.....	X
NOMENCLATURE.....	XIII
ABSTRACT.....	XIV
CHAPTER 1 : INTRODUCTION.....	1
1.1. INTRODUCTION	1
1.2. MOTIVATION OF THE RESEARCH	2
1.3. ORGANIZATION OF THE THESIS	2
CHAPTER 2 : LITERATURE REVIEW	3
2.1. ENDOTOXIN	3
2.2. ENDOTOXIN ASSAYS.....	4
2.3. INSTRUMENTAL METHODS FOR ENDOTOXINS ANALYSIS.....	6
2.3.1. Capillary Electrophoresis.....	6
2.3.2. Laser Induced Fluorescence.....	8
2.3.3. Gas Chromatography – Mass Spectrometry	9
2.3.4. Matrix-Assisted Laser Desorption Ionization-Time of Flight MS.....	12
2.3.5. Fourier Transform Ion Cyclotron Resonance Mass Spectroscopy.....	13
2.4. TECHNOLOGY AND METHOD COMPARISON	15
CHAPTER 3 : MATERIALS AND METHODS	16
3.1. MODELING AND SIMULATION OF BACTERIA AND BACTERIAL ENDOTOXIN	16
3.1.1. Bacteria and Bacterial Endotoxin Modeling	16
3.1.2. Modeled Antennas used in Simulation	18
3.1.3. The Represented Model for Simulation	22
3.2. MICROWAVE BASED MICROBIAL AND PYROGENE DETECTION PRACTICAL EXPERIMENTS.....	24
3.2.1. Microwave Experiment Setup.....	26
3.2.2. Samples preparations and measurements.....	26
3.2.3. Detection and Quantification Algorithm.....	28
3.2.4. Bacteria Classification and Mixed Containment Detection Algorithm	30
3.3. BACTERIA AND BACTERIAL ENDOTOXIN DETECTION USING RAMAN SPECTROSCOPY .	31
3.3.1. Raman Spectroscopy.....	31
3.3.2. Experiment Setup and Material for Raman Spectroscopy	33

3.3.3.	Feature Extraction Algorithm	34
CHAPTER 4 : RESULTS AND DISCUSSION		36
4.1.	SIMULATION RESULTS	36
4.2.	MICROWAVE (UWB) EXPERIMENTS RESULTS.....	39
4.3.	RAMAN SPECTROSCOPY EXPERIMENT RESULTS	55
CHAPTER 5 : CONCLUSION AND FUTURE WORKS		63
REFERENCES:		64
APPENDIX A: MICROWAVE INTERACTION WITH DIELECTRIC BIO-MATERIAL		68
A.1.	DIELECTRIC CONSTANT	68
A.2.	ELECTROMAGNETIC WAVE PROPAGATION MECHANISM.....	71
A.3.	DIELECTRIC MECHANISM	72
	Electronic Polarization.....	72
	Atomic Polarization	72
	Dipole Relaxation	72
	Ionic Relaxation.....	72
A.4.	ORIENTATION (DIPOLAR) POLARIZATION	73
A.5.	RELAXATION TIME.....	73
A.6.	DIELECTRIC DISPERSION	76
APPENDIX B: MODELING AND SIMULATION OF ELECTROMAGNETIC WAVES		77
B.1.	ELECTROMAGNETIC NUMERICAL SIMULATION.....	77

List of Tables:

Table 2.1: Gas Chromatography different detectors summary table [27]	11
Table 2.2: Technology and method Comparison in terms of process time, Accuracy and drawbacks	15
Table 3.1: the Design configuration for the simulated DRA	22
Table 3.2: Standards and Testing samples Concentrations.....	28
Table 3.3: Endotoxin Containment Concentrations.....	33
Table 4.1: Prediction output against test sample actual concentration	42

List of Figures:

Figure 2.1: Architecture Schematic Structure of an Endotoxin from enterobacteriaceae according to Rietschel et al. [6][7].	3
Figure 2.2: Schematic Diagram of Capillary Electrophoretic System.	8
Figure 2.3: Jablonski Diagram for Single State and Triplet State	10
Figure 2.4: Gas Chromatography diagram.	12
Figure 2.5: Schematic diagram For MALDI-TOF-MS.	13
Figure 2.6: Schematic Diagram for FTICR-MS	14
Figure 3.1: LPS molecule morphological and dimensions were derived from X-ray diffraction data [35]	17
Figure 3.2 : UWB microstrip patch antenna design used in the simulation, a- Top view b- graduated step and rectangular slot dimensions c- side view d- fabricated antenna [35]	20
Figure 3.3: a- DRA top view b- DRA side view.	21
Figure 3.4: UWB Microstrip patch antenna setup model	23
Figure 3.5: Dual Band DRA setup Model	23
Figure 3.6: Schematic diagram for experimental setup and instrumentation	27
Figure 3.7: Principles of Raman Effect Illustration	32
Figure 3.8: Method Process diagram	33
Figure 3.9: Input – Process –Output (IPO) for the Structural Signature	35
Figure 4.1: UWB Micro Strip Patch antenna Setup Resulted S11 signal for both Reference and Low Endotoxin Concentration.	36
Figure 4.2: UWB Micro Strip Patch antenna Setup Resulted S11 signal for both Reference and High Endotoxin Concentration	37
Figure 4.3: UWB Micro Strip Patch antenna Setup Resulted S21 signal for both Reference and Low Endotoxin Concentration.	37
Figure 4.4: UWB Micro Strip Patch antenna Setup Resulted S21 signal for both Reference and High Endotoxin Concentration	38
Figure 4.5: DRA Setup Results which show S11 and S21 signals for Reference and Endotoxin Samples	38
Figure 4.6: DRA Setup Results which show S11 and S21 signals for Reference and Endotoxin Samples that represent the most.	39
Figure 4.7: S11 power spectrum for lowest and highest endotoxin concentration against reference pyrogen free distilled water	40
Figure 4.8: S12 power spectrum for lowest and highest endotoxin concentration against reference pyrogen free distilled water.	40
Figure 4.9: Cepstrum transformation of S11 for lowest and highest endotoxin concentration against reference pyrogen free distilled water	41

Figure 4.10: Cepstrum transformation of S12 for lowest and highest endotoxin concentration against reference pyrogen free distilled water	41
Figure 4.11: Calculated Signal Energy Expansion for each endotoxins concentrations Normalized against reference sample	42
Figure 4.12: S11 signal for Different Endotoxin concentration, the circular annotation shows the specific wave form and response from 4 to 5 GHz bandwidth.....	43
Figure 4.13: S11 signal for different mixture of gram negative bacteria and distilled water with endotoxin less than 0.25 EU/ml.	44
Figure 4.14: S11 signal for different mixture of gram Positive bacteria and distilled water with endotoxin less than 0.25 EU/ml.	44
Figure 4.15: S11 signal for Sterile White soft paraffin with gram negative <i>Pseudomonas aeruginosa</i>	45
Figure 4.16: S11 signal for gamma radiated Poly ethylene glycol powder with gram negative <i>Pseudomonas aeruginosa</i>	45
Figure 4.17: a) S11 power spectrum Lowest endotoxin conc. and <i>Staphylococcus aerus</i> EPS against Reference (Pyrogen free Distilled water) b) S11power spectrum highest endotoxin conc. and <i>Staphylococcus aerus</i> EPS against Reference (Pyrogen free Distilled water).	46
Figure 4.18: Calculated Signal Energy of S11 Cepstrum for different endotoxin concentration at time gate = 0.0125 μ sec and rectangle window width of one sample (0.1 μ sec)	47
Figure 4.19: Calculated Signal Energy of S11 Cepstrum for Lowest and Highest endotoxin concentration at time gate = 0.0125 μ sec and rectangle window width of one sample (0.1 μ sec).	47
Figure 4.20: Calculated Signal Energy of S11 Cepstrum for naturally occurrence endotoxin mixed with different bacteria species at time gate = 0.0125 μ sec and rectangle window width of one sample (0.1 μ sec)	48
Figure 4.21:Normalized Detection signature's cross correlation for different bacteria species and distilled water with endotoxin less than 0.25 EU/ml.	48
Figure 4.22:Normalized Detection signature's cross correlation for different gram negative bacteria species and distilled water with endotoxin less than 0.25 EU/ml	49
Figure 4.23:Normalized Detection signature's cross correlation for endotoxin and pyrogen free distilled water.....	49
Figure 4.24: Normalized Detection signature's cross correlation for different gram positive bacteria species and distilled water with endotoxin less than 0.25 EU/ml	50
Figure 4.25: Normalized detection signature's cross correlation for mixed gram postive species	50
Figure 4.26: UWB Structural Signature for <i>Pseudomonas aeruginosa</i> in WFI	51
Figure 4.27: UWB Structural Signature for <i>Pseudomonas aeruginosa</i> in Polyethylene Glycol (PEG)	52
Figure 4.28: UWB Structural Signature for <i>Pseudomonas aeruginosa</i> in soft Parafine	52
Figure 4.29: UWB Structural Signature for <i>Salmonella typhimurium</i> in WFI.....	53
Figure 4.30: UWB Structural Signature for <i>Micrococcus luteus</i> in WFI	53

Figure 4.31: UWB Structural Signature for Staphylococcus Haemolyticus in WFI	54
Figure 4.32: UWB Structural Signature for Endotoxin in WFI.....	54
Figure 4.33:Raman Spectrum for lowest and highest endotoxin concentration against reference pyrogen free distilled water.	55
Figure 4.34:Raman Spectrum for different mixture of gram Positive bacteria and distilled water with endotoxin less than 0.25 EU/ml.....	56
Figure 4.35:Raman Spectrum for different gram negative bacteria and distilled water with endotoxin less than 0.25 EU/ml.	57
Figure 4.36: a) Normalized filtered Raman power spectrum for different bacteria species and different endotoxin concentration. b) S11 signal for Different Endotoxin concentration, the circular annotation shows the specific wave form and response from 4 to 5 GHz bandwidth [14]. c) S11 signal for different gram negative bacteria contaminant a distilled water sample with endotoxin less than 0.25 EU/ml.	58
Figure 4.37: Normalized filtered Raman power spectrum for different mixture of gram positive bacteria species against individual species.	59
Figure 4.38: Power spectrum of Raman scatters for E.coli	59
Figure 4.39: Power spectrum of Raman scatters for Salmonella typhimurium.....	60
Figure 4.40: Power spectrum of Raman scatters for Micrococcus Luteus	60
Figure 4.41: Power spectrum of Raman scatters for Staphylococcus aureus	61
Figure 4.42: Power spectrum of Raman scatters for staphylococcus haemolyticus	61
Figure 4.43: Power spectrum of Raman scatters for Endotoxin	62
Figure A0.1: Charge building on parallel plates capacitor [47].....	68
Figure A.0.2: Circuit representation of Dielectric material interact with AC source [48]	69
Figure A.0.3: Complex Permittivity Vector Diagram [48].....	70
Figure A.0.4: Material properties influence on Electromagnetic wave propagation [48]	71
Figure A.0.5: Dielectric Mechanism with respect to the frequency change [50]	74
Figure A.0.6: Dielectric Mechanism with respect to the frequency change [48]	74
Figure A.0.7: Debye relaxation of water [48].....	75
Figure B.0.1: FIT Grid complex method discreization (Primal and Dual grid)	79
Figure B.0.2: illustration of Cell from Grid G with the electric voltage e on cell's edges and the magnetic flux b on cell's surfaces.....	80
Figure B.0.3: Six magnetic facet fluxes which contribute in non-existence of magnetic charges within cell volume.....	81
Figure B.0.4: A cell V of the grid G with the magnetic grid voltage h on the edges and the electric facet flux.....	82

Nomenclature

Abbreviation	Description
ATP	Adenosine Tri Phosphate
CE	Capillary Electrophoresis
CEC	Capillary Electrochromatography
CE-LIF	Capillary Electrophoresis - Laser Induced Fluorescence
CGE	Capillary Gel Electrophoresis
CIEF	Capillary Isoelectric Focusing
CZE	Capillary Zone Electrophoresis
DRA	Dielectric Resonant Antenna
EKC	Electrokinetic Chromatography
ELISA	Enzyme Linked ImmunoSorbent Assay
FDTD	Finite-Difference Time-Domain
FE	Finite Element
FIT	Finite Integration Technique
FTICR-MS	Fourier Transform Ion Cyclotron Resonance Mass Spectroscopy
GC-MS	Gas chromatography –Mass Spectroscopy
HPLC	High Performance Liquid Chromatography
ITP	Isotachophoresis
LAL	Limulus Amebocyte Lysate
MALDI-TOF-MS	Matrix – Assisted Laser Desorption Ionization –Time of Flight Mass spectroscopy
MECC or MEKC	MicellarElectrokinetic Capillary Chromatography
MEEKC	Micro Emulsion Electrokinetic Chromatography
MoM	Method of Moments
NACE	Non-Aqueous Capillary Electrophoresis
PCR	Polymerase Chain Reaction
RPT	Rabbit Pyrogen Test
TSEE	Total Signal Energy Expansion
UWB	Ultra Wide Band

Abstract

Sepsis is a global health problem that causes risk of death. In the developing world, about 60 to 80 % of death cases are caused by Sepsis. Rapid methods for detecting its causes, represent one of the major factors that may reduce Sepsis risks. Such methods can provide microbial detection and identification which is critical to determine the right treatment for the patient. Microbial and Pyrogen detection is important for quality control system to ensure the absence of pathogens and Pyrogens in the manufacturing of both medical and food products.

This work presents two novel methods, the first method uses electromagnetic waves in the Ultra Wide Band (UWB) region of the microwave spectrum to detect and identify bacteria and bacterial endotoxins. The developed method is based on the properties of interaction between organic materials and electromagnetic waves. The interaction is measured quantitatively and qualitatively. The scattered parameters of sample network are measured and cepstrum coefficients are estimated for the analysis of the scattered parameters signals energies.

The second method based on Raman spectroscopy to detect and identify bacteria and bacterial endotoxin. It uses the frequency properties of Raman scattering through the interaction between organic materials and electromagnetic waves. The scattered intensities are measured and wave number converted into frequency, then the cepstral coefficients are extracted for both the detection and identification. The methodology depends on normalization of Fourier transformed cepstral signal to extract their classification features.

Numerical simulation and practical experiments' results proved effective identification and detection of bacteria bacterial endotoxin even with concentrations as low as 0.0003 Endotoxin unit (EU)/ml and 1 Colony Forming Unit (CFU)/ml using signal processing based enhancement techniques for both methods.

Chapter 1 : Introduction

1.1. Introduction

Sepsis and Endotoxaemia are a global health problem that causes higher risk of death. Bacterial infection is a root cause of sepsis and could lead to Endotoxaemia if it is a gram negative Bacteria. In developing world 60-80% of sepsis diagnosed patients lost their lives. The absence of an automatic system for bacteria detection and identification that provides a rapid method for bacteria and bacterial endotoxin detection complicate the choosing of suitable clinical therapeutic procedures and actions. Such a system may represent essential tool for a quality control system in pharmaceutical industry of intravenous injection products.

There are several methods for bacteria identification and classification such as gram staining, culturing and biochemical methods. Another known method such as polymerase chain reaction (PCR) used to amplify short DNA fragments (Primers) that recognize sequences of genes that encode essential molecule. In case of bacteria detection and identification, the PCR method depends on the primers of DNA sequences of bacteria genes [1]. PCR needs sophisticated equipment, several components and reagents. PCR testing takes greater than 15 minutes.

Another drawback is the difficulty to identify and detect mixed containment. One of the new and instrumental based rapid method is the adenosine triphosphate (ATP) bioluminescence. It depends on special sample preparation utilizes specific enzyme combination and surfactant. The enzyme breaks down microbial ATP and produce visible light which measured the microbial presence. One of main drawback is the non-microbial ATP reaction and false positive indication. It is time consuming method requiring 24-48 hours to be completed. There are many other instrumental based rapid methods for microbial detection [2]. However, they are rapid, they have drawbacks such as time consuming, lower specificity, false identification and quantification.

On the other hand, there are several endotoxins' assays such as Thiobarbituric acid – assay, Rabbit Pyrogen Test(RPT), Human blood test , Endotoxin Activity Assay and the well-known (gel clotting or photometric) Limulus Amebocyte Lysate (LAL) assay. Different methods and instrumentation, used in detection of endotoxin, already exist such as Capillary electrophoresis, Laser Induced Fluorescence (CE-LIF), Gas chromatography –Mass Spectroscopy (GC-MS), Matrix – Assisted Laser Desorption Ionization –Time of Flight Mass spectroscopy(MALDI-TOF-MS), Ion trap mass spectroscopy and Fourier Transform Ion Cyclotron Resonance Mass Spectroscopy [3-5]. However, since using the above mentioned instrumental methods, is very artful and complicated. This due to the fact that as these methods need samples' preparations and derivatization as preparatory step for instrument to detect the LPS. As a result, these methods are time consuming which is not preferred especially in case of severe sepsis that cause death in less than an hour. Moreover, they need complex procedures to ensure accurate measurements and have expensive implementations.