

# APPLICATIONS OF NMR SPECTROSCOPY

**Editors:**  
**Atta-ur-Rahman**  
**M. Iqbal Choudhary**

**Bentham Books**

**Applications of NMR  
Spectroscopy**  
*(Volume 8)*

**Edited by**  
**Atta-ur-Rahman, FRS**  
*Kings College*  
*University of Cambridge*  
*Cambridge*  
*UK*

**&**

**M. Iqbal Choudhary**  
*H.E.J. Research Institute of Chemistry,*  
*International Center for Chemical and Biological Sciences,*  
*University of Karachi, Karachi,*  
*Pakistan*

## **Cr r nec v q p u ' q h P O T ' U r g e v t q u e q r {**

*Volume # : 0*

Editors: Atta-ur-Rahman, *HTU* and M. Iqbal Choudhary

ISSN (Online): 2405-4682

ISSN (Print): 2405-4674

ISBN (Online): 978-981-14-3997-1

ISBN (Print): 978-981-14-3383-2

ISBN (Paperback): 978-981-14-3384-9

©2020, Bentham Books imprint.

Published by Bentham Science Publishers Pte. Ltd. Singapore. All Rights Reserved.

## **BENTHAM SCIENCE PUBLISHERS LTD.**

### **End User License Agreement (for non-institutional, personal use)**

This is an agreement between you and Bentham Science Publishers Ltd. Please read this License Agreement carefully before using the book/echapter/ejournal (“**Work**”). Your use of the Work constitutes your agreement to the terms and conditions set forth in this License Agreement. If you do not agree to these terms and conditions then you should not use the Work.

Bentham Science Publishers agrees to grant you a non-exclusive, non-transferable limited license to use the Work subject to and in accordance with the following terms and conditions. This License Agreement is for non-library, personal use only. For a library / institutional / multi user license in respect of the Work, please contact: [permission@benthamscience.net](mailto:permission@benthamscience.net).

### **Usage Rules:**

1. All rights reserved: The Work is the subject of copyright and Bentham Science Publishers either owns the Work (and the copyright in it) or is licensed to distribute the Work. You shall not copy, reproduce, modify, remove, delete, augment, add to, publish, transmit, sell, resell, create derivative works from, or in any way exploit the Work or make the Work available for others to do any of the same, in any form or by any means, in whole or in part, in each case without the prior written permission of Bentham Science Publishers, unless stated otherwise in this License Agreement.
2. You may download a copy of the Work on one occasion to one personal computer (including tablet, laptop, desktop, or other such devices). You may make one back-up copy of the Work to avoid losing it.
3. The unauthorised use or distribution of copyrighted or other proprietary content is illegal and could subject you to liability for substantial money damages. You will be liable for any damage resulting from your misuse of the Work or any violation of this License Agreement, including any infringement by you of copyrights or proprietary rights.

### ***Disclaimer:***

Bentham Science Publishers does not guarantee that the information in the Work is error-free, or warrant that it will meet your requirements or that access to the Work will be uninterrupted or error-free. The Work is provided "as is" without warranty of any kind, either express or implied or statutory, including, without limitation, implied warranties of merchantability and fitness for a particular purpose. The entire risk as to the results and performance of the Work is assumed by you. No responsibility is assumed by Bentham Science Publishers, its staff, editors and/or authors for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products instruction, advertisements or ideas contained in the Work.

### ***Limitation of Liability:***

In no event will Bentham Science Publishers, its staff, editors and/or authors, be liable for any damages, including, without limitation, special, incidental and/or consequential damages and/or damages for lost data and/or profits arising out of (whether directly or indirectly) the use or inability to use the Work. The entire liability of Bentham Science Publishers shall be limited to the amount actually paid by you for the Work.

### **General:**

1. Any dispute or claim arising out of or in connection with this License Agreement or the Work (including non-contractual disputes or claims) will be governed by and construed in accordance with the laws of Singapore. Each party agrees that the courts of the state of Singapore shall have exclusive jurisdiction to settle any dispute or claim arising out of or in connection with this License Agreement or the Work (including non-contractual disputes or claims).
2. Your rights under this License Agreement will automatically terminate without notice and without the

need for a court order if at any point you breach any terms of this License Agreement. In no event will any delay or failure by Bentham Science Publishers in enforcing your compliance with this License Agreement constitute a waiver of any of its rights.

3. You acknowledge that you have read this License Agreement, and agree to be bound by its terms and conditions. To the extent that any other terms and conditions presented on any website of Bentham Science Publishers conflict with, or are inconsistent with, the terms and conditions set out in this License Agreement, you acknowledge that the terms and conditions set out in this License Agreement shall prevail.

**Bentham Science Publishers Pte. Ltd.**

80 Robinson Road #02-00

Singapore 068898

Singapore

Email: [subscriptions@benthamscience.net](mailto:subscriptions@benthamscience.net)



# CONTENTS

PREFACE .....	i
LIST OF CONTRIBUTORS .....	ii
<b>CHAPTER 1</b> $^1\text{H}$ NMR AS A TOOL FOR DETERMINATION OF SIX COMMON SUGARS IN FOODS .....	1
<i>Y gp/Dkp' ] cpi . 'Uj w/J wgl 'Y cpi 'and' ] kVkp i 'Ej gp</i>	
<b>INTRODUCTION</b> .....	1
<b>RESULTS</b> .....	3
Workflow 1: Measurement of 6 Common Sugars in Foods .....	3
<i>Sample Preparation</i> .....	3
<i>NMR Experimental Process</i> .....	3
<i>Statistical Analysis</i> .....	4
NMR Spectral Analysis of Six Common Sugars in Beverages and Foods .....	5
NMR Spectral Analysis of Mixed Sugars in Foods .....	8
Introduction of NAIM Labeled Sugar .....	11
General Procedure for Preparation of Sugar-NAIM Derivatives .....	12
NAIM Derivatization and NMR Spectrometric Data of Aldo-Sugars .....	12
NMR Spectrometric Analysis of Mixed Sugars via NAIM Derivatization .....	14
Workflow 2: Measurement NAIM Labeled Sugars, Fru and Suc in Foods .....	15
<i>Sample Preparation</i> .....	16
<i>NMR Experimental Process</i> .....	17
<i>Statistical Analysis</i> .....	17
<b>DISCUSSION</b> .....	20
<b>CONCLUSIONS</b> .....	20
<b>ABBREVIATIONS</b> .....	21
<b>CONSENT FOR PUBLICATION</b> .....	22
<b>CONFLICT OF INTEREST</b> .....	22
<b>ACKNOWLEDGEMENTS</b> .....	22
<b>REFERENCES</b> .....	22
<b>CHAPTER 2</b> CORRELATION BETWEEN VIP SCORES AND $^1\text{H}$ NMR TO EXTRACT INFORMATION OF PSYCHOLOGICAL ATTENTION TESTS APPLIED BEFORE AND AFTER COFFEE INTAKE .....	25
<i>Okej gnTqej c 'Dcs wgw. 'Ckpg 'Eqs wgt q. 'Ngv'pek 'f g'Uqmw 'Ht wwtq/q. 'Rcwq 'J gpt kswg</i> <i>O ct ¼. 'Ht cpmiF wctvg. 'O cpwgr 'O cpf tqpg. 'Hgt weekq 'Rqk'and 'Rct 'pek 'Xcif gtt co c</i>	
<b>INTRODUCTION</b> .....	26
<b>EXPERIMENTAL</b> .....	28
Psychological Tests and Coffee Intake .....	28
Sample Extraction for $^1\text{H}$ NMR Analysis .....	30
$^1\text{H}$ NMR Analysis .....	30
Chemometrics .....	31
<b>RESULTS AND DISCUSSIONS</b> .....	32
Human Attention and Coffee Preference .....	32
Correlation between the Coffee Chemical Profile and Psychological Tests .....	35
<b>CONCLUDING REMARKS</b> .....	38
<b>CONSENT FOR PUBLICATION</b> .....	38
<b>CONFLICT OF INTEREST</b> .....	38
<b>ACKNOWLEDGEMENTS</b> .....	38
<b>REFERENCES</b> .....	39

<b>CHAPTER 3 NMR SPECTROSCOPY FOR PROBING THE STRUCTURAL DETERMINANTS OF APTAMER OPTIMIZATION AND RIBOSWITCH ENGINEERING</b> ....	42
<i>DODqt c3. " 0W wtnw: 'G00 cp3. '0 0I Āncp. 'E0" /{wtv'and'UDGxt cp</i>	
<b>INTRODUCTION</b> .....	43
Selection of Aptamers .....	43
Post-SELEX Modifications of Aptamers .....	44
Riboswitches .....	46
<b>NMR AND COMPLEMENTARY TECHNIQUES FOR APTAMERS AND RIBOSWITCHES</b> .....	48
<b>CONCLUSION</b> .....	53
<b>CONSENT FOR PUBLICATION</b> .....	53
<b>CONFLICT OF INTEREST</b> .....	53
<b>ACKNOWLEDGEMENTS</b> .....	53
<b>REFERENCES</b> .....	54
<b>CHAPTER 4 APPLICATIONS OF NMR SPECTROSCOPY IN MEDICAL DIAGNOSIS</b> .....	61
<i>Dcj ct wf kp 'Klt cj ko 'and 'Mguj co crikpKI qrcnco {</i>	
<b>INTRODUCTION</b> .....	62
<b>WORKING PRINCIPLE OF NMR IN MEDICAL DIAGNOSIS</b> .....	62
NMR in the Diagnosis of Lung Cancer .....	63
<i>Background of Lung Cancer</i> .....	63
<i>Study by Carrola et al. (2011)</i> .....	64
NMR in the Diagnosis of Alcohol Use Disorder (AUD) .....	69
<i>Background of Alcohol Use Disorder (AUD)</i> .....	69
<i>Study by Mostafa et al. (2016, 2017)</i> .....	70
NMR in the Diagnosis of Parkinson's Disease .....	73
<i>Background of Parkinson's Disease</i> .....	73
<i>Study by Ahmed et al. (2009)</i> .....	73
NMR in the Diagnosis of Other Diseases .....	76
<b>CONCLUSION</b> .....	76
<b>CONSENT FOR PUBLICATION</b> .....	76
<b>CONFLICT OF INTEREST</b> .....	77
<b>ACKNOWLEDGEMENTS</b> .....	77
<b>REFERENCES</b> .....	77
<b>CHAPTER 5 APPLICATIONS OF NMR SPECTROSCOPY IN CANCER DIAGNOSIS</b> .....	81
<i>Cuo cc 'COMc o grĀand 'Hqvqvj 'T00 cpiqwt</i>	
<b>INTRODUCTION</b> .....	82
<b>OVERVIEW OF NMR SPECTROSCOPY</b> .....	83
Types of NMR Spectroscopy Used in Cancer Diagnosis .....	83
Advantages and Disadvantages of NMR Spectroscopy .....	84
<b>TECHNICAL ASPECTS OF IN VITRO NMR APPLICATIONS</b> .....	86
<b>APPLICATIONS</b> .....	87
Brain Tumor .....	87
Breast Cancer .....	88
Ovarian and Endometrial Cancer .....	91
Prostate Cancer .....	93
Lung Cancer .....	96
Colorectal Cancer .....	101
Urinary Bladder Cancer .....	106
Oral Cancer .....	107

<b>PERSPECTIVE AND CONCLUDING REMARKS</b> .....	109
<b>CONSENT FOR PUBLICATION</b> .....	110
<b>CONFLICT OF INTEREST</b> .....	110
<b>ACKNOWLEDGEMENTS</b> .....	110
<b>REFERENCES</b> .....	110
<b>CHAPTER 6 NMR AS A TOOL FOR EXPLORING PROTEIN INTERACTIONS AND DYNAMICS</b> .....	121
<i>Sco ct'Dcuj k'and'Pcggo 'Tcuj kf</i>	
<b>INTRODUCTION</b> .....	122
Protein Dynamics and the Encounter Complex .....	123
Chemical Shift Perturbation Analysis .....	124
Target Immobilized NMR Screening .....	127
Paramagnetic Relaxation Enhancement .....	129
<b>CONCLUDING REMARKS</b> .....	132
<b>CONSENT FOR PUBLICATION</b> .....	133
<b>CONFLICT OF INTEREST</b> .....	133
<b>ACKNOWLEDGEMENTS</b> .....	133
<b>REFERENCES</b> .....	133
<b>SUBJECT INDEX</b> .....	141



## PREFACE

Nuclear Magnetic Resonance (NMR) spectroscopy has emerged as one of the most powerful techniques for the identification of materials, and for the study of their dynamic properties. As a result, the technique has found tremendous uses in almost all fields of physical, natural, and health sciences.

Volume 8 of the book series entitled *Applications of NMR Spectroscopy* is mainly focussed on the practical uses of NMR spectroscopy in solving various key problems in biomedical, health, and food sciences. The contents include NMR based analysis of common sugars, plant based constituents, nucleic acids and proteins, as well as NMR- based metabolomics and MRI for the diagnosis of chronic and acute health disorders.

The review contributed by Yang *et al.* provides an overview of the use of quantitative NMR (qNMR) techniques for the analysis of six common sugars in complex food matrices, after derivatization with naphthimidazole (NAIM). Coffee plants contain many constituents which effect cognitive functions. Valderrama *et al.* have analysed the constituents of various coffee types, and correlated them through the use of the psychological attention test. Evran *et al.* have reviewed the recent literature on the NMR-based structures of aptamers (single stranded DNA and RNA molecules) selected via an iterative process called Systematic Evolution of Ligands by Exponential Enrichment (SELEX). The applications of NMR for the identification of the ligand binding mechanisms are discussed. The review contributed by Ibrahim and Gopalsamy describes various NMR techniques used in metabolomics-based diagnosis of common diseases. The next chapter by Asmaa and Mansour provides a critical analysis of various MRI-based diagnostic approaches to study diverse human cancers. Proteins are fascinating molecules, both because of their complex structures and their interactions with other biomolecules. NMR techniques have evolved over the years to determine the structures and functions of protein molecules. This is the key theme of chapter 6 by Bashir and Rashid.

We wish to thank all the eminent scientists for their scholarly contributions. The editorial team of Bentham Science Publishers, particularly Ms. Fariya Zulfiqar (Manager Publications) and team leader Mr. Mahmood Alam (Director Publications), deserves our deepest appreciation for compiling an excellent volume in a time efficient manner. We are confident that like the previous volumes of this book series, the current treatise will also receive wide appreciation for both the readers and practitioners of NMR spectroscopy.

**Prof. Dr. Atta-ur-Rahman, FRS**  
Honorary Life Fellow  
Kings College  
University of Cambridge  
Cambridge  
UK

&

**Prof. Dr. M. Iqbal Choudhary**  
H.E.J. Research Institute of Chemistry  
International Center for Chemical and Biological Sciences  
University of Karachi  
Karachi  
Pakistan

## List of Contributors

<b>Aline Coqueiro</b>	Universidade Tecnológica Federal do Paraná, Ponta Grossa, Paraná, Brazil
<b>Asmaa A. Kamel</b>	Biochemistry Department, Faculty of Pharmacy, Tanta University, Tanta 31111, Egypt
<b>B. Bora</b>	Department of Biochemistry, Faculty of Science, Ege University, 35100 Bornova-İzmir, Turkey
<b>Baharudin Ibrahim</b>	School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia
<b>C. Özyurt</b>	Department of Chemistry and Chemical Processing Technologies, Lapseki Vocational School, Canakkale Onsekiz Mart University, Canakkale, Lapseki, Turkey
<b>E. Man</b>	Department of Biochemistry, Faculty of Science, Ege University, 35100 Bornova-İzmir, Turkey
<b>Fotouh R. Mansour</b>	Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Tanta University, Tanta 31111, Egypt Pharmaceutical Services Center, Faculty of Pharmacy, Tanta University, Tanta 31111, Egypt
<b>Ferruccio Poli</b>	Università di Bologna, Bologna, Italy
<b>Frank Duarte</b>	Faculdade União de Campo Mourão, Campo Mourão, Paraná, Brazil
<b>Keshamalini Gopalsamy</b>	School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia
<b>Leticia de Sousa FruTUozo</b>	Universidade Tecnológica Federal do Paraná, Campo Mourão, Paraná, Brazil
<b>Michel Rocha Baqueta</b>	Universidade Tecnológica Federal do Paraná, Campo Mourão, Paraná, Brazil
<b>M. Gültan</b>	Department of Biochemistry, Faculty of Science, Ege University, 35100 Bornova-İzmir, Turkey
<b>Manuela Mandrone</b>	Università di Bologna, Bologna, Italy
<b>Naeem Rashid</b>	School of Biological Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan
<b>Ö. Uğurlu</b>	Department of Biochemistry, Faculty of Science, Ege University, 35100 Bornova-İzmir, Turkey
<b>Patrícia Valderrama</b>	Universidade Tecnológica Federal do Paraná, Campo Mourão, Paraná, Brazil
<b>Paulo Henrique Março</b>	Universidade Tecnológica Federal do Paraná, Campo Mourão, Paraná, Brazil
<b>Qamar Bashir</b>	School of Biological Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan
<b>Shu-Huey Wang</b>	Core Facility Center, Department of Biochemistry, Taipei Medical University, Taipei 110, Taiwan R.O.C
<b>S. Evran</b>	Department of Biochemistry, Faculty of Science, Ege University, 35100 Bornova-İzmir, Turkey

**Wen-Bin Yang**            The Glycan Sequencing Core Facility, Genomics Research Center, Academia Sinica, Taipei 115, Taiwan R.O.C

**Yi-Ting Chen**            The Glycan Sequencing Core Facility, Genomics Research Center, Academia Sinica, Taipei 115, Taiwan R.O.C

**Ö. Uğurlu**                Department of Biochemistry, Faculty of Science, Ege University, 35100 Bornova-İzmir, Turkey

## qNMR as a Tool for Determination of Six Common Sugars in Foods

Wen-Bin Yang<sup>1,\*</sup>, Shu-Huey Wang<sup>2</sup> and Yi-Ting Chen<sup>1</sup>

<sup>1</sup> The Glycan Sequencing Core Facility, Genomics Research Center, Academia Sinica, Taipei 115, Taiwan R.O.C.

<sup>2</sup> Core Facility Center, Department of Biochemistry, Taipei Medical University, Taipei 110, Taiwan R.O.C.

**Abstract:** Nuclear magnetic resonance (NMR) spectroscopy is capable of quantifying molecules. The term so called quantitative NMR (qNMR), has been used for determination of the concentration and purity of small molecules. Carbohydrates are found in various beverages and dietary foods, including crops, milk, fruits, and vegetables. Commercial products frequently use “added sugar” in soft drinks, cookies, candies, and foods. The added sugar in beverages can be sucrose, high-fructose corn syrup (HFCS) and glucose. Here, we report a quantitative method to measure 6 common sugar ingredients in foods from a single one-dimensional <sup>1</sup>H-NMR and by using naphthimidazole (NAIM) derived sugars, which are chemically tagging aldoses with 2,3-naphthalenediamine (NADA) at the reducing ends to assist assignment of sugars. The aldoses in native sugars contain  $\alpha$  and  $\beta$  anomeric isomers, and may have overlapping signals in <sup>1</sup>H-NMR spectra. In contrast, both the anomeric isomers can be converted into a single sugar-NAIM derivative, which resolves the problem of overlapping signals to simplify the NMR quantitative analysis. This NAIM method is especially useful for identification and quantification of multiple kinds of sugars in beverages and foods. This study is to facilitate the quantification of six common sugars in beverages and foods. Our results suggest that a simple treatment of beverage and food with the NAIM labeling method provides a more extensive success rate for the quantification of sugar ingredients.

**Keywords:** Beverage, Food, Fructose, Galactose, Glucose, Lactose, Maltose, Naphthimidazole (NAIM), q-NMR, Quantitative analysis, Sugar, Sucrose, <sup>1</sup>H-NMR spectrometry.

### INTRODUCTION

Carbohydrates are found in various beverages and dietary foods, including rice, noodles, bread, meat, milk, fruit, vegetables, and drink [1, 2]. Carbohydrates are

---

\* Corresponding author Wen-Bin Yang: The Glycan Sequencing Core Facility, Genomics Research Center, Academia Sinica, Taipei 115, Taiwan R.O.C.; Tel: +886-2-27871264; E-mail: wbyang@gate.sinica.edu.tw

also used as “added sugar” in soft-drinks, cookies, candies, and many kinds of foods. For example, the added sugar in beverages can be sucrose, fructose, glucose, maltose and other sweeteners. Though carbohydrates are needed for living, an excessive uptake of sugar may induce health problems such as decayed teeth and chronic diseases [3 - 5]. In addition, foods of low glycemic index (GI) are suggested for diabetic patients. It is important to know the content and quantity of sugar in foods. Thus, developing a rapid and convenient qualitative/quantitative method for sugar measurement in foods is needed. Furthermore, many countries have introduced the sugar tax and soft-drink tax in order to reduce sugar consumption [6]. Therefore, a suitable method to verify the sugar content in foods can be provided to the government for policy implementation. The appropriate “fine sugar” or “added sugar” intake is 25 grams per day according to the scientific recommendation by the World Health Organization (WHO) [7]. Since August 2015, Taiwan Food & Drug Administration (TFDA) has proposed to regulate common sugars in foods, including glucose (Glc), galactose (Gal), fructose (Fru), lactose (Lac), maltose (Mal), and sucrose (Suc). The amounts of sugars must be labeled in the “Nutrition Facts Panel” for the products of beverages and foods. Even though the information of sugar content surely benefits consumers, this regulation will impose challenges to the food industry concerning the identification and quantification of the six common sugars in beverages and foods.

At present, high performance liquid chromatography (HPLC) and high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) are more common instrumental methods for sugar determination in foods. NMR spectroscopy is also a powerful method for identification and quantification of low molecular weight compounds. Though  $^1\text{H}$ -NMR spectra are commonly used in the routine quantitative analysis of individual sugars [8, 9], using NMR to identify each sugar in a mixture and simultaneously quantify its content is still challenging because the spectrum is usually complicated by the existence of anomeric isomers and by the similar structures of sugar components. The quantitative NMR (qNMR) technique is designed for determination of the concentration and purity of small molecules [10]. qNMR can be applied for direct quantification of multiple components in a mixture without pretreatment of sample. However, recording a qNMR spectrum would take a much longer acquisition time than a routine  $^1\text{H}$ -NMR spectrum. In another approach, we performed a simple treatment on beverages and foods with a naphthimidazole (NAIM) labeling kit to provide the sugar-NAIM derivatives for quantification by  $^1\text{H}$ -NMR spectral analysis. This method combining NAIM derivatization and NMR analysis is successfully applied to the measurement of six common sugars in foods. Our objective is to establish a convenient method for profiling and quantifying sugar ingredients in beverages and foods by using one-dimensional

$^1\text{H}$ -NMR spectroscopy *via* a simple treatment with NAIM labeling kit.

## RESULTS

### Workflow 1: Measurement of 6 Common Sugars in Foods

Using  $^1\text{H}$ -NMR for six common sugars (Glc, Gal, Fru, Suc, Mal, and Lac), the identification process was followed stepwise by sample preparation, NMR processing and statistical analysis. Fig. (1) shows the flowchart.

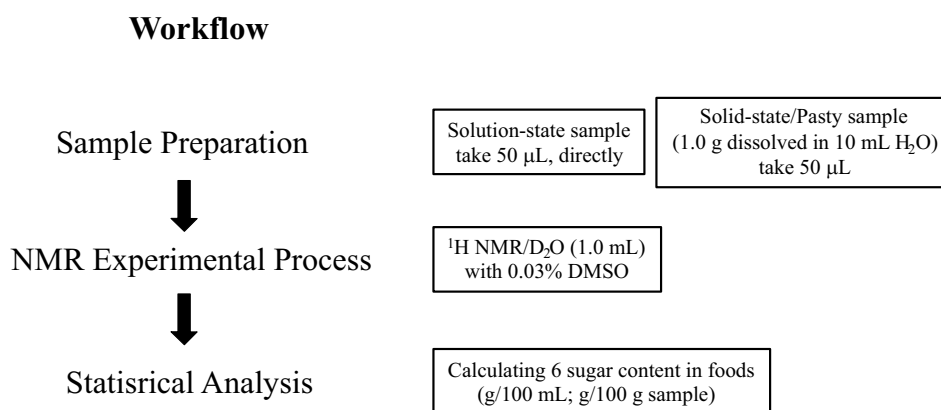


Fig. (1). Workflow of using  $^1\text{H}$ -NMR for determination of six common sugars.

#### *Sample Preparation*

Six standard sugar solutions (Glc, Gal, Fru, Suc, Mal, and Lac) were prepared in varied concentrations using 5.0, 2.5, 1.25 and 0.25 mg, respectively. The samples of beverage and food in solution-state were ready for determination without pretreatment or separation. A less than 50  $\mu\text{L}$  of sample solution was directly taken to reduce the absorption of  $\text{H}_2\text{O}$  (at 4.8 ppm) in  $^1\text{H}$ -NMR spectra. For solid-state or paste samples, 1.0 gram was dissolved in 10.0 mL of  $\text{H}_2\text{O}$ , and 50  $\mu\text{L}$  of the solution was taken for measurement. Sample solution was concentrated in vacuum (3 min), and then deuterium solution was added for NMR experiment.

#### *NMR Experimental Process*

The deuteriated water ( $\text{D}_2\text{O}$ , 99.9%, Sigma Aldrich, USA) 1.0 mL with 0.03 mol% of dimethylsulfoxide (DMSO, 99.9%, extra dry,  $\text{H}_2\text{O}$  < 50 ppm, Acros, New Jersey, USA) as internal standard was added to a dried sample in 5 mm NMR tube for recording the  $^1\text{H}$ -NMR spectrum. The  $^1\text{H}$ -NMR spectra were recorded on a Bruker AV600 MHz NMR spectrometer (Bruker BioSpin GmbH,

## Correlation Between VIP Scores and $^1\text{H}$ NMR to Extract Information of Psychological Attention Tests Applied Before and After Coffee Intake

Michel Rocha Baqueta<sup>1</sup>, Aline Coqueiro<sup>2</sup>, Leticia de Sousa Frutuozo<sup>1</sup>, Paulo Henrique Março<sup>1</sup>, Frank Duarte<sup>3</sup>, Manuela Mandrone<sup>4</sup>, Ferruccio Poli<sup>4</sup> and Patrícia Valderrama<sup>1,\*</sup>

<sup>1</sup> Universidade Tecnológica Federal do Paraná, Campo Mourão, Paraná, Brazil

<sup>2</sup> Universidade Tecnológica Federal do Paraná, Ponta Grossa, Paraná, Brazil

<sup>3</sup> Faculdade União de Campo Mourão, Campo Mourão, Paraná, Brazil

<sup>4</sup> Università di Bologna, Bologna, Italy

**Abstract:** This chapter presents the correlation between coffee compounds identified by  $^1\text{H}$  Nuclear Magnetic Resonance ( $^1\text{H}$  NMR) spectroscopy with psychological attention tests in order to verify which compounds are related to the focus and/or diffuse attention. Psychometric tests applied by a clinical psychologist, before and after coffee intake, were the focus attention AC-vector and the diffuse attention TADIM, and the focus attention TACOM-B and the diffuse attention TEDIF, respectively. Different tests to measure the attention before and after coffee intake were used to avoid learning effects. After AC-vector and TADIM tests, each volunteer consumed a total of 40 mL of coffee with different cup qualities (four different coffee blends – 10 mL per beverage) and indicated the order of preference in relation to the smell. This approach was used to create a greater metabolic variation between the samples tested, allowing to build a robust chemometric model. For each preferred coffee, a  $^1\text{H}$  NMR spectrum was obtained and a chemometric data treatment based on Partial Least Squares (PLS) regression and Variable Importance in Projection scores (VIP scores) was used to correlate the spectra with the psychological test results and to verify which metabolites of the coffee beverage could be related to the focus or diffuse attention. In general, our results showed that coffee intake attenuated diffuse attention and improved focus attention in most volunteers. The major metabolites that contributed to both diffuse and focus attention were caffeine, trigonelline, chlorogenic acids, acetate, lipids, lactate,  $\gamma$ -quinide, and polysaccharides. Among metabolites exclusively important to focus attention, formate, choline, myo-inositol, citrate, and malate were the most important. Therefore, the  $^1\text{H}$  NMR profile, in combination with chemometric tools, is interesting to assess the correlation between coffee compounds and human attention.

\* **Corresponding author Patrícia Valderrama:** Universidade Tecnológica Federal do Paraná, Campo Mourão, Paraná, Brazil; Tel:(+55) 44 3518-1525; E-mail: pativalderrama@gmail.com

**Keywords:** Attention Performance, Chemometrics, Coffee, Coffee compounds, Coffee ingestion, Coffee smell, Cup quality, Metabolomics, NMR spectroscopy, Pilot Study, PLS Regression, Psychometric Tests, VIP scores.

## INTRODUCTION

Coffee is globally one of the most widely-consumed beverages that contain over 1000 compounds responsible for its pleasant flavor and aroma [1]. Coffee is known for its stimulant, beneficial and nutritional properties leading researchers to seek to understand how these properties are correlated to their chemical composition. For example, coffee drinking habits have been associated with a decrease in the risk of developing Alzheimer's and Parkinson's disease [2], and a decrease in the incidence of cardiovascular disease and type 2 diabetes mellitus [3 - 5]. Recently, it was shown that the risk of Alzheimer's disease was lower in those who regularly consume coffee than those who do not drink it [6].

The bioactivities related to coffee consumption are related to several compounds. Of these, caffeine (1,3,7-trimethylxanthine) is the most widely studied. Caffeine is a psychoactive and neurostimulator substance that exerts most of its biological effects as an adenosine receptor antagonist, inducing a generally stimulating effect in the central nervous system [7, 8]. Studies have demonstrated the role of caffeine in improving cognitive skills, such as improving attention, increasing alertness rates, reducing tiredness and sleep duration [9, 10]. The amount of caffeine normally found in a cup of coffee can produce psychostimulant effects and increase the performance of individuals in clinical behavioral tasks [11]. However, there are hundreds of compounds in coffee, several of which with potential to contribute to coffee bioactivities directly or indirectly, for instance, some of them by interaction with caffeine [8, 9, 12].

The international coffee trade is concerned with only two coffee species: *Coffea arabica* L. and *Coffea canephora*. Both species proved to be sources of biologically active compounds, such as nicotinic acid, trigonelline, quinolinic acid, tannic acid, pyrogalllic acid, chlorogenic acids and especially caffeine [10]. Considering this, and the fact that several coffee metabolites can act on the central nervous system or potentiate the effect of caffeine, it is extremely important to develop tools to gain a complete picture of the metabolites present in the whole biological matrix, and understand which compounds in the beverage are directly related to focus and diffuse attention when performing psychological tests.

Psychological attention tests are a way of examining the level of human attention. They are used in several situations, such as psychological diagnosis assessments, personnel selection, driving permission, and to assess information processing speed. Psychometric tests to check focus and diffuse attention are the most used in



these cases. Focus attention is defined as the ability to select a source of information from all available at a given time and to be able to direct the attention (focus) to stimulus or tasks to be performed over time [13]. Diffuse attention is the mental function that focuses at the same time on various spatially dispersed stimuli, performing a quick collection of information and providing instant knowledge to the individual. Diffuse attention aims to investigate, evaluate and observe how quickly or slowly a person can discriminate against dispersed stimuli, such as a driver who drives on highways [14].

To understand which metabolites of coffee beverage can be related to human attention, a systematic method involving a wide variety of metabolites (metabolomic profiling) could be a very useful contribution. Nuclear Magnetic Resonance (NMR) spectroscopy is the analytical technique that can provide the most complete “metabolome” profile in a single analysis.  $^1\text{H}$  NMR spectroscopy is rapid, reproducible, and stable over time, requiring a very simple sample preparation, and provides both qualitative and quantitative information about the metabolites present in a sample [15, 16]. Several studies have been carried out showing the application of  $^1\text{H}$  NMR for the analysis of green and/or roasted coffee beans. For instance, this technique has been used to monitor changes in the composition of coffee during roasting [17, 18], to check adulteration in roasted coffee using corn, coffee husks, barley, and soybean [15], to discriminate coffee beans from different geographical origins [19], to differentiate coffee from different production systems [20], to evaluate the quality of green coffee or coffee beverages [17, 21], and to evaluate the anti-amyloidogenic properties of coffee and its constituents [22].

$^1\text{H}$  NMR spectroscopy can provide the “metabolome”, that is a chemical profile or fingerprint of whole tissues. Today, metabolomics constitutes a potent approach for the investigation and discovery of biomarkers in a large diversity of research domains [16, 22]. In this sense, NMR spectroscopy is a powerful tool capable of detecting a range of different types of metabolites simultaneously, providing valuable structural information with high reproducibility, although with low sensitivity (sub-millimolar concentrations) [23]. The fact that NMR has been routinely used for classical metabolic studies to characterize complex metabolite mixtures, has, in fact, made NMR the preferred technology in the field of metabolomics [24]. Thousands of metabolites in a single analysis are simultaneously monitored. The extraction of meaningful information from these large and complex datasets requires strategies, such as chemometrics tools that become essential for knowledge discovery in metabolomics [22]. In view of the above, in this study, we report the application of  $^1\text{H}$  NMR coupled to PLS regression and VIP scores to identify the coffee metabolites potentially related to the human focus and diffuse attention. The success of a metabolomics approach

## NMR Spectroscopy for Probing the Structural Determinants of Aptamer Optimization and Riboswitch Engineering

B. Bora<sup>1</sup>, Ö. Uğurlu<sup>1</sup>, E. Man<sup>1</sup>, M. Gültan<sup>1</sup>, C. Özyurt<sup>2</sup> and S. Evran<sup>1\*</sup>

<sup>1</sup> Department of Biochemistry, Faculty of Science, Ege University, 35100 Bornova-İzmir, Turkey

<sup>2</sup> Department of Chemistry and Chemical Processing Technologies, Lapseki Vocational School, Canakkale Onsekiz Mart University, Canakkale, Lapseki, Turkey

**Abstract:** Nucleic acid aptamers are single-stranded DNA or RNA molecules that can fold into unique conformations and specifically recognize various targets, such as small molecules, proteins, cells, and tissues. Aptamers are selected *in vitro* through an iterative process called Systematic Evolution of Ligands by Exponential Enrichment (SELEX). As aptamers possess several advantages over antibodies, several diagnostic and therapeutic applications have emerged in recent years. Aptamers also attract interest as they form the receptor domain of RNA-based riboswitches that function as natural modulators of gene expression. Aptamer domain of riboswitch can sense the metabolite and this binding event is transduced into a conformational change, thereby transcriptional or translational control is achieved. Riboswitch engineering has gained importance due to the potential use of artificial riboswitches in biosensors and next-generation therapeutics. Therefore, understanding the structural basis of ligand binding and conformational change is critical for the success of optimization or re-engineering of aptamers. Since crystallization of aptamer-small molecule target complexes is particularly difficult, NMR provides an indispensable tool for structural analysis. In this chapter, we first give a brief information about aptamers and riboswitches. Then, we review the NMR structures of aptamers and riboswitches reported to date. We highlight the importance of NMR for identification of ligand binding mechanism, post-SELEX optimization of aptamers, as well as for the design of artificial riboswitches. In this context, we also give some examples of aptamer studies involving a combination of NMR and other techniques.

**Keywords:** Aptamer, Aptamer-ligand interaction, NMR-guided design, Riboswitch.

\* Corresponding author Serap Evran: Department of Biochemistry, Faculty of Science, Ege University, 35100 Bornova-Izmir, Turkey; Tel: +90 232 3112304; Fax: +90 232 3115485; E-mail: serap.evran@gmail.com

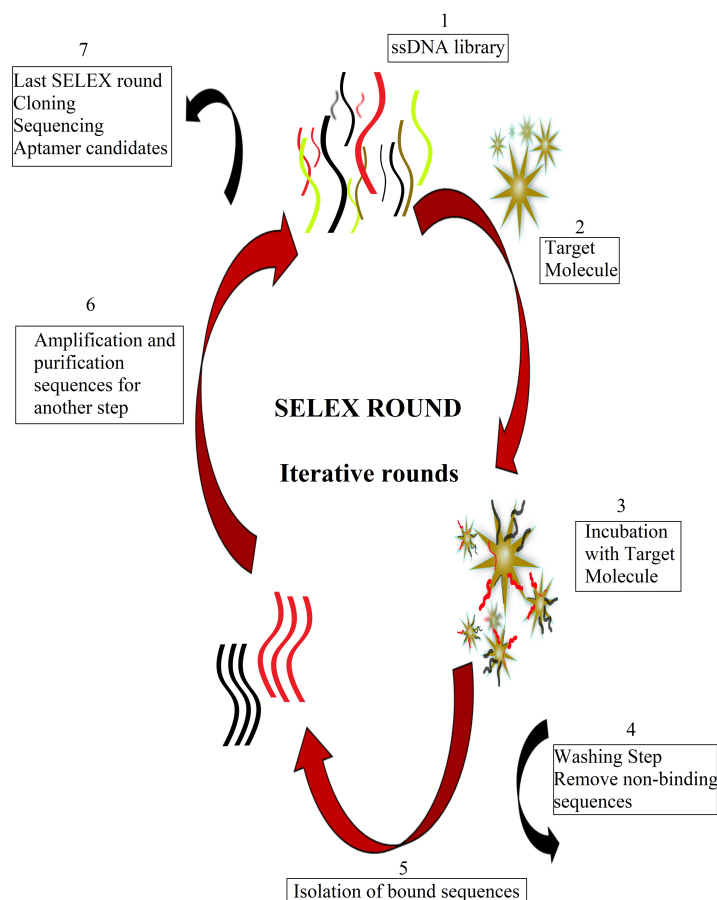
Atta-ur-Rahman and M. Iqbal Choudhary (Eds.)  
All rights reserved-© 2020 Bentham Science Publishers

## INTRODUCTION

### Selection of Aptamers

Aptamers are single-stranded DNA or RNA oligonucleotides that can bind to their targets with high affinity and specificity due to their ability to form specific three-dimensional structures [1]. Aptamers are selected by using an *in vitro* process called SELEX (Systematic Evolution of Ligands by EXponential Enrichment) [2, 3]. The SELEX process consists of three basic steps: binding, elution and amplification (Fig. 1). In the first step, the target of interest is incubated with initial DNA or RNA library. Initial library of a typical SELEX process consists of about  $10^{13}$  to  $10^{15}$  different sequences. The library is composed of chemically synthesized oligonucleotides that contain a random region of 20 to 100 nucleotides, which are flanked by specific primer binding sites at the 5' and 3' ends. Following incubation of the target and the library at pre-defined conditions, unbound sequences are removed by washing with buffer. The binding sequences are then amplified by polymerase chain reaction (PCR) *via* the primer binding sites. With repetitive cycles of binding, elution and amplification, the initial random pool of oligonucleotides is reduced to the enriched sequences that show the highest affinity and specificity to the target molecule. Binding properties of the selected aptamers depend on the molecular structure of the target, design of the initial random library, selection conditions, and the ratio of the target to the library. Typically, gradually increasing stringent conditions are applied to obtain aptamers with high affinity and specificity. The stringency can be achieved by reducing target concentration, increasing the number of wash steps, or by decreasing incubation time. Enrichment of high-affinity sequences indicates that the SELEX process can be finalized. The enriched pool is then sequenced and the aptamer candidates are chemically synthesized for further characterization of binding properties.

Aptamers bind to their targets through non-covalent interactions such as van der Waals forces, hydrogen bonding and electrostatic interactions [4]. Binding affinity and specificity of aptamers are comparable to antibodies. Moreover, aptamers are superior to antibodies due to their small size, stability, low cost, ease of chemical synthesis and unlimited target range [5]. Aptamers targeting small molecules, metal ions, peptides, proteins and cells can be developed *in vitro* by excluding the need for living systems. With these properties, aptamers have enormous potential to be used in therapeutics and diagnostics [6, 7]. Aptamers can be designed for many different purposes, such as modulating the immune system, inhibiting enzyme activity, drug transport, and blocking receptor binding [8].



**Fig. (1).** Schematic representation of SELEX.

### Post-SELEX Modifications of Aptamers

Therapeutic application of aptamers is usually limited by short half-lives due to rapid degradation by nucleases. For instance, the half-life of a 16-mer oligonucleotide in rat plasma is less than 1 minute [9]. The oligonucleotide that is promising as an anticoagulant has a limited half-life of 108 seconds [10]. Hence, post-SELEX optimization is a powerful approach to overcome therapeutic limitations since it allows modifications to the aptamer structure to improve stability and binding properties [11 - 16].

Truncation is one of the post-SELEX optimization strategies that relies on shortening the aptamer by removing the nucleotides that are not involved in target binding. The constant primer binding sites of aptamers were shown to contribute

## Applications of NMR Spectroscopy in Medical Diagnosis

**Baharudin Ibrahim\*** and **Keshamalini Gopalsamy**

*School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia*

**Abstract:** Nuclear magnetic resonance (NMR) is a special branch of spectroscopy which exploits the magnetic properties of atomic nuclei for molecular elucidation and identification. A technique that was initially developed to analyze chemical and physical molecular structure is now widely used in medical diagnosis. The non-invasiveness, non-destructiveness and simplicity of sample preparation make NMR the preferred technique for metabolomics study. Various body fluids such as urine, saliva, blood, plasma, serum and sweat have been explored to identify potential biomarkers of diseases. Psychiatric disorders, specifically alcohol-use disorder and neurological disorders such as Parkinson's disease, have been investigated with the aid of NMR spectroscopy. Cancer has been one of the most widely studied areas and the research also includes determination of biomarkers which not only could detect the presence of cancer but also potentially predict the various cancer processes in cancer cell lines. Infectious diseases including the compounds produced by the microorganisms such as in tuberculosis and pneumonia have also been explored. Besides, NMR metabolomics has also been used to establish a metabolic fingerprint for risk stratification and early detection of cardiovascular disease (CVD). The samples of subjects with the diseases were collected and the metabolites were compared against controls such as healthy individuals using complex chemometrics and multivariate data analysis such as principal component analysis, partial least square and orthogonal partial least square analyses to distinguish the potential biomarkers. In terms of the various uses of NMR metabolomics in the subject of diagnostic medicine, more improvements to overcome the analytical limitations are expected, making it one of the most notable diagnostic tools of the future. This chapter reviewed some of the published articles in cancer, psychiatric and neurological diseases to provide examples of using NMR spectroscopy in diagnosing human disorders.

**Keywords:** Cancer, Metabolomics, Neurological disorders, Nuclear Magnetic Resonance Spectroscopy, Psychiatric disorders.

---

\* **Corresponding author Baharudin Ibrahim:** School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia; Tel: 604-6535839 / 010-3664181; E-mail: baharudin.ibrahim@usm.my

## **INTRODUCTION**

Since ancient times, humans have utilized urine, saliva and other bodily fluids for the identification of various ailments. The advancement and utilization of analytical techniques for the evaluation of these biofluids have brought about the discovery of various disease biomarkers [1]. The integration of NMR, mass spectrometry (MS) and multivariate statistical techniques became the cornerstone for metabolomics-based disease diagnosis [2]. Metabolomics can be described as an in-depth study of chemical processes involving metabolites in a biological system [3]. Another terminology which is frequently used interchangeably with metabolomics is metabonomics. Metabonomics is defined as the quantitative measurement of metabolic responses of living systems against time to pathophysiological stimuli or genetic modification [4]. The primary objective of NMR-based medical diagnosis is to identify metabolites that precisely correspond to a particular disease for the early detection and treatment of said illness. In this chapter, we will analyze recent publications and highlight the advancements in experimental techniques, sample preparation, discovery and quantification of metabolites using chemometric tools used to identify biomarkers through NMR. One study for each disease will be reviewed in detail to explain this technique.

## **WORKING PRINCIPLE OF NMR IN MEDICAL DIAGNOSIS**

The principle behind NMR is that the nuclei in atoms are charged and hence is detectable by NMR, due to the formation of magnetic dipoles. When an external magnetic field is applied through NMR spectrometer, the base energy is shifted to a higher energy level. The energy transfer produces a wavelength that is measured and processed to produce NMR spectrum for the particular nucleus. With the help of chemometrics software, the area under the curve or peak height/intensity of the spectra can be calculated and used to identify significant/important compounds of diseases. This approach is known as metabolomics. The two main system used in metabolomics study are Nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). They both have different advantages and setbacks but both techniques are able to provide complementary information. The MS is usually combined with liquid chromatography (LC) or gas chromatography (GC) and has higher sensitivity compared to NMR. Thus, it is the preferred choice for metabolomics studies where a particular group of compounds are being targeted such as lipid compounds [5]. Nevertheless, MS sample preparation is extensive. It usually involves many steps such as solvent extraction, ultrafiltration, solid-phase extraction and a chemical derivatization. The presence of other chemical species may influence matrix effects, ionization suppression and enhancement and cause inconsistent results. Furthermore, samples are destroyed in the process [6, 7].

Meanwhile, NMR is fast and does not require tedious sample processing [8]. The samples are also preserved thus can be stored and re-run for further analysis. Newer NMR machines allow automation and can run samples in large quantities [9]. It is also non-selective thus is a preferred choice for bulk analysis in metabolomics studies to identify discriminating metabolites without any prior knowledge [10]. In addition to that, NMR provides insight to the molecular dynamics and mobility of a particular metabolite [11]. One major drawback of NMR is its sensitivity and resolution which is lower compared to MS [12]. However, newer NMR is continuously being developed with higher sensitivity and resolution.

Besides *in-vitro* NMR spectroscopy, *in-vivo* magnetic resonance spectroscopy is also a non-invasive method which can complement the magnetic resonance imaging (MRI) in the characterization of tissue and can be used to study metabolic changes in brain related disorders such as stroke, depression, tumors, dementia and seizures. In comparison, traditional investigational techniques such as biopsy is more invasive and has more risks and side effects. In this chapter, selected studies of NMR-based metabolomics applications with reference to specific diseases are discussed.

## **NMR in the Diagnosis of Lung Cancer**

### ***Background of Lung Cancer***

Lung cancer poses a serious health burden in most developed nations and it is estimated that there will be roughly 228,150 new cases of lung and bronchus cancer in 2019 [13]. Recent studies suggest that there may be a strong association between inherited genes and development of lung cancer. Nevertheless, there are very few genes that have been associated to lung cancer hitherto [14]. Besides hereditary factor, smoking has been shown to be one of the main risk factors for lung cancer [15]. Similar to other cancers, pathogenesis of lung cancer is induced through carcinogens, followed by a period of promotion and progression in a multistep process [16, 17]. Even though cancer risk may decrease after smoking cessation, another carcinogen may still carry on the process [18]. Table 1 shows the classification of lung cancers and its features.

Early detection is imperative to increase patient survival in lung cancer; nonetheless, available diagnostic techniques are insufficient. Diagnostic work-up for lung cancer still relies heavily on clinical perspectives and no single clinically based algorithm can be applied to all the cases [1, 19]. Definitive diagnosis of lung cancer is primarily based on the histopathological analysis of the lung cells. Due to the lack of screening tests and the onset of tumor growth generally do not display any signs or symptoms, diagnosis is frequently deferred. Therefore, to

## Applications of NMR Spectroscopy in Cancer Diagnosis

Asmaa A. Kamel<sup>1</sup> and Fotouh R. Mansour<sup>2,3,\*</sup>

<sup>1</sup> Biochemistry Department, Faculty of Pharmacy, Tanta University, Tanta 31111, Egypt

<sup>2</sup> Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Tanta University, Tanta 31111, Egypt

<sup>3</sup> Pharmaceutical Services Center, Faculty of Pharmacy, Tanta University, Tanta 31111, Egypt

**Abstract:** Cancer is a category of diseases characterized by uncontrolled cell growth and high potential to disseminate to other parts of the body. Cancer diagnosis is challenging due to the high structure similarity between normal and cancerous cells and the aggressive diagnostic procedures. Early diagnosis of cancer is crucial to increase the remission probability and avoid complications. A number of techniques have been involved in cancer diagnosis including biopsy, laboratory tests, computerized tomography (CT) scan, Ultrasonography, X-ray imaging, and nuclear magnetic resonance (NMR) spectroscopy. NMR has been applied both *in vivo* (known as magnetic resonance imaging) and *in vitro* to aid in cancer diagnosis. This chapter discusses the application of *in vitro* NMR in diagnosis and prognosis of different types of cancer with emphasis on the metabolic alterations at early stages of malignancy. The signature metabolites of brain, breast, epithelial ovarian, prostate, lung, colorectal, bladder, and oral cancers have been presented. A perspective overview of the role of NMR spectroscopy in cancer diagnosis has also been presented. This chapter shed the light on the important role of NMR spectroscopy in cancer diagnosis and treatment follow up. The applications introduced are not meant to provide a complete list of existing studies, but to present a wide overview of the current progress in this field. The chapter will cover the following topics:

**Keywords:** Applications, Bladder cancer, Brain cancer, Breast cancer, Cancer diagnosis, Colorectal cancer, Epithelial ovarian cancer, Lung cancer, Nuclear magnetic resonance (NMR) spectroscopy, Oral cancer, Perspective, Prostate cancer, Technical aspects.

\* Corresponding author Fotouh R. Mansour: Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Tanta University, Tanta 31111, Egypt; Tel: +20-10-6669-8099; Fax: +20-40-333-5466; E-mail: fotouhrashed@gmail.com



## INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy is a powerful analytical technique for both identification and quantification of analytes in solutions as well as in solid states [1]. NMR phenomenon was discovered in 1940s [2] and since then, there has been a rapid progress with regard to both method development and applications, expanding from physics to chemistry, biochemistry, pharmacy, physiology, food science, biology, and medicine [3].

In the field of medical diagnosis, NMR spectroscopy provides a non-invasive metabolic window on the biochemical processes within the body [4]. Its use is no longer restricted to research to investigate pathophysiological processes, but extends to drug assessment, personalized medicine as well as biochemical characterization and diagnosis of diseases [5]. The use of NMR-based metabolomics to aid in human disease diagnosis would give a more complete picture as it reflects the integrated functions of organs [6]. Furthermore, metabolic changes can be detected in biological fluids using NMR spectroscopy before the clinical symptoms develop, generating useful fingerprints for early diagnosis of diseases [7].

NMR spectroscopy would also help in the challenge of cancer diagnosis, especially in brain tumor, by providing another non-invasive approach besides clinical history and radiological examination [8]. The additional metabolic information provided by NMR spectroscopy can help making clinical decisions about cancer patient management without surgical diagnostic procedure [9]. NMR spectroscopy also has a great impact on metabolite-based discovery of diagnostic and prognostic biomarkers of several human diseases [10]. More sensitive biomarkers are urgently needed because traditional biomarkers of diseases are not sensitive enough and only increase after the presence of substantial diseases [6].

The use of NMR spectroscopy for medical diagnosis can be conducted both *in vitro* and *in vivo*. The biomedical applications of *in vitro* NMR include the analysis of body fluids (such as plasma or urine), extracts of tissue or small biopsy-sized specimens of intact tissues [11]. On the other hand, *in vivo* NMR spectroscopy, commonly known as magnetic resonance spectroscopy (MRS), can be done on the whole-body using a clinical magnetic resonance imaging (MRI) scanner, as an adjunct to standard examination, to obtain metabolic and functional information complementary to anatomical changes [12].

This chapter focuses on the recent applications of NMR spectroscopy in medical diagnosis and how it could offer the potential for a holistic approach to clinical medicine *via* improving disease diagnosis, biomarkers discovery as well as understanding disease mechanisms. The selected applications provide a wide

overview of the current progress in this field, and the future trends.

## OVERVIEW OF NMR SPECTROSCOPY

Since NMR was first described in 1938 by Isidor Rabi, the applications and the number of publications are steadily growing [13]. Fig. (1A) shows the total number of publications (Journal articles, book chapters, patents, conference abstracts,) with the key word “NMR” using the Semantic Scholar search engine in the last two decades. NMR is one of the most widely used techniques in metabolomic studies (Fig. 1B). The principle of NMR spectroscopy has been discussed in a number of text books [14, 15]. In this section, the types of NMR spectroscopy used in cancer diagnosis and the pros and cons of the technique will be discussed.

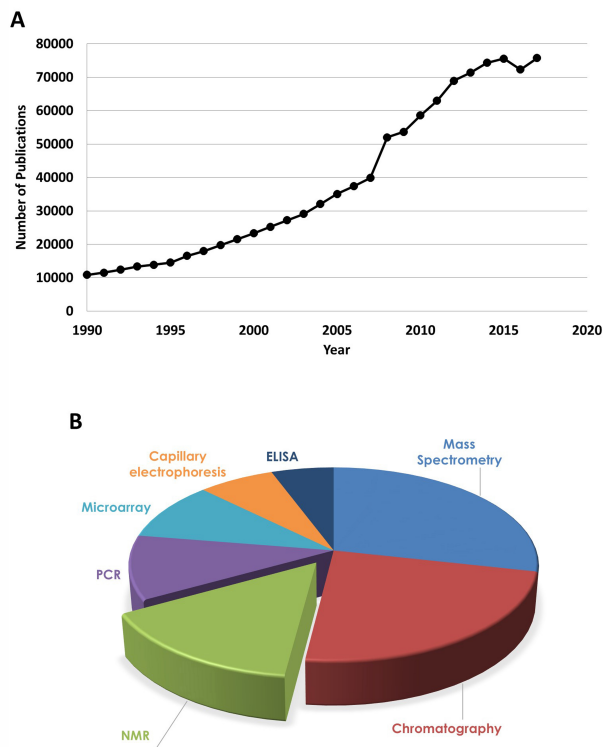


Fig. (1). A) The number of publications with keyword “NMR” in the last 20 years. B) A pie chart for the different techniques used in metabolomics.

## Types of NMR Spectroscopy Used in Cancer Diagnosis

NMR can be classified according to the number of atoms  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^{19}\text{F}$ ,  $^{31}\text{P}$ , etc,

## NMR as a Tool for Exploring Protein Interactions and Dynamics

**Qamar Bashir and Naeem Rashid\***

*School of Biological Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan*

**Abstract:** Proteins are vital players that mediate a vast majority of cellular functions. NMR spectroscopy originally developed by physicists for investigation of nuclear properties, now represents highest applications in chemistry and biochemistry. NMR has been extensively utilized by structural biologists for exploring protein-ligand interactions and by medicinal chemists for drug discovery. The ligands investigated involved small organic molecules, peptides, proteins and nucleic acids. Recently, there has been increasing interest in the dynamic studies of these protein-ligand interactions. These applications are provided by a multitude of NMR experiments ranging from the simple one-dimensional  $^1\text{H}$  spectrum to complex multidimensional NMR approaches. Chemical shift perturbation analysis allows for delineation of the binding interface, determination of the dissociation constants and estimation of ligand binding kinetics. Paramagnetic Relaxation Enhancement NMR spectroscopy has been widely used to visualize the weakly populated states and describes the process of protein complex formation. These approaches have been demonstrated for substrate binding, allostery, state equilibria and macromolecular self-association. NMR spectroscopy allows for characterization of minor conformational dynamic differences in structurally similar proteins. Target Immobilized NMR screening represents another approach to drug discovery that allows ligand screening for challenging targets. NMR spectroscopy can also be applied in combination with other techniques including X-ray crystallography and various computational methods to achieve greater coverage than any of the individual methods. This chapter is focused on the applications of NMR in exploring protein-ligand interactions and dynamics.

**Keywords:** Chemical shift perturbation, Encounter complex, Ligand binding, NMR, Paramagnetic relaxation enhancement, Protein dynamics, Protein-ligand interaction, Spectroscopy, Specific complex, Target immobilized NMR screening.

---

\* **Corresponding author Naeem Rashid:** School of Biological Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan; Tel: +924299231534; E-mail: naeemrashid37@hotmail.com

## INTRODUCTION

Protein-ligand interactions are vital for the maintenance and proper functioning of biological systems. A variety of cellular processes are carried out by proteins through interactions with multiple ligands including other proteins, small molecules and nucleic acids. These processes include signal transduction [1, 2], electron transport [3, 4], cellular metabolism [5, 6], muscle contraction [7, 8], membrane transport [9, 10], gene expression by transcription factors [11, 12], regulation of cytoskeleton [13, 14], enzymatic reactions and enzyme inhibition by intracellular inhibitors [15 - 18]. Abnormality in these interactions can lead to diseases like cancer, Alzheimer's and Creutzfeldt-Jakob disease [19, 20]. Protein-ligand interactions are the physical events, directed by the biochemical events of electrostatic forces, hydrophobic effect, hydrogen bonding, van der Waals and pi interactions. The affinity of protein-ligand interaction is a thermodynamic property described by the dissociation constant ( $K_d$ ), which is ratio of the individual rate constants of dissociation ( $k_{off}$ ) and association ( $k_{on}$ ). The  $K_d$  values can range from  $10^{-2}$  M to  $10^{-16}$  M [21, 22]. Depending on the function performed, the protein-ligand interactions are tuned in terms of the strength, specificity and life time of the final complex. On one hand are the specific and static complexes of antigens and antibodies as well as enzymes and their inhibitors. Such proteins have single partners and avoid interactions with other cellular components. In such cases, strong binding is essential to lock the complexes in a single, well-defined orientation. These complexes are characterized by their low dissociation constant ( $10^{-15}$  M to  $10^{-16}$  M), high binding energy (up to  $-21$  kcal/mol) and long life-times even up to several days [23]. On the other hand are the transient, weak complexes involved in signal transduction and electron transport. Such events require a high turnover and fast association/dissociation of the partners. Proteins involved in these interactions may recognize multiple ligands, and a high specificity in these complexes is avoided to gain a rapid dissociation. Such complexes have high dissociation constants ( $\mu$ M to mM), low binding energies and short life times of millisecond time scale [24].

The study of protein-ligand interactions is important for the understanding of mechanisms underlying the cellular processes and for drug development. Protein-ligand interactions have been studied at increasing pace by a wide range of experimental techniques [25] including UV-Visible spectroscopy [26], analytical ultracentrifugation [27], microscale thermophoresis [28], surface plasmon resonance [29], isothermal titration calorimetry [30], circular dichroism [31], dynamic light scattering [32], atomic force microscopy [33], mass spectrometry [34], differential scanning fluorimetry [35], small angle X-ray scattering [36], fluorescence microscopy [37], quartz crystal microbalance [38] and NMR [39]. These methods provide information on multiple aspects of protein-ligand

interactions including association, dissociation, conformational changes on binding, kinetic and thermodynamics parameters, with some limitations associated with each technique. It has become increasingly recognized that proteins and ligands do not behave as static objects in solution, rather they are dynamic bodies [40, 41]. There are different kinds of fluctuations, transitions, conformational changes, movements, bond vibrations and rotations going on. They correspond to the local fluctuations of chemical bonds, regional flexibility of residues relative to each other and global movements of protein domains. It also encompasses relative movements of proteins or ligands on or around the binding interface. In terms of structure, kinetics and thermodynamics, NMR is a versatile and powerful technique that presents site-specific information of protein-interactions. It comprises a number of experiments that allow for description of the binding interface, derivation of thermodynamic parameters and characterization of protein dynamics. This chapter highlights the subset of NMR experiments that can be utilized to explore protein-ligand interactions and dynamics.

### **Protein Dynamics and the Encounter Complex**

Protein dynamics cover a broad range of movements within or across the protein surface. Within living organisms, proteins are in constant motion and are interacting with other biomolecules to convey biological messages. A protein must physically interact with its ligand and form a productive complex for successful execution of the assigned task. This interaction has to be very specific to allow for binding with a specific molecule, thereby avoiding interactions with other cellular components. This is achieved by the presence of a specific binding interface that allows for selective recognition of the desired ligand. This binding interface is composed of small surface patches on the protein and ligand and is small as compared to the whole protein surface. If the ligand has to find and bind to the specific interaction interface through mere diffusion-driven collisions in solution, most of the collisions will be nonproductive due to the small chance of directly hitting the small interface. However, in most biological processes such as signal transduction cascades and electron transfer reactions, a fast association of the interacting molecules is crucial. This is achieved by the formation of a dynamic encounter complex [42] that accelerates the formation of the final specific complex by increasing the number of successful collisions, even if they are not directly on target (Fig. 1).

## SUBJECT INDEX

### A

Acetate 25, 36, 38, 74, 86, 97, 98, 100, 101, 102, 105, 108  
 formation 86  
 Acetic acid 12, 21, 72  
 Activity, inhibiting enzyme 43  
 AC-vector test 28  
 Adenocarcinoma 64, 99, 100  
 Adenosine receptor antagonist 26  
 Alcohol use disorder (AUD) 69, 70, 71, 72  
 identification test (AUDIT) 69  
 Altered pyruvate metabolism 74  
 Alzheimer's disease 26, 73, 74  
 Amino acids 94, 97, 99  
 branched-chain 94  
 Analysis 2, 8, 12, 21, 35, 48, 63, 64, 70, 87, 91, 101, 109  
 chemometric 35  
 computational 48  
 histopathological 63  
 metabolic 101  
 multivariate 64, 70, 87, 109  
 proteomic 91  
 spectral 2, 8, 12, 21  
 spectrometric 13  
 Analytes 82, 84, 110  
 Analytical ultracentrifugation 122  
 Anomeric isomers 1, 2, 12, 21  
 Aptamers 49, 52, 53  
 binding mechanisms of 49, 53  
 mutated 53  
 structured ligand-free 52  
 Atomic force microscopy 122

### B

*Bacillus cereus* fluoride riboswitch 51, 52  
 aptamer 52  
*Bacillus subtilis* cell lysate, Prepared 52  
 Background of lung cancer 63  
 Benign prostatic hyperplastic (BPH) 94  
 Benzoic acid 65

Beverages 1, 2, 3, 5, 6, 7, 8, 9, 12, 13, 18, 19, 20, 21, 25, 26, 29, 35  
 coffee milk 6, 19  
 commercial 6  
 dairy 18  
 prepared dairy 7  
 simple treatment of 1, 21  
 widely-consumed 26  
 wine 5  
 Binding 43, 46, 49, 52, 123, 128, 129  
 blocking receptor 43  
 competitive 129  
 fluoride 52  
 forces, efficient 49  
 Binding mechanisms 49, 53  
 high-affinity 49  
 Biological 71, 133  
 functions 133  
 magnetic resonance data bank 71  
 Biomarkers 61, 62, 65, 69, 70, 71, 72, 73, 82, 90, 93, 94, 95, 96, 107  
 accurate 106, 107  
 discriminatory 70  
 disease 62  
 prognostic 82  
 Biopsies 63, 81, 87, 88, 89, 104  
 invasive 104  
 prostate 93  
 Biosensors 42, 48  
 novel 48  
 Biosynthesis, bile acid 101  
 Bladder stone 106, 107  
 Bradykinesia 73  
 Brain 63, 74, 81, 82, 87, 88  
 biopsies 87  
 metastases 87  
 tumors 82, 87, 88  
 Breast cancer (BC) 81, 88, 89, 90  
 Breath condensate, exhaled 96, 99  
 Brown 6, 7, 19, 20  
 rice liquid 6, 19  
 sugarcane paste 7, 20

**C**

Caffeine 25, 26, 36, 38  
  role of 26, 38  
Caffeoylquinic acids 35  
Cancer 7, 61, 63, 65, 68, 81, 84, 88, 93, 95,  
  101, 102, 103, 106, 122  
  aggressive 95  
  bladder 81, 106  
  brain 81  
  colon 101, 103  
  colorectal 65, 81, 101  
  lethal 106  
  liver 65  
  rectal 102, 103  
Capping 46  
  biotin-streptavidin 46  
Capping of aptamers 46  
Carboxylic acids 35  
Carcinogens 63  
Cell lung carcinoma, small 99  
Cellular metabolic pathways 93  
CEST-NMR technique 52  
Chemical exchange saturation transfer (CEST)  
  51  
Chemical shift 121, 124, 125, 126, 127, 132  
  changes 125, 126, 127, 132  
  perturbation (CSP) 121, 124, 125, 126, 127,  
  132  
  Perturbation analysis 121, 124, 127  
  perturbation mapping 126  
Chemometric data treatment 25, 31  
Chlorogenic acids 25, 26, 35, 36, 38  
Chromatography 2, 11, 38, 62  
  gas 38, 62  
  high-performance anion-exchange 2  
Chromophore 20  
Chronic obstructive pulmonary disease  
  (COPD) 65, 101  
Circulating tumor cells (CTCs) 93  
Citric acid 71  
*Coffea* 26  
  *arabica* 26  
  *canephora* 26  
Coffee 8, 11, 25, 26, 27, 28, 29, 30, 34, 35, 36,  
  38, 65  
  beverages 25, 27, 29, 35, 36, 38  
  bioactivities 26  
  consumption 26, 28, 65  
  drinking habits 26

  green 27  
  ground 29, 30  
  ingestion 26  
  instant 8, 11  
  metabolites 26, 27, 35  
  roasted 27  
  tested 34  
Coffee beans 27, 29  
  roasted 27  
Collagen powder 8, 11  
Collisions 123, 124  
  diffusion-controlled random 124  
Commercial coffees 28  
Computerized tomography (CT) 81  
Computing conformational ensembles 127  
Concentrations 94, 105  
  fecal 105  
  spermine 94  
Conditions 85, 98  
  benign respiratory 98  
  ionization 85  
Consumption 72, 110  
  organic solvent 110  
Contaminants 84, 95  
Creutzfeldt-Jakob disease 122  
CSP analysis 132  
Cystoscopy 106  
  periodic 106

**D**

Definitive diagnosis of lung cancer 63  
Detection 2, 11, 64, 96, 101, 103  
  fluorescence 11  
  modalities 101  
  pulsed amperometric 2  
Detector 20  
  fluorescence 20  
Deuterium 3, 16  
  solution 3  
  solvents 16  
Diabetes mellitus 26  
Diagnosis of lung cancer 63  
Diagnosis of Parkinson's disease 73  
Dicafeoylquinic acids 35  
Diseases 26, 61, 62, 63, 68, 70, 73, 74, 76, 81,  
  82, 84, 104, 122  
  cardiovascular 26, 61, 76  
  neurological 61, 74  
  transmitted 70

Disorders 61, 63, 69, 73  
  neurological 61, 73  
  psychiatric 61  
Distinct metabolite patterns 93  
DMSO, cost-effective reagent 12  
DNA 45, 49, 42, 43, 51  
  aptamers 45, 49  
  modified 51  
  single-stranded 42, 43  
Drinking 8, 29, 32, 33, 72  
  chronic alcohol 72  
  coffee 29, 32, 33  
  non-chronic 72  
Drinks, sport 6, 18, 19  
Dynamic 85, 109, 122  
  light scattering 122  
  nuclear polarization 85, 109

**E**

Early 64, 81, 89  
  detection of lymph node metastasis 89  
  diagnosis of cancer 81  
  distant metastasis 64  
Effects 26, 52, 62, 122, 126, 130  
  hydrophobic 122, 126  
  influence matrix 62  
  negligible structural 52  
  psychostimulant 26  
  quantitative paramagnetic 130  
Electron transfer reactions 123  
Electrophoresis 11  
Electrostatic forces 122  
Endometrial cancer (EC) 91, 93  
Endometriosis 92  
Enzyme 46, 122  
  cofactors 46  
  inhibition 122  
Epithelial 81, 91, 92, 93  
  ovarian cancer (EOC) 81, 91, 92  
  -to-mesenchymal transition 93  
Epithelial cells 64, 94  
  normal prostatic 94  
Estrogen receptor 89  
Ethanol 71, 72  
  metabolism 71, 72  
  oxidation 72  
Exhaled breath condensate (EBC) 96, 98, 99  
Exonucleases 46  
Extraction 27, 30, 62, 74, 110

solid-phase 62

**F**

Factors 63, 69, 89, 103  
  decisive prognostic 103  
  extrinsic 69  
  hereditary 63  
  intrinsic 69  
  prognostic 89  
FBDD methods 129  
Fecal occult blood test (FOBT) 101, 105  
Fermented rice wine 6, 19  
Feruloylquinic acids 35  
Fingerprint 27, 76, 125  
  metabolomic 76  
Flavor 7, 18, 20, 26, 33, 35  
  cream coconut 7, 20  
  egg 7, 20  
  fish 7, 20  
  pleasant 26  
  roasted coffee 35  
  squid 18, 20  
Fluorescence microscopy 122  
Formic acid 108  
Fragment-based drug discovery (FBDD) 127,  
  128, 132  
Free induction decays (FIDs) 31  
Function 27, 42, 65, 74, 122  
  baseline lung 65  
  mental 27  
  mitochondrial 74

**G**

Gas chromatography (GC) 38, 62  
Genetic 46, 62, 73  
  modification 62  
  regulation 46  
  susceptibility 73  
Glioblastoma multiforme 88  
Glucuronic acid 12  
Glutathione metabolism 101  
Glycemic index (GI) 2  
Grape juice 6, 19  
Green coffee beans 28  
Guanosine binding 52  
Gut microbial-host co-metabolism 105



**H**

Heteronuclear single quantum coherence (HSQC) 71, 125, 130, 132  
High-fructose corn syrup (HFCS) 1  
High performance liquid chromatography (HPLC) 2, 12  
High throughput screening (HTS) 128  
Honey-plum vinegar 6, 18  
HPLC analysis 12, 20  
Human 71, 95, 96  
    expressed prostatic secretions 95, 96  
    metabolome database (HMDB) 71

**I**

Immune system 43  
Infectious diseases 61  
Inflammatory responses 73  
Interactions 26, 38, 48, 49, 50, 52, 73, 122, 123, 124, 125, 126, 127, 132, 133  
    biomolecular 127  
    complex genetic environmental 73  
    discriminative intermolecular 50  
    water-protein 124  
Invasive nature 106  
Ionization suppression 62  
Ion suppression 85  
Isothermal titration calorimetry (ITC) 49, 122

**L**

Lactic 66, 71  
    acid 66, 71  
    acidosis 71  
Leigh syndrome 74  
Ligand binding 42, 52, 121  
    affinities 52  
    kinetics 121  
    mechanism 42  
Ligand-bound solution structures 50  
Ligand nuclei 130  
Ligands 52, 128  
    cognate 52  
    low affinity FBDD 128  
Lipophilicity 128  
Liquid chromatography (LC) 2, 62, 96, 97, 98, 100  
    high performance 2

Liver cirrhosis 70  
Low-density lipoproteins (LDL) 76, 88, 97  
Lung cancer 63, 64, 65, 68, 69, 81, 96, 99, 101  
    metabolism 64  
Lymph node metastasis 89, 103

**M**

Magnetic dipoles 62  
Magnetic resonance 63, 81, 82, 84, 93, 96  
    imaging (MRI) 63, 81, 82, 84, 85, 96  
    spectroscopy (MRS) 82, 85  
Malignancy 81, 88, 93  
    common gynecologic 93  
Mestrenova software 31  
Metabolic 98, 103, 105, 107  
    biomarkers 98, 105, 107  
    fingerprinting 103  
Metabolic profiles 86, 87, 95, 97, 99, 105, 106  
    blood-based 99  
Metabolites 25, 27, 38, 62, 65, 68, 70, 71, 72, 74, 76, 81, 85, 87, 88, 89, 92, 93, 95, 99, 100, 101, 106, 109  
    choline-containing 89  
    determination and statistical analysis 70, 74  
    endogenous 72  
    signature 81, 87, 88  
    trigonelline 65  
Metabolomics 26, 27, 61, 62, 69, 72, 83, 84, 97, 109  
    based disease diagnosis 62  
    plasma-based 97  
    urinary-based 97  
Metal ions, cofactor 130  
Metalloproteins 129  
Michigan alcoholism screening test (MAST) 69  
Microscale thermophoresis 122  
Monte Carlo cross validation (MCCV) 65  
Mucin production 64  
Mucosa 102, 104, 105, 108  
    colonic 105  
    healthy 102  
    oral 108  
Muffin seasoning 18  
Multivariate data analysis 61, 97, 99, 100  
Muscle contraction 122  
Mushrooms seasoning 7, 20

**N**

NAIM derivatization and NMR 2, 12  
    analysis 2  
    spectrometric data of aldo-sugars 12  
National Institutes of Health (NIH) 70  
Natural 42, 128  
    modulators 42  
    products 128  
Neomycin 50, 52  
Neurodegenerative disorders 73, 74, 76  
Nicotinic acid 26  
NMR 3, 15, 31, 36, 49, 50, 64, 90, 100  
    fingerprinting 64  
    Mestrenova software 31  
    metabolite bioprofiling 100  
    of RNA 49  
    processing and statistical analysis 3, 15  
    profiles of coffee beverages 36  
    serum metabolomic signature 90  
    and fluorescence spectroscopy 50  
NMR-based 62, 104, 106  
    analysis of serum samples 104  
    medical diagnosis 62  
    metabolic analysis 106  
NMR-based metabolomics 63 69, 82, 89, 90,  
    91, 92, 105, 108  
    applications 63  
    analysis 90  
NMR spectra 87, 96  
    metabolic 87  
    representative high-resolution 96  
NMR spectra 72, 87  
    of cerebral gliomas 87  
    of social drinker and control 72  
NMR spectroscopic analysis 101, 106  
    of serum 101  
NMR Spectroscopy 83, 121  
    paramagnetic relaxation enhancement 121  
    used in cancer diagnosis 83  
Non-small cell lung carcinoma (NSCLC) 99,  
    101  
Nuclear magnetic resonance 1, 25, 27, 61, 62,  
    70, 76, 81, 82  
    metabolomics 76  
    spectroscopy 61  
Nucleases, vaginal 45  
Nucleic acid aptamers 42  
Nucleotides, guanine-based 53  
Nutrition facts 7

Nutritionists 8

**O**

Ochratoxin 51  
Oligonucleotides 43, 44  
Operator intervention 109  
Oral 81, 107, 108  
    cancer (OC) 81, 107  
    leukoplakia 108  
    squamous cell carcinoma (OSCC) 108  
Orthogonal partial least square 61, 70  
    analyses 61  
    discriminant analysis 70  
OSCC development 108  
Ovarian cancer 81, 91, 92, 93  
    epithelial 81, 91, 92  
Ovarian tumor 91, 92  
    malignant 91  
Over-expressed inositol 90  
Oxidative condensation 15  
Oxygen atom 131

**P**

Paramagnetic 121, 129, 130, 131  
    effects 130, 131  
    relaxation enhancement 121, 129, 130, 131  
    quantitative 131  
Parkinson's disease (PD) 26, 61, 73, 74, 76  
Partial least squares (PLS) 25, 31, 61  
Pathogenesis 63  
Phosphothionate linkage 46  
Plasma 61, 70, 72, 74, 76, 82, 87, 96, 97, 109  
    metabolites studies 72  
PLS regression 26, 27, 31, 32, 36  
    and VIP scores 27  
    model 32  
Polymerase chain reaction (PCR) 43  
Positron emission tomography 96  
Post-SELEX 44  
    modifications of aptamers 44  
    optimization strategies 44  
PRE spectroscopy 132  
Prion protein 51  
    bovine 51  
Prognosis 64, 81, 95, 96, 99  
    worst 64  
Prognostication 104

Prognostic classification capabilities 103  
Proline 87, 88, 100, 126  
  residues 126  
Prostate 81, 94  
  abnormal 94  
Prostate cancer 81, 93, 94  
  growth 94  
Prostate 93  
  -specific antigen (PSA) 93  
Prostate tumor 93, 95  
  heterogeneity 93  
Prostatic fluids 95  
Protein 99, 121, 123, 124, 127, 129, 132  
  amides 127  
  catabolism 99  
  complexes 124, 129  
  domains 123  
  dynamics 121, 123, 132  
Proton 13, 15, 20, 21, 30, 89, 92  
  NMR analysis 92  
  NMR frequency 30  
  NMR methods 89  
  signals 13, 15, 20, 21  
Pyrogalllic acid 26

## Q

Quantification of sugars 4, 17  
Quantities, stoichiometric 129  
Quantum mechanic analysis 52  
Quartz crystal microbalance 122  
Quinic acids 35, 36  
Quinolinic acid 26

## R

Random forest (RF) 90  
Reaction 12, 43  
  condensation 12  
  polymerase chain 43  
Refractive index (RI) 20  
Regression 4, 25, 31, 70  
  analysis 4  
  multivariate logistic 70  
Resistance 45, 46  
  higher 46  
  nuclease 45, 46  
Resonances 36, 49, 74, 122, 128, 129  
  chemical 36

  surface plasmon 49, 122  
Riboswitches 42, 46, 47, 48, 49, 50, 51, 52, 53  
  adenine 49  
  artificial 42, 48  
  deoxyguanosine 52  
  deoxyguanosine binding 52  
  sensing 50, 52  
  synthetic 53  
Riboswitch regulation mechanism 47  
RNA 43, 46  
  oligonucleotides 43  
  sequences ranging 46  
RNA-based 42, 46  
  intracellular sensors 46  
  riboswitches 42

## S

Saturation transfer difference (STD) 127  
Serum 90  
  metabolites 90  
  metabolomic analysis 90  
Short-chain fatty acids 105  
Sigmoidoscopy 101  
Signal(s) 1, 5, 8, 9, 14, 15, 28, 30, 31, 35, 52,  
  65, 68, 84, 86, 87  
  overlapping 1, 8  
  residual water 30, 31  
  suppression 35  
  transcription termination 52  
Signal transduction 122, 123  
  cascades 123  
Small 49, 64  
  -angle X-ray spectroscopy 49  
  -cell lung carcinoma (SCLC) 64  
Smoking 63, 64, 68, 69, 97  
  cigarette 64  
Soft independent modelling 70  
Software 4, 50, 62, 70, 74, 127  
  chemometrics 62  
  program, popular 127  
Solution NMR 50, 128  
  data 50  
  methods 128  
Soy sauce paste 7, 18, 20  
Spectrometry 13, 14, 21  
Spectroscopy 1, 3, 25, 27, 61, 62, 81, 82, 90,  
  94, 95, 100  
Squamous cell 64, 99, 100  
  carcinoma 64, 99

Status 89, 94  
  hormonal receptor 89  
  pathologic 94  
Stimuli 27, 33, 62  
  dispersed 27  
  pathophysiological 62  
Stress 73, 86  
  oxidative 73  
Structures 2, 45, 48, 51, 52, 53, 123  
  deoxyguanosine-bound X-ray 53  
  high-resolution 48  
  identical tertiary 52  
  ligand-bound 48  
  non-bound conformational 48  
Succinate 66, 74  
Sugar-NAIM derivatives 2, 9, 12, 13, 14, 16  
  preparation of 12, 16  
  procedure for preparation of 12, 16  
Sugars 1, 2, 4, 5, 6, 7, 8, 9, 12, 15, 16, 17, 18,  
  20, 21, 45  
  excessive 8  
  integration area of 4, 17  
  native 1, 18  
  non-reducing 15  
  reducing 12, 18, 21  
Surface plasmon resonance (SPR) 49, 122  
Surgical 82, 104  
  diagnostic procedure 82  
  resection 104  
Survival rates 64, 87, 89, 91, 107  
Synthetic neomycin riboswitch 50  
Syrup 1, 11, 18  
  high-fructose corn 1  
  maple sugar 8, 11

**T**

TADIM tests 25, 28  
Tannic acid 26  
Target(s) 121, 127, 128, 129, 132  
  challenging membrane protein 132  
  challenging protein 128  
  immobilized NMR screening (TINS) 121,  
    127, 128, 129, 132  
  nucleic acid 129  
  pharmaceutical drug 129  
Taurocholic acid 101, 105  
Tests 101, 105  
  blood-based DNA 101  
  fecal-based DNA 101

  fecal immunochemical 101  
  guaiac-fecal occult blood 105  
  non-invasive diagnostic 101  
Therapeutics 42, 43  
  next-generation 42  
Thermodynamic property 122  
Thrombin 51  
  binding aptamer (TBA) 51  
  conjugated DNA 51  
Thyroid-stimulating hormone 45  
TINS 128, 129  
  assay 132  
  spectrum 128, 129  
Tissue biopsies 102  
Toxicity response 51  
Transcription factors 122  
Transformation, malignant 87, 108  
Tumor(s) 63, 87, 89, 94, 97, 99, 100, 104,  
  105, 108  
  colorectal 105  
  lung 97  
  tissue 100, 104, 105

**U**

Ultrasonography 81  
Unified Parkinson's disease rating scale  
  (UPDRS) 73  
Uridine nucleotides 99  
Urinary 106  
  bladder cancer (UBC) 106  
  tract infection 106

**V**

Variable importance in projection (VIP) 25,  
  32  
Very low-density lipoproteins (VLDL) 97

**W**

Warburg effect 108  
World health organization (WHO) 2, 7, 8

**X**

X-ray 48, 81, 121  
  crystallography 48, 121  
  imaging 81



**ATTA-UR-RAHMAN, FRS**

Prof. Atta-ur-Rahman, Ph.D. in Organic Chemistry from Cambridge University (1968) has 1,232 international publications (45 international patents and 341 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 Ehrenzeitschen 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.



**M. IQBAL CHOUDHARY**

Dr. M. Iqbal Choudhary is a Professor of Organic/Bioorganic Chemistry and Director at the International Center for Chemical and Biological Sciences (H. E. J. Research Institute of Chemistry and Dr. Panjwani Center for Molecular Medicine and Drug Research) and Coordinator General of COMSTECH (OIC Ministerial Committee). He is among the most prominent scientists of Pakistan, recognized for his original contributions in the fields of natural products and bioorganic chemistry. He has written and edited 27 books, most of which have been published in USA and Europe. He is also the author of over 1000 research papers and chapters in top international science journals of the West as well as 27 US patents (H-index: 68 & Citations: 27,500). He is the Volume Editor of many international book series and journals. He has served as a visiting faculty in many prestigious universities of the world including Cornell University (New York), Purdue University (Indiana), Pennsylvania State University (Pennsylvania), Scripps Institution of Oceanography (San Diego, California), The University of Rhode Island (Rhode Island), and other top Universities.