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APPLICATIONS OF NMR SPECTROSCOPY

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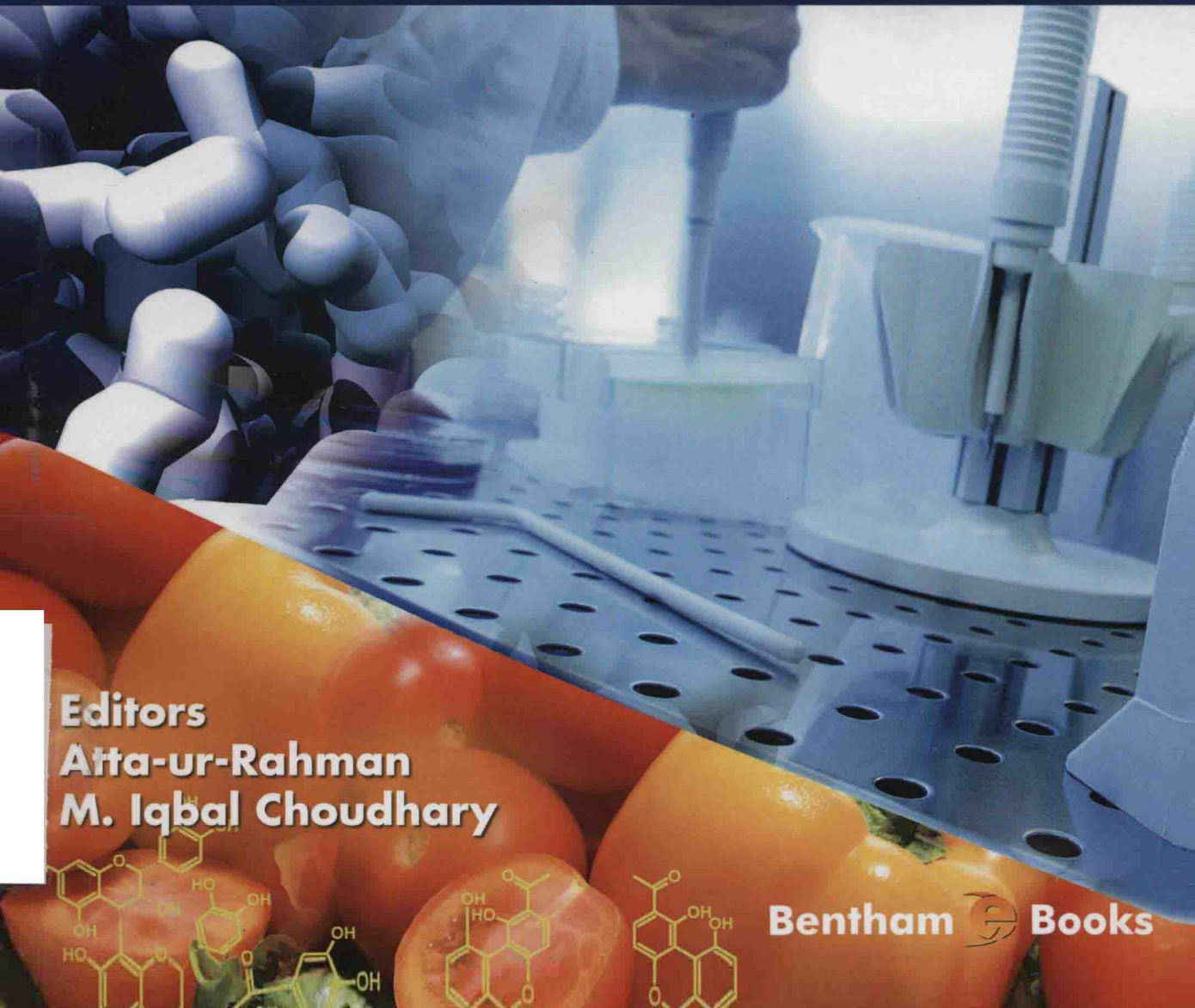

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Applications of NMR Spectroscopy
(Volume 2)

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Applications of NMR Spectroscopy

PREFACE

From its early beginning in 1940s Nuclear Magnetic Resonance (NMR) spectroscopy has evolved into a diverse, powerful, and essential analytical tool with applications in numerous fields, including structural biology, pharmaceutical sciences, material sciences, medical imaging, etc. NMR spectroscopic techniques in biomarker-based disease diagnosis, quality control of food and medicines, drug discovery and development, and materials and forensic sciences. The eBook series, "*Applications of NMR Spectroscopy*" provides comprehensive accounts of cutting edge developments in these fields. This second volume of the series is an excellent compilation of five well written reviews, contributed by several leading experts in the relevant fields.

Plant phenolics, particularly flavonoids and isoflavonoids, are known for their anti-oxidant properties. By virtue of this, plant phenolics are capable of preventing the on-set as well as progress of various diseases such as cancers, cardiovascular disorders, pre-mature ageing and neurological diseases. NMR spectroscopy is an excellent tool for the estimation, structure elucidation, conformation analyses, and study of hydrogen bonding patterns of various classes of plant phenolics in plant extracts, as well as in prepared formulations, and other health promoting products. Ziaullah and Rupasinghe have contributed an excellent review on the applications of NMR spectroscopy in the structure elucidation of polyphenols.

Magnetic Resonance Spectroscopy (MRS) is a relatively new technique which is based on the same principles as NMR spectroscopy, except that there is no read-out gradient and the frequency information is used to identify different chemical compounds and molecular compositions of the samples. Ulmer and Ahlhelm provide an *in-depth* review on the MRS technique, and its clinical applications such as brain development, noxa during pregnancy, developmental delay, stroke, infections, mitochondrial disorders, as well as brain tumours and neurological disorders.

Continuing the theme of medical and biomedical applications of NMR spectroscopy, Sing Muk Ng describes the recent developments of portable NMR-based sensors, such as NMR-MOUSE for onsite monitoring. These sensors are smaller in size and cost effective for on site *in situ* measurements. Despite their lower resolution, such portable sensors hold great promise for the diagnosis of diseases, etc, and deserve to be further developed for wide spread applications.

Airoldi *et al* have contributed a comprehensive review on various NMR techniques employed to study the molecular recognition process, including target identification and validation in drug discovery and development, ligand-receptor interactions, ligand screening and optimization, and SAR (Structure-Activity Relationship) studies. Applications, and merits and demerits of several robust NMR techniques, such as STD-NMR, trNOESY, WaterLOGSY, NOE pumping, etc are extensively discussed.

Structural biology is an important field in biomedical research. NMR has served as a key technique, along with single-x-ray diffraction, to determine the structures and dynamics of complex biological molecules, including proteins. Solution state NMR spectroscopy of proteins has numerous limitations particularly because of their insoluble nature. Membrane proteins are difficult to crystallize and also difficult to solubilize. In such cases, solid state NMR spectroscopy can serve as a viable alternative to solution NMR and X-ray crystallography. Mananga presents a

comprehensive review of key mathematical formulations which have been developed to calculate various structural parameters from solid state NMR data. These include key structural biology parameters such as torsion angles, dihedral angles, bond distances and angles, etc.

The authors who have contributed these excellent reviews deserve our special appreciation. We would also like to acknowledge the efficient management of the entire team of Bentham Science Publishers, especially Mr. Mahmood Alam (Director Publications) and Ms. Fariya Zulfiqar (Assistant Manager Publications).

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SECTION A: FOOD SCIENCES

CHAPTER 1

Application of NMR Spectroscopy in Plant Polyphenols Associated with Human Health

Ziaullah and H.P. Vasantha Rupasinghe*

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Abstract: Polyphenols have been recognized as one of the largest and most widespread groups of plant secondary metabolites with marked antioxidant properties as well as recently recognized specific biological properties associated with prevention of chronic diseases such as cardiovascular disease, various cancers and type II diabetes. Within plant kingdom, over 50,000 of structurally diverse polyphenols are present, and their characterization stands as a challenge. In recent years, renewed interest in extraction, isolation and identification of polyphenols especially flavonoids from plant sources have become the core research in food chemistry, pharmacognosy and modern medicinal biochemistry. The NMR spectroscopy, which provides a rapid and nondestructive method for profiling of polyphenols by identification of the characteristic structural features through structural assignments, has been addressed in this chapter.

Keywords: Antioxidant, Flavonoids, Lignans, Mass Spectrometry, NMR structural assignments, Phenolics, Polyphenols, Stilbenes.

1. INTRODUCTION AND GENERAL OVERVIEW

The plant derived natural products, polyphenols, were historically referred to as "vegetable tannins" as a result of the use of various plants extracts containing them in the conversion of animal skin into leather in the history of human civilization of the Ancient Greek of the archaic period (ca. 800-500 BC) [1].

The real time research on polyphenols started to address objectives beyond its use in leather manufacture during the second half of the 20th century. The first

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glimpse of "plant polyphenols" appeared in scientific literature 1957 by Theodore White, an industrial chemist, who introduced polyphenols as materials having molecular masses between 500 and 3000 Da [1].

Plant polyphenols has been a most exotic topic in modern food chemistry not only as structurally diverse and major plant secondary metabolites, but also as compounds that express a wide range of applications in various aspects of commercial as well as general public interests. Today, plant polyphenols enjoy an ever-increasing curiosity not only among scientists but also, and most remarkably, garner the general public interest because of their frequent presence in vegetables, fruits, seeds, and derived foodstuffs and beverages, whose daily consumption has been found beneficial for human health.

Polyphenols participate in the fundamental chemical reactions, by scavenging the free radicals, such as those derived from lipids and nucleic acids oxidation that highlight their importance in reducing the risk of certain age related neurodegenerative diseases. Today, it has become the fashionable trademark of "polyphenols" in latest exploitations not only by parapharmaceutic, cosmetic, agro-food industries but also by academia.

Polyphenols have gained recognition as health promoting compounds especially in relation to chronic degenerative diseases: cardiovascular diseases [2], cancers [3], aging [4], and diabetes [5]. Polyphenols are the most abundant antioxidant present in diet and therefore, their antioxidant effects can be pronounced than vitamin C or E [6]. There is evidence of polyphenols exerting biological effects *in vitro*, *in vivo* and clinical studies; other than the well-known antioxidant effect. As reviewed, major contributor to health promoting and medicinal properties of herbs, spices and medicinal plants were polyphenols [7, 8] and antioxidant, anti-inflammatory, anti-hypertensive, anti-diabetic and anti-thrombotic were some of their attributed properties [7].

Polyphenols have shown vasodilator, anti-atherosclerotic, anti-inflammatory antithrombotic effects against cardiovascular diseases [2]. These effects were directly or indirectly related to their antioxidant properties, modulation of cell signaling, *etc.* Chemopreventive polyphenols can block or reverse the

premalignant step of multistage carcinogenesis, act as antioxidants and anti-inflammatory compounds [3]. Furthermore, they can bind directly with cell signaling molecules involved in carcinogenesis and exhibit a diverse chemopreventive effect [3]. Recent discoveries suggest that dietary polyphenols may have the capacity to exert chemopreventive effects through modulating various components of the epigenetic machinery in humans [9]. Changes in the DNA methylation pattern, regulation of histone modifications and change in the expression of some non-coding RNAs were some of the epigenetic properties exerted by them. Polyphenols like resveratrol can induce and regulate autophagy; which is vital for health but decrease due to age, and thereby, contribute to lifespan extension [4]. Polyphenols, resveratrol in particular showed the capacity to reduce blood glucose levels through insulin-dependent and -independent pathways in STZ-treated type-1 diabetic animal models [5].

Plant polyphenols have been defined in different ways *e.g.*; according to the official nomenclature rules of chemical compounds by IUPAC the presence of more than one hydroxyl group on a benzene ring or other arene ring like gallic acid, pyrogallol, resorcinol, catechol and phloroglucinol—all di- and trihydroxylated benzene derivatives-are defined as "phenols". This chemical definition has been modified with some restrictions due to their biosynthetic origin and here is another proposed definition for plant polyphenols [10, 11]: *The term "polyphenol" should be used exclusively for the plant secondary metabolites which have been derived from the shikimate derived phenylpropanoid and/or the polyketide pathway(s), featuring more than one phenolic ring and is lacking any nitrogen-based functional group in their most basic structural expression.*

Today due to the increased understanding of the health benefits and chemopreventive properties of polyphenols, there continues to be significant effort dedicated to improve analytical methods for characterizing the structures of polyphenols and monitoring their levels in fruits and vegetables, isolating the active ingredients as well as developing new approaches for mapping the interactions of polyphenols with biological molecules and development of structure/activity relationships.

1.1. The Evolution of Analytical Methods

Until 1930s, methods for the determination of the constitution or the structures of new natural phenolics remained the same. In 1920s-1930s, it was Liebig that invented the first ground breaking technique for combustion analysis which was then evolved by Pregl in 1923, who introduced microanalytical technique that required only 3-4 mg of organic material [12]. Thanks to the botanists Tswett, Heftmann, D. T. Day, Lester Reed and Lederer (1900s) who have been recognized today for their pioneering contribution in the field of chromatographic separation [13, 14]. The first revolutionized report was published by Zechmeister and Cholnoky in 1931 [15]. The application of the method to colored compounds was exhibited by Karrer, Brockmann, and others, and later on developed to colorless substances, by Reichstein [16]. In 1940s, Martin and Synge became the founder of partition chromatography on paper and silica gel. The former method was widely used for isolation of plant phenolics as reported by Bate-Smith and Harborne [17]. In 1950s, the paper chromatography was extended to thin layer chromatography through the famous work of Stahl. TLC became a widespread technique after Stahl published his book in 1962 [18].

In the late 19th century, scientist started studying spectroscopy, the understanding of its theoretical basis and its application for the quantitative, qualitative analysis of organic compounds. With the passage of time each area of spectroscopy evolved and started providing valuable insights into the structure of complex molecules.

UV-visible spectroscopy was founded during 1920s-1930s. The first book entitled *Spectroscopy* by Baly [19], was published in 1924 and the first commercial spectrometer was founded in 1940s. The famous Woodward's rules were documented in a 1942 paper, while previous research in this area was extensively reviewed by Braude in 1945 [20]. Hence, UV-visible spectroscopic technique provided more reliable probe and a major tool for organic chemists until 1970s [21].

The IR spectrometer was first developed in 1905 by Coblentz in the USA, which was latter on commercialized in 1940s and 50s. *The Infrared Spectra of Complex Molecules by* Bellamy [22] and the IR correlation chart by Colthup became prerequisite to study organic chemistry [23].