Dynamic nuclear polarization enhanced nmr spectroscopy biology essay

Science, Biology

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Summary

The innovative technology of dynamic nuclear polarization enhanced nuclear magnetic resonance spectroscopy (DNP-NMR) was widely used from physics to biological system and chemistry science to determine the structure of those complex molecules. In this dissertation, the development history of dynamic nuclear polarization enhanced NMR spectroscopy will be shown from launching time to recent time. Then this technique will be described in detail including main application area, theoretical principle, innovation, relatively commercial instrument, contribution and future development. Through this dissertation, an important innovation technology of dynamic nuclear polarization enhanced NMR spectroscopy will be recognized. Since many aspects of DNP-NMR technique even have improved spaces such as THz source, polarizing agent, transmission line, DNP probe and some hardware of NMR, the DNP-NMR technology is a promising research field though it is not a very new research field. Table of contents

Introduction and background

1. 1 Background of Nuclear magnetic resonance (NMR) techniqueNuclear magnetic resonance (NMR) is a physical phenomenon that the nucleus with the magnetic moment in the magnetic field can absorb suitable electromagnetic radiation frequency. The nuclei by the spinning transition from low-energy state to high-energy state then back to equilibrium release radio frequency that performed the NMR signals. 1 NMR spectroscopy was widely used in analytical chemistry and biochemistry to characterize the structure of the molecules, as well as it was generally used in medical imaging technology area such as Magnetic Resonance Imaging (MRI) to extrapolate the internal body structure. The body tissue possesses sufficient water to provide proton signals to achieve alignment phenomenon in the powerful magnetic field that could get the signals in 3D space from specific position of human body via bio-maker metabolism, therefore, MRI was employed to distinguish diseased tissue and normal tissue in Medical area. 21. 2 Introduction of Dynamic Nuclear Polarization (DNP) enhanced NMR techniqueIn the past decades, the sensitivity of NMR was enhanced via increases in the intensities of magnetic field, besides that, dynamic nuclear

polarization technology as an essential method was utilized in NMR technique to improve the sensitivity of NMR spectrometer. After the dynamic nuclear polarization phenomenon was first demonstrated by Overhauser in 19533, the Overhauser effect as a benefit technique was employed for the structure determination of 3D molecules by measuring the distance between hydrogens. Thereafter, three different microwaves driven polarization mechanisms were recognized namely the solid effect, the cross effect and thermal mixing. Then on the basis of the spin temperature concept (DNP effect enhanced significant at low temperature), theoretical depictions of the dynamic nuclear polarization mechanisms were progressed. The microwave frequency of DNP must be matched to the magnetic fields of NMR that means if DNP experiment was operated at high magnetic field, high microwave frequency would be needed. For example DNP spectrometer performed at the magnetic field of 1. 4T (59. 6MHz), the corresponding requirement microwave frequencies were 39. 4GHz for EPR and 60MHz for 1H NMR (527GHz at 800 MHz). 18 DNP was not technically possible to generate high-frequency and only operated at low frequency as a constraint for a long time, despite this the development of DNP technique was not halted. DNP technique begin capable be performed at high magnetic field was because the fast-wave vacuum electronic device Gyrotron. It could generate high power and high frequency microwave irradiation in strong magnetic field5 that DNP application in high field was possibility. In the later stage, DNP dissolution method was discovered that DNP in liquid state NMR field presented a new breakthrough. Shortly thereafter, various polarizing agents (monoradicals6, biradicals7, and non-aqueous solvent8) were identified. With the development and application of dynamic nuclear polarization technology, the sensitivity of NMR enhanced greatly that the signal to noise ratio of NMR increased, the peak assignment optimized and the experimental acquisition time was decreased obviously. 9 Because of all above advantages, the structure of some complex biological macromolecules and functional materials could be elucidated, thus DNP enhanced NMR spectroscopy enable to widely utilize in structural biology and functional material research area. In this dissertation, the development history of dynamic nuclear polarization enhanced NMR spectroscopy will be shown from launching time to recent time. Then this technique will be described in detail including main application area, theoretical principle, innovation, relatively commercial instrument, contribution and future development. An important innovation technology of dynamic nuclear polarization enhanced NMR spectroscopy will be recognized via this dissertation.

History of dynamic nuclear polarization enhanced NMR spectroscopy

2. 1 First proposed of dynamic nuclear polarization techniqueDynamic nuclear polarization (DNP) is a useful technique utilized in nuclear magnetic resonance (NMR). In 1953, Albert E. Overhauser 3 was originally proposed DNP phenomenon which caused some criticism from well-known physicists such as Norman Ramsey, Felix Bloch, etc. However, Carver and Slichter10 confirmed the DNP phenomenon through the nuclear spin polarization in metallic lithium experiment, and then Overhauser’s theory was finally accepted. Development of DNP-NMR technique from first report to the present day1st DNP experiment Spin Amyloid NanocrystalsDNP predicted

cross-effect DNP Temperature MAS DNP 1st Gyrotron for DNP Dissolution DNPsolid-effect DNP Thermal-mixing DNP pulsed DNP/Flow DNP Biaradicals 29Si surface DNP1950 1960 1970 1980 1990 2000 2010Fig. 1 The timeline of development of DNP-NMRAs can be seen in Fig. 1, the timeline of the development of dynamic nuclear polarization in NMR technique from launching to the present day was shown clearly. Since the DNP phenomenon was predicted and first experiment was done in 1953, some other dynamic nuclear polarization mechanisms namely the solid effect, the cross effect and thermal mixing were discovered in the early 1960's. An important point is that the DNP technology was first applied to produce polarized targets for the solid-state experiments11 by Abragam in 1962. Later, another critical breakthrough occurred in the 1970's, the DNP technique was used to expand field in the solution state NMR experiments by Hausser Stehlik and Mueller Warmuth to investigate the activities and interactions of molecules in solution. 12 In the 1974, De Boer with co-workers through experiment pointed out that low temperature was a critical necessary condition for DNP builds up. 13 As can be seen in the Fig. 214, it was shown that the proportion of dynamic nuclear polarization was decreased with temperature increased, thus the concept of the weightiness of lattice temperature as low as possible was underlined. M[NCHC3[TY9IX4UROLO[)`2Figure 2: The plots of polarization (%) vs lattice temperature (K): Upper curve was electron; below curve was proton 26Since dynamic nuclear polarization mostly suitable for solid state NMR experiments, therefore more researchers focused on developing the technique of DNP in the solid state NMR experiments. For NMR spectroscopy part, it grew to even higher magnetic field, but for DNP technique part, a big problem was from the lack of microwave technology (microwave sources only could operate at low microwave frequency). However, in the period of 1980’s and 1990’s, through the experiment (an investigation of the proton DNP in coal samples15) which worked by Wind, proton signal enhancement by higher electron spin resonance (ESR) frequencies could be confirmed. The signal intensity in the magic angle spinning NMR could be enhanced through increased DNP technology. For instance, in order to selectively observe the interface of heterogeneous polycarbonate and polystyrene blends, this technique (dynamic nuclear polarization carbon-13 NMR spectroscopy) was used by Schaefer. 16 One most important event in the DNP method area was that a high frequency and high power source (gyrotron) was used in DNP-NMR spectroscopy by Griffin. 17 Gyrotrons as a free electron laser could be operated at higher microwave frequency, which means DNP method was no longer restricted at low magnetic fields. Therefore, first gyrotron (a " fast-wave" vacuum electronic device to generate the terahertz waves) started to use for DNP enhanced NMR experiment in the early stage of 1990s at 5T (212. 8MHz) magnetic field17 was a landmark in the development history. The start stage, researchers concentrated on studying structural biological by using DNP enhanced solid state NMR spectroscopy. An experiment to study membrane proteins displayed that the sensitivity of biological macromolecules in frozen glycerol-water solution could be improved by high resolution solid state NMR spectroscopy. 18 In this DNP-NMR spectroscopy, gyrotron technology was used to generate high-power and high-frequency microwave radiation (200-600 GHz) that benefit to upgrade nuclear magnetic resonance resolution and signal intensity at higher magnetic fields. 12Though DNP in liquid state NMR spectroscopy also kept on developing, a significant breakthrough was occurred in the early 2000's. The lack of low signal intensity in liquid state NMR was solved when fast dissolution DNP method was successful attempted. 1, 3-bisdiphenylene-2-phenylallyl (BDPA) as an efficient and stable free radical polarizing agent was used for polarizing hydrophobic compounds and hydrophilic substrates in fast dissolution DNP-NMR spectroscopy to enhance the signal intensity of nuclei (such as 13C, 15N, 31P, 6 Li, 29Si, and 89Y). 6 Subsequently, biradical polarizing agents were utilized such as a TEMPO-based biradical 1-(TEMPO-4-oxy)- 3- (TEMPO-4-amino) propan-2-ol (TOTAPOL), which could soluble in liquid media including salt and glycerol. Compared with the previous nitroxide-based mono-radical polarizing agent, it provided much larger DNP enhancements, so this reason allowed to TOTAPOL could widely used in structural biological sysytem. 7 Later, amyloid nanocrstals, GNNQQNY nanocrystals and amyloid fibrils was identified could produce accuracy information of biological system very efficiently. 19 Thus it was applied at later stage in order to avoid the problem caused by TOTAPOL which was so large in the aqueous channel that could not interact with bulk molecules in crystals. 20High dielectric losses of sample resulted in heating effect were a critical factor limited the development of liquid state DNP technique at high fields. In recently, a new helical double resonance (Fabry-Perot resonator) was utilized at 9. 2T (391MHz) high magnetic fields 21 to make a breakthrough in liquid state biomolecular DNP technique application area. Biradicals as efficient polarizing agents for DNP experiment only been utilized with aqueous solution, so the technique was prevented using for some moisture sensitive samples. In order to enable suit for a large scale materials, a sequence of organic solvent combined with bTbK (biradical) for DNP surface enhanced NMR spectroscopy were developed in the early 2010's. 8 1, 1, 2, 2-tetrachloroethane as a promising non-aqueous solvents for solid state DNP surface demonstrated remarkable higher sensitivity and reduction of experimental time. 82. 3 Main application of DNP-NMR techniqueSince dynamic nuclear polarization enhanced NMR spectroscopy possess excellent sensitivity that signal to noise ratio were increased and the experimental acquisition times were decreased, hence it suitable used to determined the structure of complex biological molecular systems like large soluble proteins, small peptides, nucleic acids and membrane protein. Therefore, dynamic nuclear polarization enhanced nuclear magnetic resonance spectroscopy has been widely used in structural biology22 and functional material research science23. Fig. 3 showed the measured spectrum of 13C in amino acid proline by using DNP enhanced solid state NMR spectrometer, the signal intensity of 13C increased obviously when microwave on could certify the sensitivity enhancement characteristic of this technique . For the application of functional material research aspect, Fig. 4 displayed the results of DNP experiments on Mat-Prim, the biradical polarizing agent (bCTbK) structure (a), the spectra of DNP-enhanced 13C (b), 15N (c), 1H-13C (d), 1H-15N (e) and 1H-29Si (f) demonstrated that DNP-NMR spectrometer could use to identify the characterization of organic silica mixed material (they could apply in separation, catalytic action, purification equipment and drug delivery) at molecular level, the experimental scan time was greatly reduced and the assignment of peaks were optimized. C: UsersuserAppDataRoamingTencentUsers13120657QQWinTempRichOle4M@CD6@3Y48DBL~@{%BU7PE. jpgC: Documents and SettingsAdministratorApplication DataTencentUsers13120657QQWinTempRichOleE$8T0F98T1)$$9JNOWH9)9W. jpgFig. 3 13C in amino acid proline 24 Fig. 4 DNP experiment on Mat-Prim24The hyperpolarized technique basis in the liquid state was displayed that it could be used to enhance the sensitivity of liquid-state NMR spectroscopy. The increased NMR signals of 13C, 15N, 29Si and 31P nuclei allowed that this method could be utilized in physical, chemical, biochemical and medicinal science fields. Dynamic nuclear polarization enhanced NMR possesses many advanced capability. For instance, it could rapidly monitored the cellular metabolism, determined the structure of complex bio-mixtures in metabonomics, increased signal in chemical kinetics experiments, reduced the experimental data collection time and the quantity of samples. 25

The principles of DNP-NMR technique

3. 1 Description of DNP-NMR techniqueDynamic nuclear polarization (DNP) technology is a hyperpolarization technique to boost sensitivity for NMR experiment that the NMR signal intensity of samples enhanced dramatically. In DNP enhanced solid state NMR experiments, signal to noise ratio is enhanced more than 100 times. Hence when the factors far larger than 10, 000, the experimental data collection time will much shorter, from a month decreased to just a few minutes. 5 In the previous, because of the poor microwave radiation, DNP enhanced NMR experiments just performed at low

magnetic field (magnetic field <1. 4T, the corresponding microwave frequency <40GHz for EPR and <60MHz for proton18). However, due to the improved technology in millimeter wave and terahertz, today this technique could be utilized at all magnetic fields. 53. 2 Commercially application and components of DNP-NMR systemBruker's 263 and 395 GHz dynamic nuclear polarization in NMR spectrometers are the world's first commercially available solid-state DNP-NMR system, which possess brilliant sensitivity that extend the experiment range of DNP in solid state NMR. 26 Bruker's DNP-NMR spectrometer has been used to detect a wide range of biological samples (including peptides, proteins and complex biological molecules). 24 The critical components of DNP-NMR spectrometer (Fig. 5) are THz source, transmission line, DNP probe and polarizing agent. The terahertz source is employed to generate abundant THz power to excite the electron paramagnetic resonance transitions effective; the transmission line with the capability to deliver the microwave power from THz source to the sample; the DNP probe is used to radiate the spinning sample with Rutherford and microwave power at low temperature (less than 100K); the polarizing agent is the source of the unpaired electrons. 5C: Documents and SettingsAdministratorApplication DataTencentUsers13120657QQWinTempRichOleRU9DIUU_[0EFL1S~HS~8R`O. jpgFig. 5 Components of DNP-NMR system5HyperSense is an in-vitro polarizer produced by Oxford Instruments Company, which showed dramatically increasing capability of nuclear spin polarization via the DNP technology. 25 This dissolution method9, 27 was divided three stages: first, the sample was dissolved in a glassing agent (such as DMSO/H2O), polarizing agent (proprietary radical) was added to form a sample with hyperpolarized nuclear spins and the sample was transferred in polarizer sample cup, then the sample (10-200μl) was loaded into polarizer; second, the polarization process was completed by DNP polarizer (HyperSense) automatable that includes three steps: sample conditioning (sampling in polarizing area and cooled to not less than 1. 4K), polarizing (transferred polarization of unpaired electrons via microwaves irradiation) and transfer (sample dissolution by hot solvent and transferred into a high-resolution or an image magnet); third, polarized sample was transported into high-resolution NMR and data was obtained. In the experiment, the amounts of sample were reduced to μg level and the peak assignment was improved. After polarization by this technique, acquisition detection time less than using conventional NMR. 93. 3 Theoretical principle of DNP-NMR techniqueThe basic principle of DNP is that the spin polarization of unpaired electrons are transferred from electrons spin reservoir to their neighboring nuclear spin reservoir by terahertz (microwave) irradiation at the electron paramagnetic resonance (EPR) transition frequency. 5, 28 The Fig. 6 performed the sample polarized process via microwave irradiated. Dynamic nuclear polarization mechanism was classified to two parts: one is used in liquid-state (the Overhauser effect), another is utilized in solid-state (contained the solid-effect, the cross-effect and thermal-mixing). C: Documents and SettingsAdministratorApplication DataTencentUsers13120657QQWinTempRichOle5H_46F%6T3@7`ON[E{@ %1PT. jpgFig. 6 dynamic nuclear polarization process14When DNP technology was used in the liquid state, the Overhauser effect3 causes enhanced signals from one spins type when another spins type resonance is saturated. Time-dependent coupling is requested between them though the average interaction may be zero. This effect relies on hyperfine coupling spin between an electron and a nucleus that was observed in metals and free radicals in liquid state. Increased NMR signal relies on two main factors, one is polarizer/solvent system (could solute by adding free radicals), another is pumping condition including coupling factor, leakage factor and effective saturation factor of electron paramagnetic resonance (EPR) transition with gyromagnetic ration. 29 The longitudinal (spin-lattice) relaxation time (T1) was means the waiting time of nuclei from non-equilibrium state return to initial state (90o), it depends on the NMR frequency, magnetic field intensity and the nature of compounds. 1 Long T1 relaxation times maintain polarization of the sample when it is warm-transferred to the NMR magnet. The coupling factor displays the intensity of interaction between electron spin and nuclear spin; the leakage factor can be identified from the nuclear relaxation time (T1) in the presence and in absence of radicals in the solution. 30 In addition, as T1 and T1e increased, the leakage of increased 1H polarization decreased and electron-electron cross-relaxation more influential respectively that were two main reasons for why DNP build up at low temperature more efficient, although MAS technology commonly displayed at high temperature (> 100K). 18 For example, Becerra with partners did an experiment by a DNP-MAS probe that helium was utilized as the spinning and cooling gas (the spinning velocity could reach to 5KHz and the temperatures as low as 25K), the nuclear relaxation time of 4-amino-TEMPO-water-glycerol was larger than 30s, it showed that the proton T1 enhanced more than three multiples compared to perform at temperature up 100K (≤ 10s). 18When DNP was employed in the solid state, the solid effect is13 caused by that the electron-nucleus mutual forbidden transition is stimulated in an electron-nucleus two-spin coupled system via microwave irradiation. In the two-spin coupled system, the optimal polarizing mechanism is that the electron Larmor frequency no smaller than double nuclear Larmor frequency (the inhomogeneous spread and homogenous linewidth of EPR spectrum is smaller) that avoid the cancellation of positive and negative DNP enhancements. 31 The cross effect is observed when irradiation frequency less than twice nuclear Larmor frequency (the inhomogeneous spread and homogenous linewidth of EPR spectrum is larger), so cross effect mechanism produces higher signal enhancement at high magnetic fields. 31 The thermal mixing is a process of energy exchange between electrons spin and nuclear spin. Since the stronger inter-electrons interaction, it is occurred when homogenous linewidth of EPR spectrum larger than nuclear Larmor frequency. However, it needs a higher concentration paramagnetic that it is not a promising application in NMR. 31

An appreciation of the contribution DNP-NMR technique

4. 1 The sensitivity limitation of NMRNuclear magnetic resonance (NMR) spectroscopy as a well-known technique is widely utilized in analytical chemistry and biochemistry to analyze and identify structure of molecules. However, compared to other spectroscopic technologies, it is a relatively lower sensitivity technique due to its small over population resulting in low nuclear polarization. 32 Hence, inherent low sensitivity limits its application

area such as kinetic studies, biological mixture detection, etc. However, the improvement of dynamic nuclear polarization enhanced NMR technique mends the lack of conventional NMR. 4. 2 DNP-NMR technique how to overcome limitationDNP technique through enhances the saturation of electron spin resonance to increase the DNP factor and peak intensity, optimize the peak assignment spectra and decrease the experimental acquisition time. 33 Compared to the conventional NMR, the sensitivity of DNP- enhanced NMR increased more than 100 times, some carbons significant increased after polarization. The signal-to-noise of 13C spectra was improved and that the carbon reaction centers could be observed, the spectrum of multiple molecules could be increased and the detection time of 13C, 15N and 29Si decreased greatly. 25 All the advantages of DNP-enhanced NMR lead to the analysis and detection fields of NMR were extended significantly. However, in the previous DNP-NMR development, DNP technique was restricted using at low magnetic fields due to the poor microwave radiation. The discovery of high power terahertz source (gyrotron) overcame this limitation. Gyrotron could generate sufficient THz power at high magnetic field, so it was always used in the high-field DNP enhance NMR experiments. 4. 3 The recent development of DNP-NMR techniqueOver the last ten years, key components of DNP-NMR system which impact on the enhancement level of DNP were developed actively. For the development of polarizing agent part, besides some mono- and biradicals polarizing agents was reported, a new commercially free radical polarizing agent (galvinoxyl) was proposed. A test feasibility experiment for dissolution DNP-NMR about it was done by Kovacs. It described that several-thousandfold NMR signals were increased for 13C and 15N-abundant compounds with galvinoxyl, which means it could be used in DNP-NMR to characterize various compounds well. 32 However, biaradicals only been used with liquid solution, some moisture sensitive materials were prevented, hence some tended to non-aqueous solvents field in recent years. The DNP experiment using 1, 1, 2, 2-tetrachloroethane showed that non-aqueous solvent provided same appearance as aqueous radical solvent. This evidence demonstrated that using hydrophobic biradicals for dissolution DNP-NMR experiments extended the application range to developing functional materials area to characterize structure of materials. 8For microwave transmission line part, large diffraction loss in previous transmission line resulting in sample was irradiated inefficient, thus corrugated waveguides instead of rectangular waveguide was typically used in recent DNP-NMR experiment. Thorsten Maly with his partner presented simplified THz instrumentation concept that used TE01 circular waveguide mode as critical transmission mode to deliver the microwave irradiation from gyrotron source to the samples could get larger enhancement factors. 5. 4. 4 Future development of DNP-NMR techniqueDynamic nuclear polarization enhanced NMR is not a new research area, but because of excellent contribution in many various analytical fields, researchers still focus on the development of this technology. With more and more fresh and preeminent improved methods are found, more and more limitations are overcame, then the application range for using in complex biomolecules and materials will more wide and the development of DNP-NMR technique are going to better and better. On 18th of March, 2013, Bruker Company announced that the world's highest frequency field DNP-NMR spectrometer (527GHz solid state DNP-NMR spectrometer) was set up successfully at University of Utrecht (Holland). Compared with the Bruker 263 and 395 systems, this machine delivered much higher frequency microwave by novel gyrotron and 800 MHz wide bore superconducting magnets was used. 34 These improved hardware of solid state DNP-NMR spectrometer allowed better to using in complex molecules and materials science. That demonstrated that DNP enhanced NMR technology also have many progressive space. Fig. 7 displayed the relationship between the DNP-NMR technology literature publication quantity and relative research periods. The publication quantity tended to sharply increase, especially after 2008. The literature quantity nearly twice of the total amount of before 2008 hinted that more researchers focused on DNP-NMR technology, therefore, DNP-NMR technology is a promising development technology research area in the recent and future. Fig. 7 Plot of Literature quantity vs yearIn addition, through keep on improving the dynamic nuclear polarization (DNP) technology and related DNP enhanced NMR spectroscopy (DNP-NMR) technique, DNP-NMR spectroscopy is growing to a critical technology for applying to a wider scope of materials. In the recent, many new areas for research to enhanced NMR spectroscopy methods are continuing as well.

Conclusion

In conclusion, the innovative technology of dynamic nuclear polarization enhanced nuclear magnetic resonance spectroscopy (DNP-NMR) was discussed. Through above description concerned this technique in history, principle and contribution aspects, and the basic information for DNP-NMP was already known. Instead of the conventional NMR spectroscopy, DNP approach enhanced the sensitivity of NMR via sample polarization. As a result of improved NMR sensitivity, DNP-NMR technology was widely used in biological system and materials science field to determine the structure of those complex molecules. Though DNP-NMR technology was not a very new research field, it stills a promising research field. Besides the reason of the weightiness in analytical science area, many respects of DNP-NMR even have improved spaces such as THz source, polarizing agent, transmission line, DNP probe and some hardware of NMR also an important reason. Therefore, the development of DNP-NMR technique will be going on.