EXTENDING THE APPLICATIONS OF LONG-LIVED STATES AND LONG-LIVED COHERENCES IN LIQUID STATE NMR

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by

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Dedicated to my Parents and Supervisor.

Certificate

I certify that the thesis entitled **EXTENDING THE APPLICATIONS OF LONG-LIVED STATES AND LONG-LIVED COHERENCES IN LIQUID STATE NMR** submitted by **Mr. UPANSHU GANGWAR**, to Indian Institute of Technology for the award of **Doctor of Philosophy** is a record of his bonafide research work. He has worked under my guidance and supervision. To my knowledge, he has fulfilled the requirements for thesis submission, which has reached the requisite standard. The results presented in this thesis have not been submitted in part or whole to any other University or Institute for the award of any degree or diploma.

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Upanshu Gangwar

Abstract

Nuclear magnetic resonance (NMR) has proved to be an efficient tool for studying molecular level interactions and dynamics. It has a wide range of applications from structure elucidation of large and small molecules, molecular dynamics, chemical kinetics, quantum computing to MRI (Magnetic Resonance Imaging). The longer timescale of relaxation times in NMR, known as longitudinal (T_1) and transverse relaxation (T_2), is one of the main reasons for extensive usage of NMR. Earlier, it was believed that these relaxation lifetime set the upper limit to applications of NMR but Long-lived states (LLS) and Long-lived coherences (LLC) provided a way to achieve higher lifetime than T_1 and T_2 in scalar coupled two-spin systems.

LLS was mainly exploited to study slow dynamic processes, i.e., singlet exchange NMR spectroscopy, to provide a long timescale in diffusion experiments, and drug screening boosted by hyperpolarization. The application of LLC involved determination of hidden coupling constant and line narrowing. Further extension of their applications was limited by the restriction to a single two-spin system. A trial to overcome these restrictions forms the central part of this thesis entitled **"Extending the Applications of Long-lived states and Long-lived coherences in liquid state NMR"**.

First chapter introduces important concepts of solution state NMR and relaxation theory, i.e., Hamiltonian, operators, superoperators, product operator formalism, and Redfield relaxation theory. An introduction to various bases, LLS, LLC and Pure shift NMR techniques.

Chapter 2 examines the theory of LLS and its relaxation in the case of in-

termolecular interactions. The systems studied here are supra-molecular drug assemblies of β-cyclodextrin (β-CD). Often these complexes lies in the intermediate motional regime where studying the intermolecular interactions may be a difficult task due to the limit $\omega\tau_c \approx 1$. Usual studies may provide a limited answers to the structural details. Relaxation studies may provide the information on a deeper level. In this chapter, an attempt has been made to apply the relaxation theory of long-lived spin order to these supra-molecular drug assemblies. Experiments are performed by creating the LLS in pure drug molecules and a mixture of drug:β-CD and observing the sustenance of LLS with locking pulse. The change in relaxation lifetime of LLS indicates the structural and environmental changes. Computational and theoretical calculations validates these observations.

Chapter 3 discusses 2-dimensional LLC techniques as a filter for coupled spins. Although, NMR provides a great insight to structural and dynamical information, especially biological samples and metabolic mixtures but 1D ^{1}H NMR spectroscopy is burdened by overlapping resonances leading to the unavoidable challenge in matabolite identification and quantification. 2D NMR seems to be an answer but despite the better signal acquisition and processing methods there are several cases where this tecchnique seems to have some limitations. Spectral and relaxation editing is another major technique for these kind of problems. In this chapter the idea of LLC has been utilized for relaxation editing of 2D correlation NMR techniques. A pulse sequence for LLC-TOCSY and COSY has been proposed and results on some of the metabolomic mixtures have been shown.

Chapter 4 of the thesis introduces a pure shift technique to create broadband LLC in a mixture of two spin systems. One of the limitations to application of LLC is its restriction to a single two-spin system. Pure Shift NMR provides a new way of exciting the coherence between singlet and triplet states by using a selective pulse simultaneously applied with a weak magnetic field gradient. This study shows that it is possible to create $I_{1x} - I_{2x}$ simultaneously for multiple two spin systems in a sample and record the LLC. Broadband LLC can be utilized in sub-spaces of large proteins and macro-molecules. The relaxation lifetime and oscillation frequency of LLC depends upon the choice of sustaining pulse. MLEV16 appears to be the most suitable choice. The applications of this method are yet to be explored as low signal to noise ratio due to pure shift method makes it hard to be utilized in complex pulse sequences. This may seem a small step for creating the LLC in mixtures and multiple spin systems but it may be a necessary one. It allows to combine the idea of long relaxation lifetime with pure shift technique which might be useful in high resolution studies.

Chapter 5 improves the understanding of different behavior of LLS and LLC under distinct composite pulses. Here, a numerical simulation method is used to elucidate the behavior of LLS and LLC with off-resonance effect. For the purpose of sustaining LLS and LLC, continuous wave(CW) was used starting from the first demonstration. It works well when the offset of the locking pulse is exactly in center of frequencies of the two spins. However, for sustaining the LLS and LLC with off-resonance locking CW seems less suitable as their characteristic properties diminish when the offset is moved away. Whenever there is an attempt to create LLS and LLC in broadband manner, this problem is encountered. One alternative is to use composite pulses for locking instead CW. Several decoupling sequences and a few 2D NMR technique, i.e., TOCSY, and ROESY have utilized different composite pulses suitable to their requirement. Very few attempts have been made to find better composite pulses for LLS and LLC off-resonance sustaining. In this chapter, the previous ideas of off-resonance locking for CW is extended to the composite pulses using numerical analysis.

Appendix A includes two parts for NMR studies on Bispidine diamide derivatives.

First part (**1**) shows the application of selective inversion technique applied to a bispidine-peptide conjugate for conformational studies. Bispidine serves as a versatile scaffold for nucleating secondary structures such as reverse turns, helices, and sheets in peptides. These derivative show unique type of conformational exchange.

The rate of this conformational change is measured using NMR. Selective and non-selective inversion recovery experiments were performed on the interested sites with varying mixing delay at each temperature. The rate constant was measured fitting the selective inversion data to CIFIT program. The exchange rates obtained at different temperatures were used to find out the activation energy barrier ($\Delta E\dagger$) and enthalpy of activation ($\Delta H\dagger$).

Second part (**2**) gives insight into the DOSY technique utilized for validation of vesicles formation by bispidine derivative. Often at higher concentration or depending upon solvent nature these derivatives tend to form spherical, cylindrical, and planar assemblies. DOSY can be useful for studying macro-assembly formation and finding out the diffusion of these assemblies through solvent. A concentration dependent DOSY is performed with Methanol as solvent. As the concentration of compound is increased the diffusion constant is decreased suggesting the restricted motion of molecules through solvent.

सारांश

नाभिकीय चुंबकीय अनुनाद (एन. एम. आर.) आणविक स्तर की परस्पर क्रिया और गतिशीलता के अध्ययन के लिए एक कुशल उपकरण सिद्ध हुआ है। इसके अनुप्रयोगों की एक विस्तृत श्रृंखला है जो कि बड़े और छोटे यौगिको की संरचना व्याख्या, आणविक गतिकी, रसायन गतिकी, क्वांटम कंप्यूटिंग से लेकर एम. आर. आई. (चुंबकीय अनुनाद इमेजिंग) तक फैले हैं। एन. एम. आर. के अत्यधिक उपयोग कि वजह इसकी उत्तेजित अवस्था के लम्बे जीवन काल हैं, जिन्हें अनुदैर्ध्य (T_1) और अनुप्रस्थ जीवन काल (T_2) के नाम से जाना जाता है। कुछ समय पूर्व, इन्हें ही विश्राम की ऊपरी सीमा माना जाता था लेकिन लॉग–लिव्ड स्टेट्स (एल एल एस) एवं लॉग–लिव्ड कोहेरेंस (एल एल सी) ने अदिश युग्मित दो–स्पिन निकाय में और भी अधिक लंबा जीवन काल प्राप्त करने का मार्ग प्रदान किया है।

एल एल एस का उपयोग प्रमुख रूप से धीमे गतिशील प्रक्रियाओं , जैसे सिंगलेट एक्सचेंज एन एम आर स्पेक्ट्रास्कपी में, प्रसार प्रयोगों में लंबा कालक्रम देने में , और अति ध्रुवीकरण से दवाओ के जांच एवं निस्पंदन को प्रोत्साहन प्रदान करने के लिए किया गया। अद्रश्य युग्मन स्थिरांक का निर्धारण एवं रेखा संकुचन आदि एल एल सी के अनुप्रयोगों में संयुक्त हैं। परंतु आगे इनके अनुप्रयोगों के विस्तार केवल एक दो–स्पिन निकाय के लिए ही परिसीमित थे । यह शोध प्रबंध जिसका शीर्षक ”तरल अवस्था एन एम आर में लॉग–लिव्ड स्टेट्स (एल एल एस) एवं लॉग–लिव्ड कोहेरेंस (एल एल सी) के अनुप्रयोगों का विस्तारण” है , इन्हीं प्रतिबंधों को अभिभूत करने का प्रयत्न है ।

पहला अध्याय तरल अवस्था एन एम आर के महत्वपूर्ण अवधारणाओ एवं विश्राम सिद्धांत, जैसे की, हैमिल्टनीयन, ऑपरेटरों , सुपर ऑपरेटरों , ऑपरेटर उत्पाद नियम, एवं रेडफ़ील्ड विश्राम सिद्धांत के बारे में परिचित करता है।

अध्याय २ अंतर आणविक परस्पर क्रियाओं के लिए एल एल एस के सिद्धांत एवं इसके विश्राम का अध्ययन करता है। यहाँ अध्ययन किए गए निकायों में सुपरा आणविक औषधि–β–सायिक्लोडेक्स्ट्रिन समूह शामिल हैं। प्रायः ये समूह मध्यवर्ती गतिवान सीमा में स्थित होते हैं जहाँ अंतर आणविक परस्पर क्रियाओं को अध्ययन करना कठिन कार्य हो सकता है क्योंकि $\omega\tau_c \approx 1$। प्रायिक विधिओं से संरचनात्मक

विवरण के विषय में केवल परिसीमित उत्तर ही प्राप्त हो सकते हैं । विश्राम अध्ययन इस विषय में गहन जानकारी प्रदान कर सकते हैं। इस अध्याय में लॉग–लिव्ड स्पिन क्रम के विश्राम सिद्धांतों को इन सुपरा आणविक औषधि समूहों पर प्रयोग किया गया है। शुद्ध औषधि अणुओ एवं औषधि–β–सायिक्लोडेक्स्ट्रिन के मिश्रण में एल एल एस का निर्माण करके एवं पाशन स्पंद के दौरान उनके निर्वहन के प्रयोगों को प्रदर्शित किया है। एल एल एस के विश्राम जीवन काल में परिवर्तन संरचनात्मक एवं वातावरण में बदलाव को दर्शाता है। संगणकीय एवं सैद्धांतिक परिगणनाएं इन पर्यवेक्षण की पुष्टि करते हैं।

अध्याय ३ एक २–आयामी प्रविधि के बारे में विनिमय करता है जो की संयुग्मित स्पिनो के निस्पंदन के लिए प्रयोग किया जा सकता है। यद्यपि एन एम आर संरचनात्मक एवं गतिकीय जानकारी के संदर्भ में बेहतर अंतर्दृष्टि प्रदान करता है, खासकर जैविक एवं उपापचायी नमूनों के लिए, लेकिन १–आयामी ^{1}H- एन एम आर स्पेक्ट्रास्कपी अतिव्यापी अनुनादों की वजह से उनके पहचान एवं परिमाणन अपरिहार्य चुनौतियों से पीड़ित है। २–आयामी एन एम आर इसका एक उत्तर प्रतीत होता है लेकिन बेहतर सिग्नल अधिग्रहण एवं प्रसंसकरण विधिओ के बावजूद भी कुछ स्थितिओ में ये सीमित है। इस तरह की समस्याओं को दूर करने के लिए वर्णक्रम संबंधी एवं विश्राम संबंधी सम्पादन एक और प्रमुख विधि है। इस अध्याय में एल एल सी के इसी अवधारणा का उपयोग २–आयामी सह–संबंध एन एम आर विधिओ के विश्राम संबंधी सम्पादन के लिए किया गया है। एल एल सी –टॉक्सी एवं एल एल सी कोज़ी के स्पंद अनुक्रम एवं उनके उपापचयी मिश्रण पर कुछ अनुप्रयोग प्रदर्शित किए गए हैं।

इस शोध प्रबंध का अध्याय ४ दो–स्पिन निकाय के मिश्रण में एक प्युर शिफ्ट एन एम आर विधि के द्वारा ब्रॉडबैंड एल एल सी बनाने को परिचित करता है। एल एल सी के अनुप्रयोगों में एक परिसीमा यह है की ये केवल एक दो–स्पिन निकाय तक मान्य है। प्युर शिफ्ट एन एम आर ने एक चयनात्मक स्पंद के साथ साथ दुर्बल चुंबकीय क्षेत्र प्रवणता का उपयोग करके सिंगलेट एवं ट्रिप्लेट अवस्थाओं के बीच कोहेरेंस को उत्तेजित करने का नया मार्ग प्रदान करता है। ये अध्ययन ये दिखाता है की एक से अधिक दो–स्पिन निकायों में एक साथ $I_{1x} - I_{2x}$ को बनाना संभव है। ब्रॉडबैंड एल एल सी को विशाल प्रोटीन्स एवं बड़े अणुओ के उपस्पेस में प्रयोग किया जा सकता है। इसका विश्राम जीवन काल एवं दोलन आवृत्ति उसे संभालने वाले स्पंद के विकल्प पर निर्भर करता है। इस विधि के अनुप्रयोगों का अन्वेषण करना अभी भी बाकी है क्योंकि प्युर शिफ्ट एन एम आर की वजह से कम सिग्नल का नॉइज़ से अनुपात इसे दुर्लभ बनाता है। मिश्रण में एल एल सी बनाने का भले ही ये एक क्षुद्र चरण है परंतु ये एक आवश्यक चरण है। ये लंबे विश्राम जीवन काल को प्युर शिफ्ट एन एम आर को संयुक्त करने की अनुमति प्रदान करता है जिसे अति रेसोल्यूशन अध्ययन में उपयोग कर सकते हैं।

अध्याय ५ एल एल एस एवं एल एल सी के अलग अलग सम्मिश्रित स्पंदों के दौरान उनके अलग

अलग व्यवहार की समझ को उत्तम बनाता है। यहाँ एक संख्यात्मक सतत अनुकरण विधि का उपयोग करके दूर के वाहक आवृत्ति के अंतर्गत एल एल एस एवं एल एल सी के व्यवहार को स्पष्ट किया गया है। एल एल एस एवं एल एल सी को संभालने के उद्देश्य के लिए शुरुआत से ही कन्टिन्यूअस वेव (CW) का उपयोग किया जाता है। जब पाशन स्पंद का अंतर्लम्ब बिल्कुल दो स्पेक्ट्रल शिखरों के बीच में होता है तो यह बहुत अच्छा काम करता है। हालांकि दूर के अंतर्लम्ब के दौरान एल एल एस एवं एल एल सी को संभालने के लिए CW का उपयोग अनुचित प्रतीत होता है क्योंकि इनके वैशिष्ट्य गुण अंतर्लम्ब को दूर करने पर घट जाते हैं। जब भी ब्रोडबैंड एल एल एस एवं एल एल सी को बनाने का प्रयत्न किया जाता है, इस समस्या का सामना करना पड़ता है। एक विकल्प यह है की CW के स्थान पर सम्मिश्रित स्पंदों का उपयोग किया जाए। कई असयुग्मन अनुक्रम एवं कुछ २–आयामी एन एम आर विधियाँ, जैसे की , टॉक्सी एवं रोज़ी उनके उद्देश्य के अनुसार अलग अलग सम्मिश्रित स्पंदों का उपयोग करते हैं। एल एल एस एवं एल एल सी के दूर अंतर्लम्ब वाहक आवृत्ति के दौरान बेहतर विकल्प की खोज के बहुत ही कम प्रयत्न किये गए हैं। इस अध्याय में CW के दौरान दूर अंतर्लम्ब वाहक आवृत्ति की पूर्व अध्ययन को संख्यात्मक विश्लेषण के द्वारा सम्मिश्रित स्पंदों तक लागू किया है।

परिशिष्ट A के अंतर्गत दो भाग शामिल हैं जिनमें बिसपिडीन के व्युत्पन्न पर एन एम आर के द्वारा अध्ययन किया गया है।

प्रथम भाग (१) एक चयनात्मक चुंबकन उलटने की विधि का अनुप्रयोग दिखाता है जो की बिसपिडीन–पेप्टाइड के गठनात्मक अध्ययन के लिए उपयोग किया है। बिसपिडीन एक माध्यमिक संरचना, जैसे की, रिवर्स टर्न , हेलिक्स, एवं पेप्टाइड में शीट्स के लिए वध–मंच की तरह उपयोग किया जाता है। इनके व्युत्पन्न एक अद्वितीय प्रकार का संरचना विनिमय दिखते हैं। इस विनिमय की गति को एन एम आर के द्वारा मापा सकता है। प्रत्येक तापमान पर मिश्रण समय को बदल के चयनात्मक एवं गैर चयनात्मक चुंबकन उलट उगाही प्रयोगों को हितबद्ध स्थानों पर लागू किया गया। गति स्थिरांक को सिफिट योजना की मदद से प्रयोगों के डेटा को फिट करके मापा गया। अलग अलग तापमान पर पाए गए विनिमय गति स्थिरांकों को उपयोग करते हुए सक्रियण ऊर्जा अवरोध $(\Delta E^{\dagger})$ एवं सक्रियण तापीय धारिता $(\Delta H^{\dagger})$ का पता लगाया गया।

द्वितीय भाग (२) डोजी विधि के लिए अंतर्दृष्टि प्रदान करता है जो की बिसपिडीन के व्युत्पन्न द्वारा पुटिका के बनने का पुष्टीकरण कर सकता है। प्रायः अधिक संकेन्द्रण पर या विलायक के व्यवहार पर ये व्युत्पन्न गोलाकार , बेलनाकार, एवं तलीय आणविक समूह का निर्माण करते हैं। डोजी इन बड़े आणविक समूह की संरचना के अध्ययन में एवं विलायक के अंदर प्रवाह स्थिरांक के निर्धारण में उपयोगी साबित हो सकता है। एक संकेन्द्रण आधारित डोजी को मेथेनोल विलायक के लिए प्रदर्शित किया गया। जैसे जैसे

व्युत्पन्न की संकेंद्रता बढ़ती है वैसे वैसे प्रवाह स्थिरांक का मान कम होता जाता है जो की ये दर्शाता है की अणुओ का विलायक के अंदर गति सीमित होती जा रही है।

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List of symbols

Γ	Relaxation superoperator
γ	Magneto gyric ratio
$\hat{\mathscr{H}}$	Hamiltonian
$\hbar$	Planck's constant
μ	Nuclear magnetic moment
ν	Frequency
ρ	Density operator
τ	Relaxation lifetime or delay
B	Main magnetic field
B_0	Main magnetic field along z-axis
J	Coupling constant
k	Rate constant
k_B	Boltzmann's constant
R	Gas constant
T_1	Longitudinal relaxation lifetime
T_2	Transverse relaxation lifetime