

**DEVELOPMENT OF NMR METHODS USING LONG-LIVED  
STATES AND COHERENCES**

**MANINDER SINGH**



**DEPARTMENT OF CHEMISTRY  
INDIAN INSTITUTE OF TECHNOLOGY DELHI  
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**DEVELOPMENT OF NMR METHODS USING LONG-LIVED  
STATES AND COHERENCES**

by

**MANINDER SINGH**

Department of Chemistry

Submitted

in fulfillment of the requirements of the degree of Doctor of Philosophy

to the



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**Dedicated to my  
Parents**

## Certificate

I certify that the thesis entitled *Development of NMR methods using Long-Lived States and Coherences* submitted by **Mr.Maninder Singh** to Indian Institute of Technology Delhi for the award of **Doctor of Philosophy** is a record of bona fide research work done by him. He has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis which to my knowledge has reached the requisite standard. The results presented in this thesis have not been submitted in part or full to any other University or Institute for the award of any degree or diploma.

**Dr.Narayanan D. Kurur**

**(Professor)**

Department of Chemistry

Indian Institute of Technology Delhi

New Delhi 110016

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**Maninder Singh**

# Abstract

As Nuclear Magnetic Resonance (NMR) has evolved over the last few decades, it has become one of the most versatile analytical techniques for molecular structure and dynamics determination. The principal factor contributing to all utilities of NMR is the relative long lifetime, compared to all other forms of spectroscopies, of nuclear spin order. Thus, methods that further increase the lifetime of nuclear spins are of great interest. This thesis mainly focuses on further exploring methods like Long-Lived States (LLS) and Coherences (LLC) to extend the nuclear spins lifetime much beyond the Zeeman magnetization. Besides, some more efficient methods are developed for measurement of LLS and LLC under various sample or experimental conditions which were restricted by earlier methods.

This thesis is composed of five chapters. First chapter is the introductory chapter where the basic tools such as angular momentum, operators, Hamiltonians, density matrix and product operator formalism are defined that are necessary for understanding the theory of the following chapters. Besides, the theory behind the creation of LLS and LLC in a coupled two spin system is explained which are the central topics of this thesis. And finally, the chapter ends with showing the organization of the rest of the thesis.

It is well known that the phenomenon of nuclear relaxation plays a prominent role in various applications of NMR especially in MRI. Thus, it is quite interesting to study the behaviour of both LLS and LLC in the presence of paramagnetic ions. In chapter 2, we have studied the relaxation behavior of LLC in the presence of paramagnetic species like  $\text{Cu}^{2+}$  and  $\text{Mn}^{2+}$  ions. The molecule, 2,3,6-trichlorobenzaldehyde, containing a pair of coupled aromatic protons is used as a test molecule for LLC excitation in this study. The effect of paramagnetic ions on the relaxation

rates is quantized by external random field (ERF) model using random field fluctuations of the partially correlated local fields acting on the nuclear sites. We observed that in presence of paramagnetic substances, the rate of relaxation of the LLC's is faster than transverse relaxation for both  $\text{Cu}^{2+}$  and  $\text{Mn}^{2+}$ . Besides, the external random fields acting at the two nuclear sites (i.e. at two coupled protons) are found to be strongly correlated with each other.

The chapter 3 describes an improved technique for fast and reliable measurement of lifetimes of long-lived coherences (LLC's) in solution NMR. The normal procedure to obtain LLC relaxation time is lengthy, laborious and may contain fitting errors. Also, the method may prove to be inefficient for *in vivo* studies when changes that occur at faster rate than total required experimental time are monitored. We report here a new pulse sequence to monitor the relaxation of LLC as an exponentially decaying function free from any type of modulation. This method works perfectly for weakly coupled two spin system at Boltzmann equilibrium. In contrast, for strongly coupled spin system, the method is imperfect. The utility of the new sequence is demonstrated in a weakly coupled two spin-1/2 systems, the nucleotide Uridine-5'-monophosphate (UMP) and 2,3,6-trichlorobenzaldehyde (TCB). The signal in this method is a pure exponential in contrast with the existing method which has decaying oscillatory signal. Hence, only a few experiments are required which effectively reduces the experimental time from several hours to few minutes. Simulations also reveal the same results as observed experimentally.

So far, LLS has been observed in homogeneous magnetic fields, which precludes applications to many biological samples that are inherently inhomogeneous. Thus in chapter 4, we present a method for the measurement of lifetimes of LLS in inhomogeneous field. The method combines established sequences for the excitation of LLS with their conversion into LLC followed by windowed acquisition. The method is applied to a pair of diastereotopic scalar-coupled protons

in a solution of the dipeptide Alanine-Glycine (Ala-Gly). The observations were made both in homogeneous and inhomogeneous static magnetic fields. The values of LLS lifetimes obtained are nearly same, i.e.,  $T_{LLS} = 22.4 \pm 0.7$  s in a homogeneous field and  $T_{LLS} = 21.1 \pm 0.6$  s in an inhomogeneous field. The method may find applications in measuring LLS in NMR in gas phase, *in-cellulo* NMR, *in-vivo* MRI and in other studies where static field is compromised.

The last chapter 5 is about using LLS and LLC in relaxation editing in NMR. The range of dispersion of  $T_1$ ,  $T_2$  and  $T_{1\rho}$  within a molecule is unfortunately restricted from some *ms* to few seconds. Any enhancement in this range of distribution of lifetimes further for effective suppression of signals facilitating signal assignment and characterization is welcome. We report here a new strategy in this direction. LLS and LLCs are utilized here to enhance the dispersion of lifetimes of nuclei in molecules much more than any earlier reported methods. The characteristic range of  $T_{LLS}$  or  $T_{LLC}$  values within the sample containing a mixture of components or components within a large macromolecule can be exploited in spectral edited NMR experiments for the stepwise filtering of complicated spectra. The resulting spectra benefits from reduced signal overlap unravelling signal assignment and characterization. The LLS and LLC filtering strategies are demonstrated in weakly and strongly coupled spin systems separately which is extended to molecules containing two or three pairs of weakly and strongly coupled spins. The potential of these strategies was further substantiated by an application to metabolomic mixture and several 1D and 2D NMR experiments (INEPT, COSY and HSQC). A comparison is also made with earlier methods of relaxation editing using relaxation time  $T_1$  and  $T_2$ .

## सामान्य सारांश

जिस प्रकार नाभिकीय चुंबकीय अनुनाद (एनएमआर) पिछले कुछ दशकों से विकसित हुआ है, यह आणविक संरचना और गतिशीलता निर्धारण के लिए सबसे बहुमुखी विश्लेषणात्मक तकनीकों में से एक बन गया है। एनएमआर की सभी उपयोगिताओं में योगदान करने वाला प्रमुख कारक, नाभिकीय स्पिन ऑर्डर का अन्य सभी प्रकार के स्पेक्ट्रोस्कोपी की तुलना में लंबा जीवनकाल है। अतः वर्तमान काल में जो विधियाँ, एनएमआर के जीवनकाल में पुनः वृद्धि करती हैं, रूचि का केन्द्र बन चुकी हैं। यह शोध-प्रबन्ध मुख्य रूप से जीमन मैग्नेटाईसेशन के परे नाभिकीय स्पींस को जीवनकाल के विस्तार के लिए लॉग-लिब्ड स्टेट्स (एलएलएस) और कोहेरेन्सेस (एलएलसी) जैसे अन्य तरीकों की खोज करने पर केंद्रित है। इसके अलावा, एलएलएस और एलएलसी के विभिन्न नमूने या प्रयोगात्मक परिस्थितियों में माप के लिए कुछ और प्रभावी तरीके विकसित किए गए हैं, जिन्हें पहले के तरीकों से प्रतिबंधित किया गया था।

यह शोध-प्रबन्ध पांच अध्यायों से बना है, पहला अध्याय प्रारंभिक अध्याय है जहां मूल उपकरणों जैसे कोणीय संवेग ऑपरेटर, हैमिल्टोनियन, घनत्व मैट्रिक्स और उत्पाद ऑपरेटर की औपचारिकता परिभाषित की गई हैं, जो निम्नलिखित अध्यायों के सिद्धांत को समझने के लिए आवश्यक है। इसके अलावा एक युग्मित दो स्पिन सिस्टम में एलएलएस और एलएलसी के निर्माण के पीछे के सिद्धांत को समझाया गया है जो इस शोध-प्रबन्ध का केंद्रीय विषय हैं। अन्ततः यह अध्याय शोध-प्रबन्ध के संगठन को प्रदर्शित करने के साथ समाप्त होता है।

यह अच्छी तरह से ज्ञात है कि नाभिकीय रिलेक्सेशन की घटना एनएमआर के विभिन्न अनुप्रयोगों में, विशेष रूप से एमआरआई में एक प्रमुख भूमिका निभाती है। इस प्रकार, पैरामैग्नेटिक आयनों की उपस्थिति में एलएलएस और एलएलसी दोनों के व्यवहार का अध्ययन करना काफी रुचिकर है। अध्याय 2 में, हमने  $\text{Cu}^{2+}$  और  $\text{Mn}^{2+}$  आयनों जैसे पैरामैग्नेटिक प्रजातियों की उपस्थिति में एलएलसी के विश्राम व्यवहार का अध्ययन किया है। इस अध्ययन में एलएलसी निर्माण हेतु अणु, 2,3,6-ट्राइक्लोरोबेंज़ाल्डहाइड के युग्मित एरोमैटिक प्रोटॉन की एक जोड़ी का प्रयोग किया गया है। नाभिक पर कार्य करने वाले आंशिक रूप से सहसंबद्ध स्थानीय क्षेत्रों के यादृच्छिक क्षेत्र में अस्थिरता का प्रयोग करने वाले बाहरी यादृच्छिक क्षेत्र (एआरएफ) मॉडल के द्वारा एलएलसी रिलेक्सेशन दर पर पैरामैग्नेटिक आयनों के प्रभाव को परिमाणित किया गया है। हमने पाया है कि पैरामैग्नेटिक पदार्थों,  $\text{Cu}^{2+}$  और  $\text{Mn}^{2+}$  की उपस्थिति में, एलएलसी की रिलेक्सेशन की दर अनुप्रस्थ रिलेक्सेशन की

दर से तेज है। इसके अतिरिक्त यह भी पाया गया कि दोनो युग्मित नाभिकीय स्थलों पर कार्य करने वाले बाहरी यादृच्छिक क्षेत्र दृढ़तापूर्वक सहसम्बद्ध हैं।

अध्याय 3 में एलएलसी के जीवन काल के परिमाणन हेतु एक शीघ्र एवम् विश्वसनीय तकनीक का विवरण दिया गया है। एलएलसी रिलेक्सेशन-समय प्राप्त करने की सामान्य प्रक्रिया लंबी, श्रमसाध्य है और इसमें फिटिंग त्रुटियां हो सकती हैं। इसके अलावा सामान्य विधि in vivo अध्ययनों में, जहाँ पर परिवर्तन सम्पूर्ण प्रयोगात्मक समय से पूर्व हो रहा हो, अप्रभावी सिद्ध हो सकता है। हम यहां एलएलसी रिलेक्सेशन के निरीक्षण हेतु एक नई पल्स अनुक्रम का विवरण दे रहे हैं जो कि किसी भी प्रकार के मॉड्यूलन के बिना सामान्यतः घातांकीय क्षयकारी फलन से फिट किया जा सकता है। बोल्टज़मैन संतुलन में यह विधि कमजोर युग्मित दो स्पिन सिस्टम के लिए पूरी तरह से काम करती है। इसके विपरीत, दृढ़तापूर्वक युग्मित स्पिन सिस्टम के लिए यह विधि अपूर्ण है। नई अनुक्रम की उपयोगिता एक कमजोर युग्मित दो स्पिन-1/2 प्रणालियों, न्यूक्लियोटाइड यूरिडीन -5'-मोनोफोस्फेट (यूएमपी) और 2,3,6-ट्राइक्लोरोबेंज़ाल्डहाइड (टीसीबी) में प्रदर्शित होती है। यह पद्धति विद्यमान विधि, जो कि क्षयकारी दोलनीय संकेत है, के विपरीत एक शुद्ध घातांक है। इसलिए केवल कुछ प्रयोगों की आवश्यकता होती है जो प्रायोगिक समय को प्रभावी ढंग से कई घंटे से कुछ मिनट तक कम कर देता है। सिमुलेशन प्रयोगात्मक रूप से बनाए गए समान परिणामों को भी प्रकट करते हैं।

अब तक, एलएलएस को सजातीय चुंबकीय क्षेत्र में देखा गया है, जो कि कई जैविक नमूनों के परीक्षण के लिए अप्रभावी है। इस प्रकार अध्याय 4 में हम एलएलएस के जीवनकाल को विजातीय चुंबकीय क्षेत्र में नापने की विधि प्रस्तुत करते हैं। यह विधि एलएलएस को बनाने के लिए एलएलसी में अपने रूपांतरण के साथ स्थापित किए गए अनुक्रमों को जोड़ती है, जो कि विंडोड अधिग्रहण के बाद होता है। डायपेटाइड एलनिन-ग्लाइसीन (एला-ग्लाइ) के समाधान में डायस्टेरेयोटोऑपिक स्केलर-युग्मित प्रोटॉन की एक जोड़ी के लिए विधि लागू की गई है। यह अवलोकन सजातीय एवं विजातीय स्थैतिक चुंबकीय क्षेत्रों में किये गये। प्राप्त एलएलएस जीवन काल के मान लगभग समान हैं,  $T_{LLS} = 22.4 \pm 0.7$  एक सजातीय क्षेत्र में और  $T_{LLS} = 21.1 \pm 0.6$  है जो एक विजातीय क्षेत्र में हैं। यह विधि गैसीय अवस्था में एलएलएस को मापने में, इन-सेल्युलो एनएमआर में, इन-विवो एमआरआई में और अन्य क्षेत्रों में जहां स्टैटिक फील्ड से समझौता किया गया है, में प्रभावी सिद्ध हो सकती है।

आखिरी अध्याय 5, एनएमआर में रिलेक्सेशन संपादन में एलएलएस और एलएलसी का उपयोग करने के विषय में है। अणुओं में  $T_1$ ,  $T_2$  और  $T_{1\rho}$  की सीमा कुछ मिसे० से कुछ सेकंड तक सीमित होती है। इस समय सीमा में वृद्धि कर संकेत अभिहस्तांकन में सुविधा पहुँचाने हेतु किसी भी विधि

का उपयोग स्वागतयोग्य है। हम यहां इस दिशा में एक नई रणनीति प्रस्तुत कर रहे हैं। एलएलएस और एलएलसी का उपयोग यहां अणुओं में नाभिक के जीवन काल के फैलाव को बढ़ाने के लिए किया जाता है।  $T_{LLS}$  या  $T_{LLC}$  के विलक्षण परास मानों का उपयोग मिश्रित घटकों या विशाल अणुओं के घटकों के जटिल स्पेक्ट्रा के चरणबद्ध निस्पंदन में किया जा सकता है। परिणामतः स्पेक्ट्रा अधिव्यापन की कमी से संकेत अभिहस्तांकन और निस्पण को अत्यन्त सुविधाजनक बनाया गया है। एलएलएस और एलएलसी फ़िल्टरिंग रणनीतियों को कमजोर और दृढ़तापूर्वक युग्मित स्पिन सिस्टम में प्रदर्शित किया गया है। इस विधि को पुनः ऐसे अणुओं पर उपयोग किया गया जिनमें कमजोर एवं दृढ़तापूर्वक युग्मित स्पिन सिस्टम एकसाथ उपस्थित हैं। इन रणनीतियों की क्षमता को मेटाबोलोमिक मिश्रण और कई 1 डी और 2 डी एनएमआर प्रयोगों (INEPT, COZY और एचएसक्यूसी) द्वारा आगे बढ़ाया गया। विश्राम अवधि  $T_1$  और  $T_2$  का उपयोग करके रिलेक्सेशन संपादन के पहले तरीकों से भी तुलना की गई है।

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