
THE NUCLEAR OVERHAUSER EFFECT IN STRUCTURAL AND CONFORMATIONAL ANALYSIS

Second Edition

Methods in Stereochemical Analysis

Series Editor

Alan P. Marchand, Denton, Texas, USA

Advisory Board

- A. Greenberg, Charlotte, North Carolina, USA
I. Hargittai, Budapest, Hungary
A. R. Katritzky, Gainesville, Florida, USA
J. F. Liebman, Baltimore, Maryland, USA
E. Lippmaa, Tallinn, Estonia
L. A. Paquette, Columbus, Ohio, USA
P. von R. Schleyer, Athens, Georgia, USA
S. Sternhell, Sydney, Australia
Y. Takeuchi, Tokyo, Japan
F. Wehrli, Philadelphia, Pennsylvania, USA
D. H. Williams, Cambridge, UK
N. S. Zefirov, Moscow, Russia

Other Books in the Series:

Motohiro Nishio, Miroru Hirota, and Yoji Umezawa

The CH/ π Interaction: Evidence, Nature, and Consequence

David A. Lightner and Jerome E. Gurst

Organic Conformational Analysis and Stereochemistry from Circular Dichroism Spectroscopy

Jacek Waluk

Conformational Analysis of Molecules in Excited States

Eiji Ōsawa and Osamu Yonemitsu

Carbocyclic Cage Compounds: Chemistry and Applications

Janet S. Splitter and Frantisek Turecek (editors)

Applications of Mass Spectrometry to Organic Stereochemistry

William R. Croasmun and Robert M. K. Carlson (editors)

Two-dimensional NMR Spectroscopy: Applications for Chemists and Biochemists. Second Edition

Jenny P. Glusker with Mitchell Lewis and Miriam Rossi

Crystal Structure Analysis for Chemists and Biologists

Kalevi Pihlaja and Erich Kleinpeter

Carbon-13 NMR Chemical Shifts in Structural and Stereochemical Analysis

Louis D. Quin and John G. Verkade (editors)

Phosphorus-31 NMR Spectral Properties in Compound Characterization and Structural Analysis

Eusebio Juaristi (editor)

Conformational Behavior of Six-Membered Rings: Analysis, Dynamics and Stereoelectronic Effects

THE NUCLEAR OVERHAUSER EFFECT IN STRUCTURAL AND CONFORMATIONAL ANALYSIS

Second Edition

David Neuhaus
Michael P. Williamson



A John Wiley & Sons, Inc., Publication

New York / Chichester / Weinheim / Brisbane / Singapore / Toronto

This book is printed on acid-free paper. ∞

Copyright © 2000 by Wiley-VCH, Inc. All rights reserved.

Published simultaneously in Canada.

No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, scanning or otherwise, except as permitted under Sections 107 or 108 of the 1976 United States Copyright Act, without either the prior written permission of the Publisher, or authorization through payment of the appropriate per-copy fee to the Copyright Clearance Center, 222 Rosewood Drive, Danvers, MA 01923, (978) 750-8400, fax (978) 750-4744. Requests to the Publisher for permission should be addressed to the Permissions Department, John Wiley & Sons, Inc., 605 Third Avenue, New York, NY 10158-0012, (212) 850-6011, fax (212) 850-6008, E-Mail: PERMREQ @ WILEY.COM.

Library of Congress Cataloging-in-Publication Data:

Neuhaus, David, 1956–

The nuclear Overhauser effect in structural and conformational analysis / David Neuhaus and Michael P. Williamson.—2nd ed.

p. cm. — (Methods in stereochemical analysis)

Includes bibliographical references and index.

ISBN 0-471-24675-1 (alk. paper)

1. Conformational analysis. 2. Overhauser effect (Nuclear physics) I. Williamson, Michael P., 1957– II. Title. III. Series.

QD481 .N46 2000

541.2'23—dc21

99-049630

Printed in the United States of America.

10 9 8 7 6 5 4 3 2 1

CONTENTS

PREFACE	xv
ACKNOWLEDGMENTS	xvii
PREFACE TO THE FIRST EDITION	xix
SYMBOLS, ABBREVIATIONS, AND UNITS	xxi
INTRODUCTION	xxv
 PART I. THEORY	 2
CHAPTER 1. BACKGROUND	3
1.1. Energy Levels, Populations, and Intensities / 3	
1.2. Relaxation, T_1 and T_2 / 8	
1.3. The Nature of Relaxation / 12	
1.4. The Local Field and Dipole–Dipole Relaxation / 13	
1.5. Pulses and Saturation / 15	
References / 22	

CHAPTER 2. THE STEADY-STATE NOE FOR TWO SPINS**23**

- 2.1. The Origin and Form of the NOE / 23
 - 2.1.1. Qualitative Considerations / 23
 - 2.1.2. The Solomon Equations / 27
- 2.2. Dependence of the NOE on Molecular Motion / 30
 - 2.2.1. Correlation Times, Spectral Density Functions, and Transition Probabilities / 31
 - 2.2.2. Anisotropic Tumbling / 38
- 2.3. What the Symbols Mean for Two Spins and for Many Spins / 38
 - 2.3.1. Relaxation Rates / 39
 - 2.3.2. T_1 Measurements and Cross-Relaxation / 42
- 2.4. Effects of Other Relaxation Sources / 46
 - 2.4.1. The External Relaxation Rate ρ^* / 46
 - 2.4.2. Intermolecular Dipole–Dipole Relaxation / 50
 - 2.4.3. Quadrupolar Relaxation / 52
 - 2.4.4. Chemical Shift Anisotropy (CSA) Relaxation / 53
 - 2.4.5. Scalar Relaxation / 53
 - 2.4.6. Spin–Rotation Relaxation / 53
- 2.5. The Heteronuclear NOE / 54
- 2.6. An Extension to the Solomon Equations / 59
 - References / 60

CHAPTER 3. THE STEADY-STATE NOE IN RIGID MULTISPIN SYSTEMS**62**

- 3.1. The Equations / 63
 - 3.1.1. The Solomon Equations for More Than Two Spins / 63
 - 3.1.2. Cross-Correlation / 66
 - 3.1.3. Two General Solutions to the Multispin Solomon Equations / 68
 - 3.1.3.1. Saturation of One Spin / 68
 - 3.1.3.2. Saturation of All Spins Except One / 69
 - 3.1.4. Internuclear Distances and Steady-State NOE Enhancements / 71
- 3.2. What the Equations Mean / 72
 - 3.2.1. General: Direct Enhancements and Spin Diffusion / 72
 - 3.2.2. Interpretation at the Extreme Narrowing Limit ($\omega\tau_c \ll 1$) / 76

3.2.2.1.	Direct Effects / 76
3.2.2.2.	Indirect Effects / 79
3.2.2.3.	When Do Indirect Effects Matter? / 80
3.2.2.4.	Magnetic Equivalence / 82
3.2.2.5.	T_1 and the 3/2 Effect / 84
3.2.2.6.	Chemical Equivalence / 85
3.2.3.	Away from the Extreme Narrowing Limit / 86
3.3.	In Practice / 91
3.3.1.	Incomplete Saturation / 92
3.3.2.	Failure to Reach Steady-State / 93
3.3.3.	Competition From Other Relaxation Sources / 94
	References / 97

CHAPTER 4. THE KINETICS OF THE NOE

98

4.1.	Introduction / 98
4.1.1.	Types of Kinetic NOE Experiment / 99
4.1.2.	Overview / 100
4.2.	Theory of the Kinetic NOE in a Two-Spin System / 105
4.3.	Theory of the Kinetic NOE in Multispin Systems / 108
4.3.1.	Multispin Kinetics in Transient NOE Experiments / 108
4.3.2.	Multispin Kinetics in TOE Experiments / 111
4.4.	Estimating Internuclear Distances / 111
4.4.1.	The Initial Rate Approximation / 112
4.4.2.	Distances From Enhancement Ratios / 113
4.4.3.	Errors in Distance Measurements Using the Initial Rate Approximation / 115
4.4.4.	Spin Diffusion, Nonlinear NOE Growth, and Interpretation / 117
4.5.	More About Experiments / 122
4.5.1.	Symmetry in Kinetic NOE Experiments / 122
4.5.2.	T_1 Values as an Aid to Interpretation / 124
	References / 127

CHAPTER 5. THE EFFECTS OF EXCHANGE AND INTERNAL MOTION

129

5.1.	Transfer of Saturation / 131
5.2.	General Equations for the NOE in Systems of Two-Site Exchange / 136

5.2.1.	Exchange in a Two-Spin System /	136
5.2.2.	Exchange in Dimethylformamide /	143
5.3.	Applications to More Complicated Cases of Exchange /	148
5.3.1.	Averaging of Rates Rather than Enhancements /	148
5.3.2.	Analyzing Conformational Equilibria /	150
5.3.2.1.	Olefinic Methoxy Conformations /	150
5.3.2.2.	Nucleotide Conformations /	151
5.3.2.3.	A Statistical Approach /	156
5.4.	Estimating Flexibility Using Heteronuclear Relaxation Analysis /	158
5.5.	How Internal Motions Average Internuclear Distances /	167
5.5.1.	Internal Motions Slower Than Overall Tumbling: “ r^{-6} Averaging” /	171
5.5.2.	Internal Motions Faster Than Overall Tumbling /	172
5.6.	Allowing for Averaging /	174
5.7.	The Transferred NOE /	178
5.8.	Intermolecular NOE Enhancements Involving Water /	185
	References /	187

CHAPTER 6. COMPLICATIONS FROM SPIN-SPIN COUPLING **190**

6.1.	Decoupling /	190
6.2.	Selective Population Transfer /	191
6.2.1.	Theory /	192
6.2.2.	Consequences /	199
6.3.	Effects of Cross-Correlation /	201
6.4.	Strong Coupling /	204
6.4.1.	$A\{B\}$ Enhancements /	206
6.4.2.	$AB\{X\}$ Enhancements /	206
6.4.3.	Scalar Relaxation /	213
6.4.3.1.	Scalar Relaxation of the First Kind /	213
6.4.3.2.	Scalar Relaxation of the Second Kind /	216
	References /	217

PART II. EXPERIMENTAL **219**

CHAPTER 7. ONE-DIMENSIONAL EXPERIMENTS **221**

7.1.	Sample Preparation /	221
7.1.1.	Solvent /	221
7.1.2.	Solute Concentration /	224
7.1.3.	Sample Purification /	225

7.2.	Setting Up the Steady-State Difference Experiment /	227
7.2.1.	Introduction to the Difference Experiment /	227
7.2.2.	Minimizing Subtraction Artifacts /	230
7.2.3.	Automatic Multiple Experiments /	234
7.2.4.	Irradiation Power and Selectivity /	235
7.2.5.	Multiplet Irradiation and SPT Suppression /	240
7.2.6.	Timing /	243
7.3.	Processing, Display, and Calculation of Results /	247
7.3.1.	General /	247
7.3.2.	Reference Deconvolution /	249
7.4.	Other 1D Experiments Employing Continuous Saturation /	254
7.4.1.	CW Steady-State Integration /	254
7.4.2.	The Truncated Driven NOE (TOE) Experiment /	255
7.5.	Transient Experiments /	258
7.5.1.	Selective Pulses /	259
7.5.2.	Non-Gradient Transient NOE Experiments /	260
7.5.3.	Gradient-Assisted Transient NOE Experiments /	261
7.5.3.1.	Gradient Selection /	262
7.5.3.2.	DPFGSE-NOE /	264
7.5.3.3.	GOESY /	267
7.5.3.4.	Variations /	272
7.5.3.5.	Applications and Practicalities /	274
7.5.4.	Doubly Selective Experiments /	276
	References /	279

CHAPTER 8. THE TWO-DIMENSIONAL NOESY EXPERIMENT 282

8.1.	One Dimension or Two? /	282
8.1.1.	The Negative NOE Regime ($\omega\tau_c > 1.12$) /	283
8.1.2.	The Positive NOE Regime ($\omega\tau_c < 1.12$) /	283
8.2.	Basic Principles /	285
8.3.	Acquiring a NOESY Spectrum /	293
8.3.1.	Fixed Delays and Pulse Widths /	294
8.3.2.	Acquisition Times t_1 and t_2 and Spectral Widths SW_1 and SW_2 /	296
8.3.3.	Quadrature Detection in F_1 and F_2 /	299
8.3.4.	Phase-Sensitive NOESY /	304
8.4.	Phase-Cycling, Signal Selection, and Artifact Suppression /	307
8.4.1.	Rejection of Nonlongitudinal Contributions During τ_m : J -Peak Suppression /	309

- 8.4.2. Other Forms of *J*-Peaks: Zero Quantum Coherences and Pulse Angle Effects / 310
- 8.4.3. Axial Peaks / 313
- 8.4.4. Quadrature Images / 313
- 8.4.5. t_1 Noise / 314
- 8.5. Data Processing / 316
 - 8.5.1. Zero Filling / 317
 - 8.5.2. Window Functions and Linear Prediction / 318
 - 8.5.3. Symmetrization and t_1 Noise Removal / 320
 - 8.5.4. Integration / 321
- 8.6. Variations / 322
 - 8.6.1. Semi-Selective and Network-Edited Experiments / 322
 - 8.6.2. Other Variants / 327
- References / 328

CHAPTER 9. OTHER DEVELOPMENTS

331

- 9.1. Heteronuclear NOE Enhancements / 331
 - 9.1.1. Non-Specific Heteronuclear NOE Experiments / 331
 - 9.1.2. Specific Heteronuclear NOE Experiments / 335
- 9.2. Editing and Spectral Simplification of NOE Experiments / 341
 - 9.2.1. Editing Using the NOE Itself / 341
 - 9.2.2. Editing Using Heteronuclear Scalar Couplings / 346
 - 9.2.2.1. X-Filtered NOE Experiments / 347
 - 9.2.2.2. X-Separated NOE Experiments / 350
 - 9.2.3. Homonuclear Three-Dimensional NOE Experiments / 359
 - 9.2.4. Editing Using Something Else / 362
- 9.3. Rotating Frame NOE Experiments / 364
 - 9.3.1. Theory / 365
 - 9.3.1.1. Spin-Locking / 365
 - 9.3.1.2. Spin-Locked Transverse Dipole-Dipole Relaxation / 367
 - 9.3.1.3. Other Effects During Spin-Locking / 371
 - 9.3.2. Practicalities / 379
- 9.4. Manipulation of $\omega\tau_c$ / 382
- References / 384

PART III. APPLICATIONS **389****CHAPTER 10. APPLICATIONS OF THE NOE TO
STRUCTURE ELUCIDATION** **391**

- 10.1. General Considerations / 391
 - 10.1.1. Why Structural and Conformational Problems Are the Same / 391
 - 10.1.2. Spectra and Assignments / 393
 - 10.1.3. Reporting Results and Interpretation / 395
 - 10.1.4. Miscellaneous / 397
- 10.2. Aromatic Substitution and Ring Fusion Patterns: Simple Cases / 398
- 10.3. Aromatic Substitution and Ring Fusion Patterns: More Complex Cases / 406
 - 10.3.1. Petroporphyrins / 407
 - 10.3.2. Isoquinoline and Related Alkaloids / 409
- 10.4. Double Bond Isomers / 415
- 10.5. Saturated Ring Systems: Simple Cases / 420
 - 10.5.1. Substituent Stereochemistry / 422
 - 10.5.2. Ring Fusion Stereochemistry / 432
- 10.6. Saturated Ring Systems: More Complex Cases / 442
 - 10.6.1. Pulvomycin / 444
 - 10.6.2. Penitrem A / 447
 - 10.6.3. Other Examples / 450
- References / 453

**CHAPTER 11. APPLICATIONS OF THE NOE TO
CONFORMATIONAL ANALYSIS** **456**

- 11.1. General Considerations / 456
 - 11.1.1. Why Structural and Conformational Problems are Different / 456
 - 11.1.2. Multiple Conformations / 458
- 11.2. Local Conformational Detail in Small Molecules / 459
 - 11.2.1. Slowly Exchanging Equilibria / 461
 - 11.2.2. Rapidly Exchanging Equilibria: A Hypothetical Example, X-CH₂OH / 461
 - 11.2.3. Rapidly Exchanging Equilibria: Real Examples / 463
- 11.3. Conformational Analysis of Medium-Sized Molecules / 472
- References / 484

CHAPTER 12. CALCULATING STRUCTURES OF BIOPOLYMERS 485

- 12.1. Introduction / 485
- 12.2. Restraints / 486
 - 12.2.1. Assigning NOE Restraints / 487
 - 12.2.1.1. Using Only (^1H , ^1H) NOESY Data / 488
 - 12.2.1.2. Using Preliminary Structural Data / 489
 - 12.2.1.3. Using Heteronuclear Labeling / 490
 - 12.2.2. Measuring NOE Restraints / 491
 - 12.2.3. Calibrating NOE Restraints / 493
 - 12.2.4. Averaging in Equivalent Groups / 499
 - 12.2.4.1. Pseudoatom Corrections / 500
 - 12.2.4.2. Multiplicity Corrections / 503
 - 12.2.4.3. r^{-6} Summation / 506
 - 12.2.5. Stereoassignments and Torsion Angle Restraints / 506
 - 12.2.6. Other Types of Restraints / 511
- 12.3. Calculating Structures / 515
 - 12.3.1. Distance Geometry Calculations / 517
 - 12.3.1.1. The Exact Case / 518
 - 12.3.1.2. Distance Geometry Applied to NMR Structure Determination / 519
 - 12.3.1.3. Distance Geometry in Torsion Angle Space / 522
 - 12.3.2. Restrained Molecular Dynamics Calculations / 523
 - 12.3.3. Simulated Annealing Calculations / 527
 - 12.3.4. Other Methods / 529
- 12.4. Assessing and Describing NMR Structures / 530
 - 12.4.1. Global Precision: Overall Root Mean Squared Deviations / 531
 - 12.4.2. Local Precision: Local RMSD and Angular Order Parameters / 533
 - 12.4.3. Assessing the Quality of Structures / 536
- 12.5. Refinement / 540
 - 12.5.1. General / 541
 - 12.5.2. Specific Protocols for Refinement / 543
- References / 546

CHAPTER 13. BIOPOLYMERS**550**

- 13.1. Peptides and Proteins / 550
 - 13.1.1. Assignment: Heteronuclear Methods / 554

13.1.2.	Assignment: (^1H , ^1H) NOE-Based Methods /	555
13.1.3.	Structure Determination of Protein Monomers /	556
13.1.3.1.	Small Rigid Unlabeled Proteins /	556
13.1.3.2.	Larger Rigid Labeled Proteins /	558
13.1.3.3.	Conformationally Mobile Proteins /	560
13.1.4.	Structure Determination of Symmetric Protein Oligomers /	562
13.1.5.	Through-Space Connections by Solid-State NMR Experiments with Proteins /	563
13.2.	Polynucleotides /	566
13.2.1.	Structures and Conformations /	567
13.2.2.	Assignment /	574
13.2.2.1.	Duplex DNA /	574
13.2.2.2.	RNA /	576
13.2.2.3.	Other Nucleotides /	577
13.2.3.	Structure Calculation /	577
13.2.3.1.	Sequence Dependent Conformation in Duplexes /	577
13.2.3.2.	Non-Helical Conformations /	581
13.3.	Oligosaccharides /	583
13.3.1.	Sequence and Linkage Determination /	584
13.3.2.	Conformation /	585
13.4.	Complexes /	587
13.4.1.	Drug–Protein Complexes /	589
13.4.2.	Drug–DNA Complexes /	589
13.4.3.	Protein–Nucleic Acid Complexes /	590
	References /	592

APPENDIX I. EQUATIONS FOR ENHANCEMENTS INVOLVING GROUPS OF EQUIVALENT SPINS	596
--	------------

APPENDIX II. QUANTUM MECHANICS AND TRANSITION PROBABILITIES	599
--	------------

INDEX	611
--------------	------------

PREFACE

When we completed the first edition of this book, just over 10 years ago, we did not expect to undertake rewriting it for a long time to come. It is certainly true that the theory behind the NOE has altered little in the last 10 years (as reflected by the fact that Chapters 1, 2, 3, and 6 are largely unchanged). However, there has continued to be a very rapid growth in the range of applications, particularly in the macromolecular area.

To a large extent, this has dictated the changes we have made to the book. The biggest change from the first edition is the inclusion of a completely new chapter (Chapter 12), which deals with the way NOE enhancements are used to calculate structures of biomolecules. Chapter 13, which discusses applications of the NOE to calculations of macromolecular structures, has been largely rewritten. Much the same is true for Chapter 4, which deals with the kinetics of the NOE; although the theory is largely the same, we have now placed much more emphasis on transient NOE experiments (such as 2D and gradient-selected experiments), which have seen a major increase in use over the last 10 years.

Interest in internal motions in macromolecules was the main driving force behind the changes we have made to Chapter 5 (which also includes a new section on NOE enhancements involving water, and a major update on the transferred NOE), and a large number of new approaches have been incorporated into Chapters 7 (particularly gradient enhanced experiments) and 8 (network editing). Chapter 9 (“Other Developments”) remains the home for “miscellany” and contains a lot of new material, much of it concerned with the new experiments that became available in the wake of isotope labeling.

We have not significantly changed the content of the chapters dealing with applications to small molecules (Chapters 10 and 11), partly because neither

of us is still active in that field, but more importantly because we feel the examples we gave in the first edition still represent a balanced selection that demonstrates coherently what is usefully possible. It is true that some types of structural problem are now more frequently solved by other means (e.g., positional isomers on aromatic rings might now be more frequently distinguished using long-range ^{13}C – ^1H correlation than by using the NOE), but we felt illustration of the utility of such NOE experiments was still important.

Overall, we have thus broadened the scope of the first edition somewhat, feeling that to exclude (for example) applications of the NOE to relaxation measurements, because it does not relate directly to structure or conformation, was unnecessarily restrictive.

One of the aims we set ourselves in writing this second edition was to make the book shorter. The most casual glance will reveal that we have achieved the opposite of success in this regard. The reason, unfortunately, is simple: as George Bernard Shaw (or was it Blaise Pascal?) is supposed to have said, “I am sorry to have written you a long letter, but I did not have time to write you a short one.” For this and other reasons, we feel it is safe to predict that there will be no third edition.

David Neuhaus
Michael P. Williamson

ACKNOWLEDGMENTS

We are deeply indebted to Dr. James Keeler, who has made many valuable comments on the manuscript and contributed much to the theory sections. His work on extending aspects of NOE theory remains a major influence on this book. We would also like to thank Dr. Deirdre Hickey for all her invaluable help and encouragement in preparing the manuscript, Prof. Dudley Williams, who instigated the project, Prof. Gareth Morris, for a number of useful observations and figures, and Prof. Ruth Lynden-Bell for her advice on Appendix II. Thanks also to all those who contributed ideas, comments, figures, or data, including (for the second edition) Drs. Mike Bernstein, David Case, Bob Diamond, Bob Dutnall, Philip Evans, Mark Fletcher, Charlie Hoogstraten, Richard Lewis, Beat Meier, Michael Nilges, Carol Post, and John Schwabe and (for the first edition) Andy Derome, Jeremy Everett, Duncan Farrant, Maurice Guéron, Peter Hore, Laurence Kruse, Forrest McKellar, Andrew Lane, Werner Leupin, Jeremy Sanders, Maurice Shamma, Richard Sheppard, Alan Whittle, and David Widdowson, as well as the authors and publishers who gave permission to reproduce published material. We thank all those at Wiley involved in the production of the book, particularly Barbara Goldman and Christine Punzo, both of whom showed patience well beyond the call of duty when waiting for the text, and, subsequently, in coping with all our various requests. Finally, we thank our families for putting up with so many lost evenings and weekends (again!).

PREFACE TO THE FIRST EDITION

In the past ten years a quiet revolution in the applications of NMR to organic chemistry and biochemistry has occurred. One of the main changes has been a great increase in the use of the nuclear Overhauser effect (NOE) to solve structural and conformational problems, which in turn was largely due to experimental developments such as the advent of NOE difference spectroscopy and the two-dimensional NOE experiment (NOESY). In this book we have tried to bring chemists and biochemists who are not NMR specialists up to date with these trends. The book aims to provide readers with sufficient background information to enable them to apply the NOE successfully within their own work, and also assess critically other papers in which the NOE is used to solve structural problems.

Because the NOE is transmitted directly through space, it is uniquely well suited to revealing the spatial arrangements of nuclei within molecules, but its interpretation is particularly vulnerable to the overapplication of inappropriate equations. For this reason, we have tried above all to emphasize a clear understanding of the underlying concepts, since only through an awareness of these can the most useful NOE experiments be devised and interpreted. Unavoidably, we have had to use a certain amount of mathematics to do this, but it is kept to a minimum, consistent with a clear explanation. Perhaps contrary to first appearances, most of the expressions in this book require no more than elementary mathematical skills.

As the title implies, our treatment is deliberately restricted to those aspects of the NOE that are relevant to the study of stereochemistry and conformation; topics such as dipolar interactions in solids, and the use of the NOE to study relaxation phenomena, kinetics, or exchange, have therefore been omitted.

The book is organized into three parts, dealing with theory, experimental practice, and an illustrative overview of applications of the NOE to problems in chemistry and biochemistry.

SI units have been used throughout, as far as possible. This results in occasional numerical factors in some expressions (e.g., the factor $\mu_0/4\pi$, which appears in expressions relating relaxation rates to internuclear distances).

David Neuhaus
Michael P. Williamson

SYMBOLS, ABBREVIATIONS, AND UNITS

Throughout, subscript letters are used to denote spin states and transitions; thus, N_α is the number of molecules in spin state α , and R_I is the spin–lattice relaxation rate of spin I . Superscript letters are used to denote different molecules or conformations; thus, S^a is the signal from spin S in molecules with conformation a, and R_S^b is the spin–lattice relaxation rate of spin S in molecules with conformation b. Vector quantities are written in bold.

ADC	analog-to-digital converter
AQ	acquisition time
$\gamma\mathbf{B}/2\pi$	field strength in Hz
$\gamma\mathbf{B}$	field strength in rad s^{-1}
\mathbf{B}_0	applied magnetic field (tesla)
\mathbf{B}_1	irradiating magnetic field (tesla)
CW	continuous wave
$d_{AB}(i, j)$	distance between proton A in residue i and proton B in residue j in a peptide or protein
D	dwelt time in t_2 (s)
1D	one-dimensional
2D	two-dimensional
3D	three-dimensional
dB	decibels
DG	distance geometry
DPFGSE	double pulsed field gradient spin echo
DQF-COSY	double-quantum filtered 2D correlation spectroscopy
$f_I\{S\}$	fractional NOE enhancement of I on saturating S

FID	free induction decay
FT	Fourier transform
$g(\tau)$	correlation function, usually $\exp(-\tau/\tau_c)$
GOESY	gradient enhanced NOE spectroscopy
h	Planck's constant = 6.626×10^{-34} Js
\hbar	Planck's constant divided by 2π = 1.055×10^{-34} Js
HMQC	heteronuclear multiple quantum correlation (also coherence)
HSQC	heteronuclear single quantum correlation (also coherence)
I	nuclear spin quantum number, <i>or</i> general symbol for a nucleus; in expressions concerning NOE experiments, I generally represents the nucleus at which the enhancement is measured.
I_z	longitudinal component of I magnetization
I_z^0	equilibrium value of I_z
IN	t_1 increment in a 2D experiment (s)
ISPA	isolated spin pair approximation
J	spin–spin coupling constant (Hz)
$J(\omega)$	spectral density function, usually $2\tau_c/(1 + \omega^2\tau_c^2)$
k	Boltzmann's constant = 1.38×10^{-23} J K ⁻¹
M	macroscopic magnetization vector (J T ⁻¹)
M_z	z component of M
M_{xy}	transverse component of M
MAS	magic angle spinning
N_i	number of molecules (population) in state i
n_i	population deviation from equilibrium of state i , namely $N_i - N_i^0$
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser effect correlation spectroscopy
R_I^{DD}	dipolar contribution to the spin–lattice relaxation rate of I , defined as $W_{0IS} + 2W_{1I} + W_{2IS} + \sum_x (W_{0IX} + W_{2IX})$ (cf. Eqs. 2.31 and 3.8) (s ⁻¹)
$R_I \equiv R_{1I}$	longitudinal relaxation rate of $I = R_I^{\text{DD}} + \rho_I^*$, roughly equal to the inverse of the selective T_1 value (cf. Section 2.3.1) (s ⁻¹)
R_2	transverse relaxation rate (s ⁻¹)
r	internuclear distance
r_{Tropp}	effective internuclear distance sensed by the NOE in the presence of rapid internal motion; most commonly used to denote the effective distance to a methyl group (cf. Section 5.5.2)
RF	radiofrequency
rmsd	root mean squared deviation (or difference); usually implies root mean squared <i>atomic</i> deviation when comparing structures
ROE	rotating frame NOE; also known as CAMELSPIN
ROESY	rotating frame 2D NOE spectroscopy
S	general symbol for a nucleus; in expressions concerning NOE experiments, S generally represents the nucleus which is saturated (S can also represent the electron spin quantum number).

Also, generalized order parameter for describing the geometrical extent of internal motions relative to the molecular frame (cf. Section 5.4)

S^2	square of the generalised order parameter (cf. Section 5.4)
S^{ang}	angular order parameter (cf. Section 12.4.2)
S_z	longitudinal component of S magnetization
S_z^0	equilibrium value of S_z
SPT	selective population transfer
SW_1	spectral width in F_1 of a multidimensional experiment (Hz)
SW_2	spectral width in F_2 of a multidimensional experiment (Hz)
SW_3	spectral width in F_3 of a multidimensional experiment (Hz)
t_1	incremented time in 2D experiments; first incremented time in multidimensional experiments
$t_{1 \text{ max}}$	maximum time reached by t_1 in the last increment of a 2D or multidimensional experiment (s)
t_2	detection period in 2D experiments; second incremented time in multidimensional experiments
$t_{2 \text{ max}}$	maximum time reached by t_2 (s)
t_3	detection period in 3D experiments
$t_{3 \text{ max}}$	maximum time reached by t_3 (s)
T	temperature (K)
T_1	normally used to mean $T_1^{\text{nonselective}}$ (see below)
$T_1^{\text{nonselective}}$	nonselective longitudinal relaxation time, obtained by following the course of longitudinal relaxation after a nonselective pulse; approximately given by $(R_l + \sigma_{IS} + \sum_X \sigma_{IX})^{-1}$ (s) (cf. Section 2.3.2)
$T_1^{\text{selective}}$	selective longitudinal relaxation time, obtained by following the course of longitudinal relaxation after a selective pulse; approximately equal to R_l^{-1} (s)
$T_{1\rho}$	spin–lattice relaxation time in the rotating frame (s)
T_2	spin–spin, or transverse, relaxation time (s)
$T_{2\rho}$	spin–spin relaxation time in the rotating frame (s)
T_2^*	decay constant for free precession, which is shorter than T_2 because of inhomogeneous broadening (s)
$T_{2\rho}^*$	decay constant for free precession in the rotating frame (s)
t_D	relaxation delay (s)
TMS	tetramethylsilane
TOCSY	total correlation spectroscopy
TOE	truncated driven NOE
TPPI	time-proportional phase incrementation
TRNOE	transferred NOE
VDW	van der Waals
VT	variable temperature
W	transition probability (s^{-1})

X	general symbol for a nucleus; in expressions concerning NOE experiments in this book, X represents all nuclei other than I and S (when not italicized, X can also refer to a non-hydrogen nucleus)
X_z	longitudinal component of X magnetization
X_z^0	equilibrium value of X_z
α, β	spin states for a spin 1/2 nucleus
γ	gyromagnetic ratio ($\text{rad T}^{-1} \text{s}^{-1}$)
η	viscosity (cP)
η_{max}	maximum enhancement attainable in a two-spin system at a given value of τ_c and ω
μ	magnetic dipole moment (J T^{-1})
μ_0	permeability constant in a vacuum = $4\pi \times 10^{-7} \text{ kg m s}^{-2} \text{ A}^{-2}$
ν	precession rate (Hz)
ν_0	reference frequency of rotating frame (Hz)
ρ_{IS}	direct dipole–dipole relaxation rate between I and S , defined by $\rho_{IS} = W_{0IS} + 2W_{1I} + W_{2IS}$ [Eq. 2.30] (s^{-1})
ρ^*	external spin–lattice relaxation rate (s^{-1})
ρ_1	alternative notation for ρ_{IS} , useful when comparing the NOE with the ROE
ρ_2	direct relaxation rate in the rotating frame (s^{-1})
σ_I	chemical shift tensor for nucleus I (cf. Sections 5.4 and 6.3)
σ_{IS}	cross-relaxation rate between I and S , defined by $\sigma_{IS} = W_{2IS} - W_{0IS}$ (Eq. 2.29) (s^{-1})
σ_1	alternative notation for σ_{IS} , useful when comparing the NOE with the ROE
σ_2	cross-relaxation rate in the rotating frame (s^{-1})
τ_c	rotational correlation time (for overall molecular tumbling) (s)
τ_e	correlation time for an internal motion (cf. Section 5.4) (s)
τ_m	mixing time in a 2D or multidimensional NOE experiment (s)
τ	buildup time in kinetic NOE experiment; also general symbol for a fixed delay other than τ_m (s)
ω_0	Larmor precession frequency due to \mathbf{B}_0 (rad s^{-1})
ω_1	Larmor precession frequency due to \mathbf{B}_1 (rad s^{-1})
$\{ \}$	denotes irradiated spin
$\langle \rangle$	denotes average or expectation value <i>or</i> conformational average
\langle , \rangle	Dirac notation for a wave function (ket) and its complex conjugate (bra), respectively

INTRODUCTION

The fundamentals of the NOE were described very early in the history of NMR, in a classic paper by Solomon published in 1955.¹ This paper included the first experimental demonstration of the NOE. Solomon's work followed studies on nuclear spin relaxation by Bloembergen, Purcell, and Pound,² and Overhauser's original prediction that saturation of electrons in a metal would produce a large polarization of the metal nuclear spins.^{3,4}

Solomon's work then lay almost dormant for some years until the advent of double resonance techniques^{5,6} led to the more widespread availability of spectrometers having a decoupler. Papers appeared by Kaiser on distance estimation using the intermolecular $^1\text{H}\{^1\text{H}\}$ NOE,⁷ and by Lauterbur on signal enhancement in ^{13}C spectra.⁸

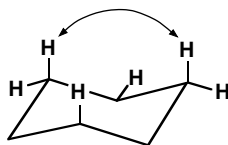
The first paper to demonstrate the power of the NOE in structural problems was by Anet and Bourn in 1965.⁹ The next few years saw a surge in the number of papers reporting applications of the NOE, especially after publication of the book by Noggle and Schirmer.¹⁰ On the CW spectrometers of the day, these experiments involved comparison of integrals to measure steady-state NOE enhancements.

Noggle and Schirmer's book surveyed the field as it stood in 1971, and for some years there was little that needed to be added. There were two major advances in the decade or so following 1971. The first was the application of the NOE to large molecules. In 1972, Balaram et al.¹¹ observed a negative NOE enhancement between a protein and a peptide binding to it, and they showed that negative enhancements are a general property of large molecules. Further applications to large molecules soon revealed the problem of spin diffusion, and the truncated driven NOE (TOE) experiment was suggested by Wagner and Wüthrich¹² as a means of getting around this difficulty.

The second major advance was the introduction of the two-dimensional NOE experiment, largely by Ernst's group. The first use of the experiment to measure NOE enhancements (as opposed to chemical exchange) was published in 1980,^{13,14} and the experiment has come to play a central role in work with biological macromolecules. This area has burgeoned still further with the arrival in the late 1980s of isotopically labeled proteins and the many multidimensional NOE experiments that can be applied to them.

Both these advances could not have had the impact they did without developments in instrumental techniques. The first FT NMR spectrometers became available around 1970, and brought much improved sensitivity. To observe small NOE enhancements, one needs not only sensitivity but also instrumental stability, and this was provided by superconducting magnets, which arrived at the end of the 1970s. The third in this trio of instrumental advances was the advent of dedicated computers, which could control the cycling of decoupler frequencies and gating. A direct result of these changes was the NOE difference experiment, which emerged at about this time. A much more recent development is the use of pulsed field gradients, which have found applications in almost all parts of NMR. Gradients provide little benefit for 2D NOESY experiments, but promise to revolutionize 1D measurements.¹⁵

Although the theory of the NOE as applied to organic molecules has scarcely changed since 1971, its application has increased dramatically. Using the techniques available in 1971, enhancements smaller than 5% could be seen only with difficulty. Now it is not particularly difficult to detect enhancements of less than 1%, or even 0.1% using newer gradient assisted experiments. Hall and Sanders¹⁶ point out that the maximum possible size of NOE enhancement seen between 1,3 diaxial protons in a cyclohexane chair is about 3%. This size of enhancement was just below the threshold of detection in the CW era, but it is well within modern instrumental capabilities.



REFERENCES

1. Solomon, I. *Phys. Rev.* 1955, 99, 559.
2. Bloembergen, N.; Purcell, E. M.; Pound, R. V. *Phys. Rev.* 1948, 73, 679.
3. Overhauser, A. W. *Phys. Rev.* 1953, 89, 689.
4. Overhauser, A. W. *Phys. Rev.* 1953, 92, 411.
5. Freeman, R.; Anderson, W. A. *J. Chem. Phys.* 1962, 37, 85.
6. Hoffman, R. A.; Forsén, S. *Prog. Nucl. Magn. Reson. Spectrosc.* 1966, 1, 15.
7. Kaiser, R. *J. Chem. Phys.* 1963, 39, 2435.

8. Lauterbur, P. C.; quoted in Baldeschweiler, J. D.; Randall, E. W. *Chem. Rev.* 1963, 63, 81.
9. Anet, F. A. L.; Bourn, A. J. R. *J. Am. Chem. Soc.* 1965, 87, 5250.
10. Noggle, J. H.; Schirmer, R. E. "The Nuclear Overhauser Effect; Chemical Applications," Academic Press, New York, 1971.
11. Balaram, P.; Bothner-By, A. A.; Dadok, J. *J. Am. Chem. Soc.* 1972, 94, 4015.
12. Wagner, G.; Wüthrich, K. *J. Magn. Reson.* 1979, 33, 675.
13. Macura, S.; Ernst, R. R. *Mol. Phys.* 1980, 41, 95.
14. Kumar, A.; Ernst, R. R.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* 1980, 95, 1.
15. Stott, K.; Stonehouse, J.; Keeler, J.; Hwang, T.-L.; Shaka, A. J. *J. Am. Chem. Soc.* 1995, 117, 4199.
16. Hall, L. D.; Sanders, J. K. M. *J. Am. Chem. Soc.* 1980, 102, 5703.