Annual reports on
NMR Spectroscopy

Volume 66

Annual Reports on
NMR SPECTROSCOPY

VOLUME **66**

Edited by

GRAHAM A. WEBB
Royal Society of Chemistry
Burlington House
Piccadilly, London, UK

ELSEVIER

Amsterdam • Boston • Heidelberg • London • New York • Oxford
Paris • San Diego • San Francisco • Singapore • Sydney • Tokyo
Academic Press is an imprint of Elsevier

Academic Press is an imprint of Elsevier
Linacre House, Jordan Hill, Oxford OX2 8DP, UK
84 Theobald's Road, London WC1X 8RR, UK
Radarweg 29, PO Box 211, 1000 AE Amsterdam, The Netherlands
30 Corporate Drive, Suite 400, Burlington, MA 01803, USA
525 B Street, Suite 1900, San Diego, CA 92101-4495, USA

First edition 2009

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

ISBN: 978-0-12-374737-2
ISSN: 0066-4103

Printed and bound in Great Britain
09 10 11 12 10 9 8 7 6 5 4 3 2 1

CONTRIBUTORS

David L. Bryce
Department of Chemistry and Centre for Catalysis Research and Innovation, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5

Rebecca P. Chapman
Department of Chemistry and Centre for Catalysis Research and Innovation, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5

Martin Goez
Institut für Chemie, Martin–Luther-Universität Halle–Wittenberg, Kurt–Mothes-Str. 2, D-06120 Halle/Saale, Germany

Harri Koskela
Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN), University of Helsinki, A.I. Virtasen aukio 1, FIN-00014, Finland

Ryan T. McKay
National High Field Nuclear Magnetic Resonance Centre (NANUC), University of Alberta, Edmonton, AB, Canada T6G 2E1

V. Mikhaltsevitch
Qrsciences Ltd., Cannington WA 6107, Australia

Cory M. Widdifield
Department of Chemistry and Centre for Catalysis Research and Innovation, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5

PREFACE

It is my pleasure to introduce Volume 66 of *Annual Reports on NMR Spectroscopy*. As is usual with this series of reports, a number of diverse areas of science are covered in the contributions found in the present volume.

The first contribution is on 'Quantitative 2D NMR Studies' by H. Koskela; this is followed by an account from R. T. McKay on 'Recent Advances in Solvent Suppression for Solution NMR: A Practical Reference'; the third chapter is on 'Photo-CIDNP Spectroscopy' by M. Goez; this is followed by a discussion of 'Techniques Used in 14N NQR Studies' by V. Mikhaltsevitch; finally, there is a review of 'Chlorine, Bromine and Iodine Solid-State NMR Spectroscopy' by C. M. Widdifield, R. P. Chapman and D. L. Bryce.

It gives me great pleasure to thank all of these authors for their interesting and timely contributions.

G. A. Webb
Royal Society of Chemistry
Burlington House
Post Room
Piccadilly
London, WIJ OBA
England

CONTENTS

CHAPTER 1

Quantitative 2D NMR Studies

Harri Koskela

Contents

Abstract

Nuclear magnetic resonance (NMR) spectroscopy is regarded as one of the most important analytical techniques in chemistry for characterization of molecular structure. In addition to the structural information, NMR spectroscopy also gives quantitative information about the sample constituent. The induced current in the coil can be regarded as linearly dependent on the concentration of the nucleus in the sample. Therefore the resonance integrals in a simple one-dimensional spectrum measured with the excitation–acquisition scheme offer a way to measure absolute amounts of the chemicals present in the sample. Recently, the need for quantitative analysis of highly complex samples has led to a situation where resonance overlap in

Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN), University of Helsinki, A.I. Virtasen aukio 1, FIN-00014, Finland

Annual Reports on NMR Spectroscopy, Volume 66
ISSN 0066-4103, DOI: 10.1016/S0066-4103(08)00401-8

one-dimensional spectra can compromise or even prevent accurate quantification of sample compounds. Two-dimensional NMR offers improved resolution of resonances, and therefore the use of two-dimensional NMR experiments in determination of sample constituent has gained interest in many fields of research where quantification of compounds in complex samples is needed. Concepts of the quantitative two-dimensional NMR and recent applications are discussed.

Key Words: Quantitative 2D NMR, 2D *J*-resolved NMR, COSY, TOCSY, HSQC, 2D INEPT, Natural products, Metabolomics, Oil fractions, Food analysis.

1. INTRODUCTION

There are several analytical instrumentation techniques which offer qualitative details about the sample. Some of those use electromagnetic radiation to measure absorption of energy on electron orbitals (visible and ultraviolet spectroscopy[1]) or bond vibrations (infrared spectroscopy[2]). Mass spectrometry, which can be used to determine the mass/charge ratio of ions, is recognized as a technique that can give accurate data from the molecular ions and fragments.[3] While these techniques can also be applied to extract quantitative information of the constituent of a sample, only one technique, nuclear magnetic resonance (NMR) spectroscopy, can give a detailed view to the molecular structure as well as intrinsically quantitative information.

The use of ^{1}H NMR spectroscopy in quantification has extended in many fields of chemistry. Most important field of application is in synthetic organic chemistry, where ^{1}H NMR experiment is used in structural analysis for determination of the number of protons in a molecule, and further to study mixture samples with high precision and accuracy.[4] Application of quantitative ^{1}H NMR has also taken its place in new, emerging fields of research, like metabolomics.[5]

The experimental set-up for quantitative ^{1}H NMR is quite straightforward, which has made its use so widespread. A typical procedure is to use a 90° excitation pulse, and the repetition delay is set equal to or more than five times the longest T_1 of protons to ensure sufficient relaxation of the magnetization before the next scan.[6] The peak integrals in the obtained ^{1}H NMR spectrum can then be used to determine the analyte concentrations with high accuracy. The principle of reciprocity, which states that the NMR signal strength is inversely proportional to the 90° pulse length,[7] offers also ways to accurately compare constituents of separate samples. This approach has been applied in ^{1}H NMR quantification of algal toxins[8] and protein concentrations[9] with external standards.

The quantification is not restricted to proton detection, as there are number of other NMR observable nuclei, like carbon which is common in organic molecules. While the major isotope, carbon-12, is not usable by NMR, the carbon-13 with 1.1% natural abundance can be observed with NMR spectroscopy. When the sample concentration is not the limiting factor for the analysis, quantitative ^{13}C{^{1}H} NMR is very useful in analysis of organic compounds. The advantage of quantitative

$^{13}C\{^1H\}$ NMR is the wider chemical shift range, which makes it less likely that the resonances of interest are overlapping. A typical procedure for quantification with $^{13}C\{^1H\}$ NMR spectroscopy is to use inverse-gated proton decoupling[10] to avoid the uneven nuclear Overhauser effect (NOE) contribution to the signal intensity.[11] Also, a shorter excitation pulse angle (30°–45°) can be applied so that adequate recovery of magnetization can be achieved in a shorter time period than five times the longest carbon-13 T_1. This approach provides no significant advantage in total acquisition time for a required signal-to-noise ratio, as discussed by Traficante.[12] However, since the excitation range of the radiofrequency (RF) pulse is the reciprocal of its length, the use of short pulses is valuable for nuclei with a wide spectral range to minimize the offset effects to the signal intensities.[13] Another common approach for keeping the repetition delays reasonably short is to use relaxation reagents.[14–18] A new quantitative $^{13}C\{^1H\}$ NMR experiment which applies a precise analysis of NOE enhancement and T_1 relaxation to speed up the acquisition has also been recently reported by Giraudeau and Baguet.[19]

Inverse-gated $^{13}C\{^1H\}$ NMR lacks sensitivity due to the absence of NOE. Therefore, polarization transfer experiments, such as distortionless enhancement by polarization transfer (DEPT) and insensitive nuclei enhanced by polarization transfer (INEPT), which offer higher sensitivity in carbon detection, have also been adapted for quantification[20–22]. Moreover, since these methods detect proton polarization transferred to carbon, the repetition rate is dictated by the T_1 relaxation of protons, not carbons. The drawback of polarization transfer methods is that quaternary carbons are not usually visible in the spectrum. However, a DEPT-based pulse sequence dubbed as DEPTQ[23,24] has been proposed that enables the detection of quaternary carbons in the spectrum. The study of evolution of magnetization during DEPTQ by Özdogan and Orbay[25] revealed a good agreement between theoretical and experimental results, suggesting that the experiment has potential in quantification.

Overlapping of signals often hampers reliable quantification with one-dimensional (1D) NMR experiments. In liquids the resonances are narrow due to the isotropic medium, but the complexity of the sample may still result in insufficient resolution of signals. Some experimental set-ups are reported to diminish the instrumental contribution of $^{13}C\{^1H\}$ NMR linewidths below 0.003 Hz,[26–28] which can help analysis of complex mixture samples. Several processing methods for the analysis of spectra with overlapping resonances, such as deconvolution[29,30] and singular value decomposition,[31] can help quantification to some degree, but even these methods have their limits with highly crowded spectra.

The idea of two-dimensional (2D) NMR spectroscopy was introduced in 1971 by Jeener,[32] and several experimental demonstrations were soon reported by Ernst and co-workers.[33–36] Since then many new 2D NMR experiments have been designed to give higher resolution of the resonances and information about the spectral parameters, and therefore the structural details, that would be inaccessible or at least more laborious to determine with 1D NMR experiments. The improved resolution in 2D NMR experiments has paved the way for general acceptance that NMR spectroscopy is a valid analytical technique in analysis of complex samples.[37,38]

2D NMR is, however, still comprehended more of qualitative than quantitative technique. If quantitativity is associated with 2D NMR, it is usually in contexts of measurement of exchange of magnetization through chemical exchange or cross relaxation. 2D exchange spectroscopy (EXSY) experiment[39,40] is useful in mapping exchange networks and rate constants in molecules.[41] With a different mixing time, the same experiment can be used also to study NOE mediated via cross relaxation. The 2D nuclear Overhauser effect spectroscopy (NOESY) spectrum[39,42] can be used to establish intra- and intermolecular spatial arrangements. One important application of this type of experiments is in determination of three-dimensional structure of biological macromolecules.[43,44] As the research of increasingly complex systems has gained interest, quantification of chemicals present in these samples has become a difficult task to perform just with 1D NMR experiments. In recent years more and more publications have emerged where the 2D NMR spectroscopy has been applied not only in structural elucidation but also in quantification of the concentration of the studied chemicals. The following sections concern with concepts of the quantitative 2D NMR, i.e. 2D NMR in quantification of the amount of analytes, and in some recent applications where 2D NMR has given significantly higher accuracy compared to quantitative 1D NMR.

2. CONCEPTS

Quantification by 2D NMR is not straightforward, as the factors that contribute to the volume of correlation peaks are numerous (e.g. relaxation rates, multiplicity and magnitude of coupling constants). However, the key issue in the use of 2D NMR experiments in quantification is that the observed cross peak volume can be regarded as linear with respect to the concentration of the analyte. Therefore, the quantification of chemicals can be based on determination of a calibration curve which has been defined by several standard samples containing known amounts of analytes within the expected concentration range. This calibration curve can then be used with the real samples to correlate the cross peak volume with the actual concentration of the analyte(s). A more universal and accurate quantification can be obtained, if the coherence pathways and polarization transfer efficiencies of the applied 2D NMR experiment are understood. The efficiency of the polarization transfer can be estimated by inspecting the function of the pulse sequence theoretically. Product operator formalism,[45–49] a concise version of density matrix calculations, provides tools to follow magnetization evolution through pulse sequences by simple transformations of spin operators. The density functions of the magnetization give information of the polarization transfer efficiency, and by that, offer a way to correct integration data so that observed cross peak volumes can be directly used to estimate the concentration of analytes. In the following sections, the polarization transfer in some 2D homo- and heteronuclear shift correlation experiments is demonstrated with product operator calculations. Additional aspects that affect the cross peak volume, like RF pulse imperfections and relaxation, are also discussed.

2.1. Homonuclear experiments

The simplest 2D NMR experiment, which offers enhanced resolution, is the homonuclear *J*-resolved experiment.[50] The experiment is basically a spin-echo sequence,[51,52] where the echo period is incremented. During this t_1 period the chemical shift evolution is refocused with a 180° pulse, but homonuclear *J* couplings are evolved. If we consider a proton *H* coupled to proton(s) *H'*, the density function of the observed proton magnetization is modulated by these couplings accordingly

$$\sigma(H) \propto \prod_{k=1}^{n} \cos\left(\pi J_{HH'_k} t_1\right) \tag{1}$$

The result is that the lines in a proton resonance multiplet are dispersed along both the F_1 and F_2 dimension, giving a tilted shape to the cross peak. With further processing the tilt can be straightened, so that the F_2 projection of the spectrum shows only single line for each resonance, and the multiplet structure is given in the F_1 dimension. In this way partially overlapping resonances can be separated, and integration is easier to accomplish. The *J*-resolved spectra are typically processed in magnitude mode, but there are also many reports of various approaches how a phase-sensitive spectrum can be obtained.[53–58] A F_2 projection of the 2D *J*-resolved spectrum can also be used to produce a quantitative "proton-decoupled" 1D spectrum.[59]

The second way to improve the resolution of proton resonances is with homonuclear correlation spectroscopy, or COSY, experiment.[36,60–63] In the basic form of the experiment the magnetization is excited with a 90° pulse. During the consequent t_1 period the chemical shift and homonuclear *J* couplings evolve, until a second 90° pulse is applied prior to acquisition. This pulse causes polarization transfer to take place between protons that are coupled to each other. If we consider a proton *H* coupled to another proton *H'*, then the observable terms of the proton *H* magnetization for the COSY experiments are

$$\sigma(H) \propto H_x \sin\left(\omega_H t_1\right) \cos\left(\pi J_{HH'} t_1\right) - H_y H'_z \sin\left(\omega_{H'} t_1\right) \sin\left(\pi J_{HH'} t_1\right) \tag{2}$$

The term H_x gives the diagonal peak, whereas the term $H_y H'_z$ corresponds the polarization transferred from the remote proton *H'* with coupling $J_{HH'}$ to the observed proton. This term is responsible of the off-diagonal cross peak. Two resonances that are overlapping in the 1D spectrum can be separated by their off-diagonal cross peaks if they are coupled to separate protons with distinctly different chemical shifts. However, the nature of the off-diagonal term results in several implications. First of all, the splitting of the active *J* coupling is in anti-phase, i.e. the lines appear with 180° phase difference. If the line separation is comparable to the linewidth, self-cancellation[64] of lines will take place, diminishing the absolute-value cross peak volume. This can also introduce significant variation of the cross peak absolute-value volume, if the linewidths vary between the spectra. If COSY is performed in phase-sensitive mode,[65] or COSY is acquired with double-quantum filtering (DQF-COSY),[66–69] the total volume (the sum of

positive and negative lines) of any off-diagonal cross peak is, by default, zero. This can be of course circumvented by integrating only either positive or negative part of the cross peak volume. Due to aforementioned reasons, quantification with the COSY-type experiments is best to do using a calibration curve which has been defined by several standard samples. This approach can still give very accurate results as demonstrated by Giraudeau et al.[70] They made a comparative study of quantitativity between DQF-COSY and 2D *J*-resolved NMR with tropine–nortropine mixtures. The authors found a slightly better accuracy for DQF-COSY over 2D *J*-resolved NMR (errors were 2 and 3%, respectively), with standard deviation under 1%. There are also some reports on how a COSY-type spectrum can be acquired with in-phase cross peaks,[71–74] which should be much easier to integrate. It should be noted, that the evolution of magnetization is more complex in these experiments, and additional lineshape distortions or other limitations might be introduced.

Another related 2D NMR experiment, total correlation spectroscopy, or TOCSY,[75,76] can be applied to obtain in-phase off-diagonal cross peaks. In this experiment the chemical shift evolves during the t_1 period. The following spin-lock period t_m, which can be produced with e.g. composite pulse trains,[77–79] leads to an isotropic mixing condition bringing about homonuclear Hartmann–Hahn transfer of magnetization through *J* couplings.[80] If we consider a proton *H* coupled to another proton *H′*, the observable *in-phase* terms of the *H* proton magnetization are as follow[75]

$$\begin{aligned}\sigma(H) \propto\ & 1/2H_x \sin(\omega_H t_1)\left[1 + \cos\left(2\pi J_{HH'} t_m\right)\right] \\ & + 1/2H_x \sin\left(\omega_{H'} t_1\right)\left[1 - \cos\left(2\pi J_{HH'} t_m\right)\right]\end{aligned} \tag{3}$$

The first half of the equation gives the diagonal cross peak, whereas the second half produces the cross peak. With two-spin system the highest intensity for the cross-peak term is obtained when t_m is set for $1/(2J_{HH'})$. When more complex spin systems are involved, the setting of the optimal mixing time needs some trade-off to give a satisfactory intensity for cross peaks. This also means that quantification with TOCSY is best to do using a calibration curve which has been defined by several standard samples. Zero-quantum coherences are also produced during the mixing time,[75] which are responsible of distortions observed in the lineshapes. A number of techniques have been proposed to eliminate these zero-quantum distortions[81–84] which should enable more accurate integration.

2.2. Proton detected heteronuclear experiments

Methyl (CH_3), methylene (CH_2) or methine (CH) groups are common in organic chemicals. Compared to proton, carbon has a much larger chemical shift range, so higher separation of the CH_n proton resonances is achieved with ^1H–^{13}C heteronuclear correlation spectroscopy.[85] Therefore this approach can facilitate quantification even with highly complex mixtures.

Bodenhausen and Ruben reported in 1980 a 2D shift-correlated experiment designated as heteronuclear single-quantum coherence, or HSQC[86] (Figure 1).

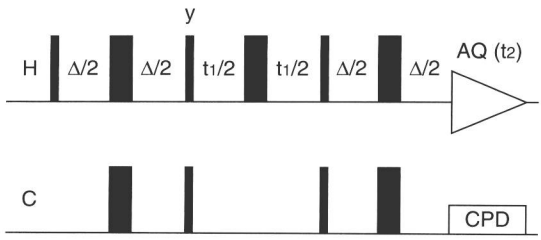

Figure 1 A basic pulse sequence for HSQC experiment. Narrow and wide bars represent 90° and 180° RF pulses, respectively. Pulse phase is *x*, if not stated otherwise.

The method employs inverse detection, thus giving superior sensitivity over the 2D methods with direct detection.[87]

HSQC starts with an INEPT step[88] which transfers the proton polarization to carbon. Then the polarization is modulated by the carbon chemical shift during the t_1 evolution period. Finally, a reverse-INEPT step[89] returns the polarization back to proton for observation. Pulsed field gradients can also be incorporated into HSQC[90] for coherence selection. Due to single-quantum coherence states, evolution of homonuclear coupling during t_1 evolution period has no effect to cross peak shape in HSQC. The evolution of polarization during the HSQC experiment is easy to predict, facilitating quantitative use of the experiment. The evolution period itself should only give the carbon chemical shift modulation to the signal, and therefore should not affect the quantitativity. The product operator analysis can be started with evolution time $t_1 = 0$ and then calculate what is the magnetization state after INEPT and reverse-INEPT steps. The density function after the pulse sequence is

$$\sigma(CH_3, CH_2, CH) \propto \sin^2(\pi J_{CH}\Delta) \tag{4}$$

As can be seen, the density function is the same for all CH_n groups. The cross peak volume is naturally dependent on proton number in the CH_n group, i.e. the CH_3 cross peak is generally three times more intense than CH group. The CH_2 cross peak intensity follows also the number of protons present, so if the protons are equivalent, the cross peak intensity is twice the intensity of the CH cross peak. The magnetization intensity is maximum whenever Δ equals $1/(n|J|)$, where n is a positive *even* integer. From the point of relaxation, the first solution, $\Delta = 1/(2|J|)$, is the most practical one. The effect of INEPT optimization can be more easily followed if the polarization transfer delay Δ is defined as $1/(2J_{opt})$

$$\sigma(CH_3, CH_2, CH) \propto \sin^2\left(\frac{\pi}{2}\frac{J_{CH}}{J_{opt}}\right) \tag{5}$$

The maximum intensity is met when the J_{CH}/J_{opt} ratio equals 1, and the intensity decreases if the J_{CH}/J_{opt} ratio is higher or lower than 1 (Figure 2). From the point of sensitivity it is best to set J_{opt} to the average of J_{CH}s of the sample. When the difference of J_{CH}s is exceptionally large, e.g. with a sample containing both alkane ($^1J_{CH} \approx 125$ Hz) and alkyne ($^1J_{CH} \approx 250$ Hz)[91] functionality, the cross peak

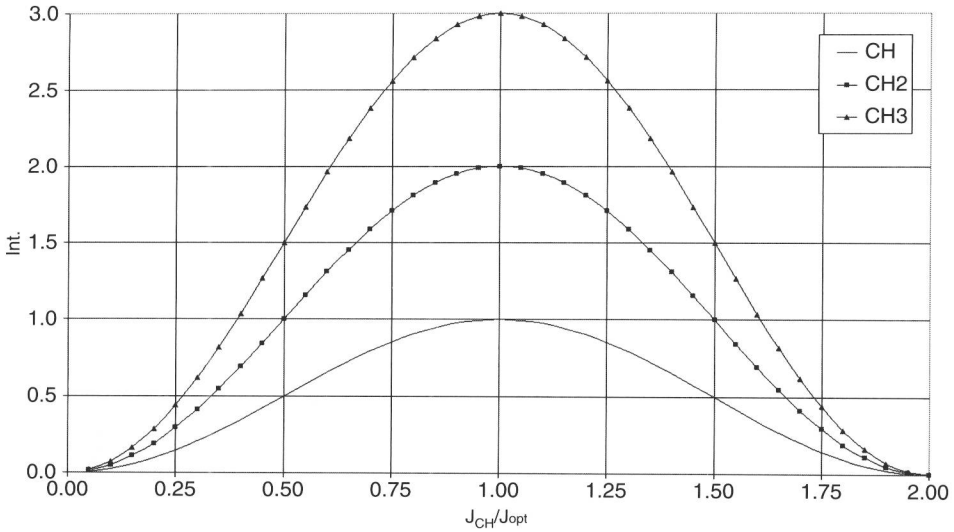

Figure 2 Relation of signal intensity and ratio J_{CH}/J_{opt} in the HSQC (see Equation (5)). The maximum intensities for CH_3, CH_2 and CH groups are 3, 2, and 1, respectively.

volumes for these protons will be 75% from the maximum attainable if the J_{opt} is set for average of the couplings. When the J_{CH}s of the sample are known, suitable coefficients can be formulated to correct the volumes to take this effect into account, and by that improve the accuracy of quantification.

As always, homonuclear coupling evolution during the pulse sequence affects the polarization transfer to the desired coherence and therefore affects the quantitativity. Homonuclear coupling evolution is active in HSQC experiment during both INEPT and reverse-INEPT periods. We can consider a CH group, with one remote proton H' coupled to the CH group proton. During both the INEPT and reverse-INEPT period a part of the polarization from the CH group proton ends up to the H' through a COSY-type transfer. Therefore, the density function for this spin system is

$$\sigma\left(\mathrm{CH}-H'\right) \propto \sin^2\left(\frac{\pi}{2}\frac{J_{\mathrm{CH}}}{J_{\mathrm{opt}}}\right)\cos^2\left(\frac{\pi}{2}\frac{J_{HH'}}{J_{\mathrm{opt}}}\right) \tag{6}$$

The loss of polarization is illustrated in Figure 3.

The loss of intensity can be significant, when the $J_{HH'}$ approaches the value of J_{CH}. Therefore HSQC experiment is most effective in detecting $^1J_{CH}$ correlations, whereas its usefulness in detecting $^{2\text{–}3}J_{CH}$ correlations is limited. If there are a large number of coupled protons, the general density function for a CH group with n remote coupled protons is

$$\sigma\left(\mathrm{CH}-nH'\right) \propto \sin^2\left(\frac{\pi}{2}\frac{J_{\mathrm{CH}}}{J_{\mathrm{opt}}}\right)\prod_{k=1}^{n}\cos^2\left(\frac{\pi}{2}\frac{J_{HH'_k}}{J_{\mathrm{opt}}}\right) \tag{7}$$

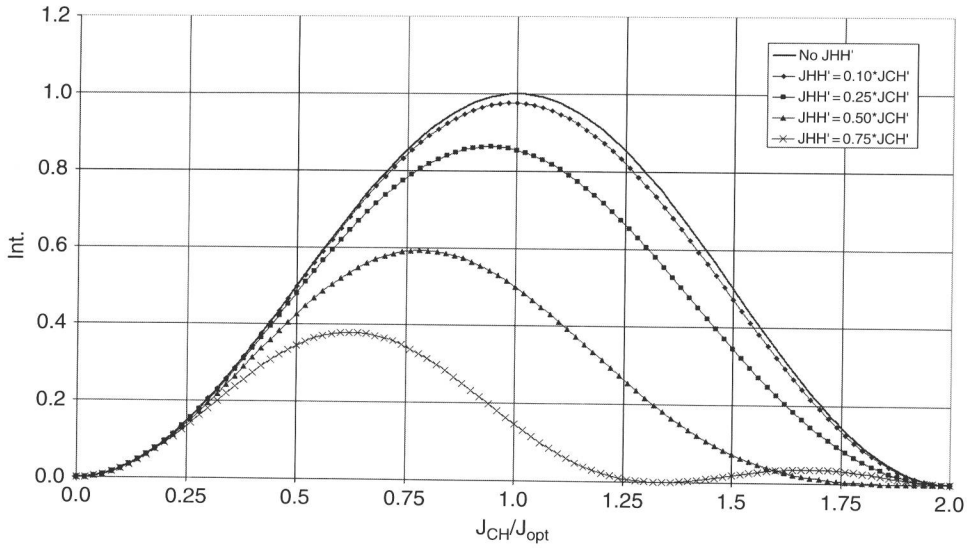

Figure 3 Signal loss in respect of homonuclear coupling evolution in the HSQC (see Equation (6)).

If $J_{HH'}$ is about 5% of J_{CH} (corresponds roughly to the typical values of $^3J_{HH}$ versus $^1J_{CH}$), the intensity loss is insignificant if only few protons are coupled (Figure 4). However, e.g. the methine group in the isobutyl fragment can have eight coupled protons, leading to 5% loss of intensity. More importantly, the cross peak would be split to nine lines (if equal J_{HH}s are assumed), which makes reliable integration of the cross peak difficult. If high accuracy in quantification is needed, these factors must be taken into account.

Recently, there have been attempts to refine the HSQC experiment to improve the quantitativity. Heikkinen et al.[92] reported an interesting variation of the experiment called as Q-HSQC. In this experiment, uniform polarization transfer over a range of J_{CH} couplings was accomplished with use of constant-time INEPT periods with varied J_{CH} evolution times. In the subsequent paper the lineshape problems associated with the J_{HH} coupling evolution during the long constant-time INEPT periods were corrected by Q-CAHSQC using the CPMG-INEPT approach, and the effects of carbon pulse offset were also discussed.[93] A solution to the necessity in Q-HSQC and Q-CAHSQC to measure a multiple of four scans per transient was proposed by Peterson and Loening.[94] Their QQ-HSQC experiment elegantly exploited localized manipulation of the sample volume in a way that the different parts of the sample were optimized for different polarization transfer evolutions, resulting reduced number of scans per transient and shorter total acquisition time. The principle concerning the spatial encoding of the sample volume with pulsed field gradients has also been employed in a number of applications, e.g. for measuring diffusion coefficients, homonuclear correlations and longitudinal relaxation rates.[95–97]

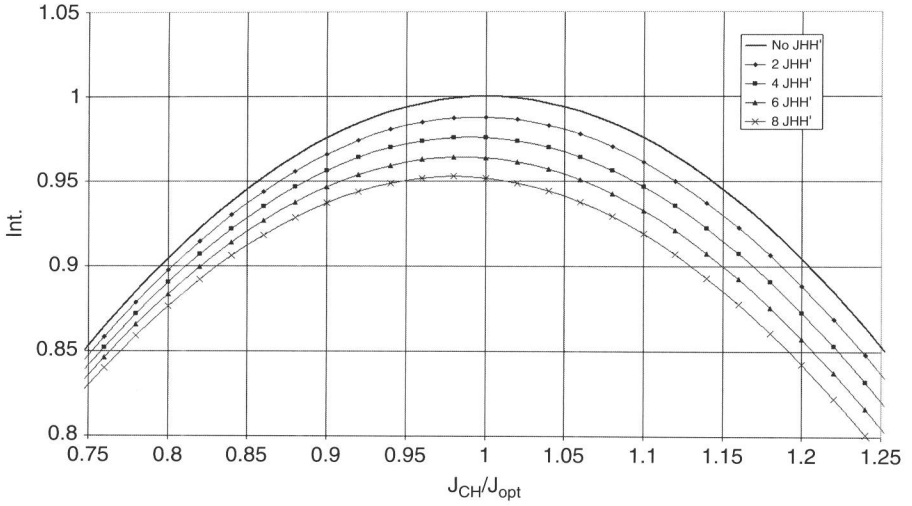

Figure 4 Signal loss in respect of multiple homonuclear couplings in the HSQC when $J_{HH'}$ is presumed to be 5% of the J_{CH} (see Equation (7)).

The other common inverse-detection method, heteronuclear multiple quantum coherence (HMQC)[98–101] relies on multiple-quantum coherence transitions during the pulse sequence. Due to the multiple-quantum coherence transitions it is more laborious to theoretically follow the course of magnetization, and the cross peak will be broader in the F_1 dimension due to the J_{HH} evolution.[102,103] Unlike HSQC, HMQC can also be optimized for[2–3] J_{CH} couplings. This heteronuclear multiple bond correlation experiment, or HMBC,[104,105] has lower sensitivity than HMQC/HSQC experiments, and the $^1J_{CH}$ correlations can appear as artefacts in the spectrum. However, the cross peak volume should follow the concentration of analyte, so with proper method validation HMQC and HMBC should also be applicable for quantification.

2.3. Carbon-detected heteronuclear experiments

INEPT[88] is used in multinuclear NMR spectroscopy to enhance sensitivity of nuclei with low gyromagnetic ratio and/or low natural abundance.[106–108] When used in direct observation of an insensitive heteronucleus like carbon, the standard INEPT has some shortcomings. The most prominent is the anti-phase multiplet structure of the resonances, which prevents proton decoupling during acquisition. These shortcomings have been addressed in several papers, and some improvements like possibility for multiplicity editing have been published.[109–112] There are also several examples how the INEPT experiment can be expanded to a 2D heteronuclear shift correlation experiment called as 2D INEPT.[113–115] Bendall et al.[113] have presented a thorough Heisenberg vector analysis how polarization is transferred in various versions of the 2D INEPT