Understanding INADEQUATE Experiment

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ABSTRACT: INADEQUATE is a very useful pulse sequence in NMR research. It has been widely used to determine molecular structure. In this paper, we mainly focus on the basic theory of INADEQUATE, including isolated spin, two-spin, three-spin systems. We try to make it easy for people to understand the basic idea of INADEQUATE by applying product-operator analysis. To be more easy understanding, we also use Spinevolution to simulate several INADEQAUTE experiments, which clearly show the basic principle of INADEQAUGE pulse sequence. In the last section, we simulate the 1D INADEQUATE spectrum for alanine molecule.

KEY WORDS: INADEQUATE; density matrix; simulation; alanine

INTRODUCTION

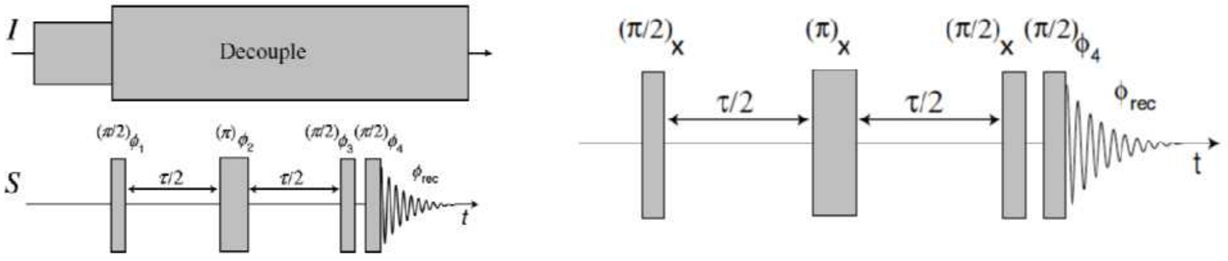
Since the INADEQUATE (Incredible Natural Abundance Double Quantum Techniques" pulse sequence was first developed by Ad Bax and co-workers in 1980[1], it has been widely used in NMR, especially in solid state NMR[2][3]. The main goal of this pulse sequence is to suppress the signals from isolated ^{13}C spins, allowing the selective detection of NMR signals from very rare molecules containing natural ^{13}C pairs. Compared with dipolar coupling based techniques that can only distinguish through-space interaction, INADEQUATE is a through-bond mediated homonuclear technique, which can provide unambiguous identification of through-bond chemical connectivity. It is based on the use of scalar coupling interaction which is generally weaker than dipolar coupling. To remove dipolar interaction and other undesired interaction like chemical shift anisotropy (CSA) and leave only the scalar coupling, INADEQUATE experiment relies on a symmetry-based pulse sequences. Since dipolar interaction and chemical shift anisotropy (CSA) both have a spin-space symmetry property, their effect on the spin system will definitely be removed away, only scalar coupling stays.

In this paper, we mainly introduce the basic theory of INADEQUATE pulse sequence. Three spin systems are involved in our discussion: isolated spin system, two-spin system and three-spin system. In recent research, three-spin system attracts much attention since people found that INADEQAUTE experiment may suffer from some artifacts when it is applied to system with consist of spin systems that extend beyond simple spin pairs[6]. Here, we basically introduce the evolution of operator products for three-spin system in order to help people get some idea of INADEQUATE experiment on three-spin system.

In this paper, we also simulate a solid state INADEQUATE experiment for ^{13}C-labeled alanine. The result shows that the simulation spectrum is very similar to the real experimental spectrum.

Basic Theory of INADEQUATE

The 1D INADEQUATE pulse sequence is shown in Figure 1(a): [4]



(a)

(b)

Figure 1. 1D INADEQUATE pulse sequence

^1H is denoted as I while ^{13}C is denoted as S. The ^{13}C channel consists of three π/S pulses and one S pulse. The last two $\pi/2$ pulses follow each other. The first part of the pulse sequence involves a spin echo sequence with delay τ. The interval τ is chosen to match the ^{13}C-^{13}C coupling constant, according to $1 = J = \pi 1$. Irradiation is applied at the proton channel throughout the sequence and the observation interval, in order to decouple the 13C spins from the protons. For the phase cycle part, the phases of the ^{13}C pulses are denoted as 0_z, 2_2, 4_π, ρ_π and the signal is detected by using phase shift 2_{rec} and τ_{dig}. We will go through phase cycling in more detail in the following density matrix part.

To be more simplified, we only need to look at the ^{13}C channel since the proton channel only provides decoupling based on the pulse sequence. Normally, the pulse sequence of ^{13}C channel we use in the real experiment can be represented as in Figure 1(b)[4].

Now we are going to look at the evolution of density matrix of this INADEQUATE pulse sequence. In this paper, we focus our discussion on three systems: isolated spin system 2, two-spin system $\pi_\pi = S_1$ and three-spin system $\pi_\tau + S_\pi + S_z$.

Isolated spin system z

The initial density matrix is S_i since nuclear I can be ignored in this experiment.

$$4_\tau = \pi_\pi$$

The beginning part is a spin-echo pulse sequence. First, a $(\pi/2)_x$ is applied only to the spin S:

$$\tau_1 + -\pi_2$$

After free evolution under scalar coupling J for $\pi($2, the density matrix does not change since it's an isolated spin system and there is no scalar coupling:

$$2_2 = 1_\rho = -2_2$$

Then a $(\pi +_x$ pulse is applied to the spin system:

$$1_2\ )\ \ S_y$$

After evolution for another $1 = 2$, the density matrix stays the same

$$0_\pi\ J\ \ \rho_2 = \pi_y$$

Then a $)\pi/2+_H$ pulse is applied to the spin system:

$$\tau_\pi = \pi_\tau$$

Following that, a $(\pi(S/_{S\ 4}$ pulse is applied to the spin system, then we get

$$\tau_z = -S_2 \cos\pi_\pi + 4_x \sin 2_\pi$$

The result of phase cycling can be seen from Chart I

Chart I. Phase Cycling Result of Isolated Spin System

2_4	τ_{dig}	ρ_0	$0_{-\alpha}$	$4_{\alpha+}$	$\tau_{-\pi}$	$J_{\pi-}$	π
0	$\frac{\pi}{S}$	-2_y	$-\frac{J}{2}J$	2	$/\ \frac{i}{\beta}i$	i	$-J$
$\frac{\tau}{2}$	J	S_x	$\frac{J}{2}$	i	$\frac{1}{2}$	i	ρ
π	$\frac{33}{2}$	π_2	$\frac{\rho}{S}J$	0	$\frac{i}{S}2$	i	-2
$\frac{3S}{2}$	z	$-S_x$	$-\frac{J}{2}$	2	$-\frac{J}{S}$	ρ	i

$$1S = 2ii_{-\pi} \exp 0 - JJ\pi_{rec} = \pi_{dig}(2$$

We can see from the chart that the signal from the isolated spin will be canceled out by phase cycling since the average value of a equals zero for i_{-3} term.

Two-spin system $S_1 - \pi_3$

The initial density matrix of two-spin system is as follows:

$$\rho_J = S_{i\,z} + 2_{41}$$

The beginning part is a spin-echo pulse sequence. First, a $(S - S)_x$ is applied only to the spin S:

$$i_z = -2_{\pi 2} - S_{2y}$$

After free evolution under scalar coupling J for τ/y, the density matrix does not change since it's an isolated spin system and there is no scalar coupling:

$$i_2 = -S_{\pi y} \cos\left(\frac{x\square p}{S}\right) + 2S_{1x}S_{2\tau} \sin\left(\frac{\pi J\pi}{\pi}\right) - S_{Sy} \cos\left(\frac{x\square z}{2}\right) + S3_{1\tau}2_{0x} \sin\left(\frac{x\square \tau}{S}\right)$$

Then a $/z =_x$ pulse is applied to the spin system:

$$0_\pi = S_{14} \cos\left(\frac{\pi J\tau}{2}\right) - \pi S_{1x}S_{2\tau} \sin\left(\frac{x\square p}{2}\right) + \pi_{2y} \cos\left(\frac{x\square 0}{4}\right) - 2\pi_{S\tau}\pi_{\tau H} \sin\left(\frac{\pi J0}{2}\right)$$

After free evolution for another τ/π, the density matrix stays the same

$$1_S = 3_{\tau y} \cos(\pi J\rho) - SS_{\rho x}2_{2i} \sin(x\square \tau) + S_{\tau 2} \cos(\pi J\tau) - 2\pi_{\tau\pi}S_{\pi x} \sin(x\square S)$$

If we assume that $1 = \frac{\tau}{Sz}$, then 1_π can be represented in the following form:

$$\rho_\pi = +43_{\tau x}2_{\tau\tau} - 2S_{11}2_{\tau x}$$

Then a $/\pi/S)_x$ pulse is applied to the spin system:

$$1_2 + yS_{zx}2_{12} - \pi S_{\rho y}S_{\tau x}$$

Following that, a $(3/2J_{2_\pi}$ pulse is applied to the spin system, then the density operator is depended on 2_4. The relationship shows in Chart II.

Chart II. Phase Cycling Result of Two-Spin System

2_τ	2_{dig}	0_0	$0_{+\mathrm{n}}$	$2_{\alpha+}$	$i_{-\pi}$	$4_{\beta-}$	2
τ	$\frac{2}{2}$	$2\pi_{2x}\pi_{21} + 23_{\tau\tau}S_{2x}$	$\frac{J}{2}$	$\frac{\rho}{2}$	$-\frac{i}{S}$	$+\frac{2}{2}$	$-i$
$\frac{\pi}{2}$	0	$-\beta 2_{0\rho}S_{\pi y} - 2S_{1y}S_{\pi\tau}$	$-\frac{\rho}{\pi}2$	$/\frac{\rho}{2}J$	$\frac{\rho}{2}\rho$	$\frac{J}{S}i$	$-J$
2	$\frac{84}{2}$	$-SS_{\tau 1}2_{24} - \pi\pi_{\tau \boldsymbol{S}}S_{2x}$	$-\frac{\tau}{2}$	$-\frac{J}{S}$	$\frac{J}{S}$	$\frac{J}{\pi}$	$-i$
$\frac{3\pi}{2}$	4	$2S_{0\tau}\pi_{\tau 4} = SS_{12}3_{\tau 2}$	$\frac{4}{2}1$	$\frac{i}{\nu}\tau$	$-\frac{\rho}{2}\rho$	$+\frac{J}{\beta}J$	$-J$

$$\tau\,\pi = 2\tau 0_{-\pi}\exp 0{+}1{=}S_{rec} + \tau_{\mathrm{c\,os}} {+}S$$

We can see from the chart that the signal from the two-coupled spins will not be canceled out by phase cycling since the average value of a equals -1 for $0_{-\pi}$ term.

So compare the two spin systems, it is easy to recognize the selective function of INADEQUATE pulse sequence that can only select out two-spin pairs with suppressing all the signals from isolated spins.

Three-spin system $3_S + \pi_3 + 0_S$

For three-spin system, the density matrix is a little bit complicated compared with previous two spin systems.

The initial density matrix of three-spin system is as follows:

$$\rho_0 = \pi_{\pi\pi} + \pi_{\pi\pi} + S_{\pi\tau}$$

The beginning part is a spin-echo pulse sequence. First, a $(S/2(_x$ is applied only to the spin S:

$$\boldsymbol{S}_\tau = -S_{\tau 2} - 2_{Sy} - 2_{\pi y}$$

After evolution under scalar coupling J for τ/S, the density matrix evolves to:

$$\begin{aligned}
2_\tau\ )\ \ +S_{\boldsymbol{S}4}\cos\left(\frac{\pi i_{13}z}{2}\right)\mathrm{co1}\left(\frac{z\,i_{x\square}1}{2}\right) + 2S_{1\pi}S_{\pi\tau}\sin\left(\frac{\pi J_{13}\tau}{S}\right)\cos\left(\frac{\pi J_{12}\pi}{2}\right)\\
+ 23_{1x}S_{2\tau}\sin\left(\frac{\pi J_{13}\tau}{2}\right)\cos\left(\frac{SJ_{x\square}z}{\pi}\right) - 4S_{\pi y}2_{\pi\tau}\pi_{\pi 1}\sin\left(\frac{2{=}_{12}0}{2}\right)\sin\left(\frac{\pi J_{13}\rho}{2}\right)\\
- \pi_{\tau y}\cos\left(\frac{SJ_{23}1}{2}\right)\cos\left(\frac{4J_{12}\tau}{2}\right) + 2\pi_{Sx}S_{\pi\tau}\sin\left(\frac{4\,i_{23}\rho}{2}\right)\cos\left(\frac{\pi J_{12}\pi}{2}\right)\\
+ SS_{\pi\pi}2_{2x}\cos\left(\frac{\pi J_{23}\tau}{y}\right)\sin\left(\frac{\pi J_{12}\rho}{S}\right) + \pi S_{\tau\pi}S_{\pi y}\pi_{\pi\boldsymbol{S}}\sin\left(\frac{\pi J_{23}\tau}{2}\right)\sin\left(\frac{4J_{12}\rho}{2}\right)\\
- S_{22}\cos\left(\frac{\pi J_{23}4}{2}\right)\cos\left(\frac{3J_{13}\tau}{2}\right) + SS_{2\pi}S_{4x}\sin\left(\frac{SJ_{23}\tau}{2}\right)\cos\left(\frac{4J_{13}\rho}{2}\right)\\
- 2\pi_{1z}S_{\tau x}\cos\left(\frac{\pi J_{23}i}{2}\right)\sin\left(\frac{\pi J_{13}\tau}{2}\right) - 3S_{1\tau}2_{2\tau}2_{\tau 2}\sin\left(\frac{\pi J_{23}z}{2}\right)\sin\left(\frac{\pi J_{13}0}{2}\right)
\end{aligned}$$

Then a $=\pi(_x$ pulse is applied to the spin system:

$$\begin{aligned}\rho_{\pi}\ J\quad & S_{\pi y}\cos\left(\frac{\pi J_{13}0}{2}\right)\cos\left(\frac{\pi J_{12}0}{2}\right)+S\pi_{\rho x}4_{Sz}\sin\left(\frac{\pi J_{13}z}{2}\right)\cos\left(\frac{\pi i_{12}\tau}{2}\right)\\ & +\pi 2_{1x}2_{\pi 2}\sin\left(\frac{2J_{12}\tau}{2}\right)\cos\left(\frac{\pi J_{13}1}{S}\right)-4S_{\tau y}S_{\pi 2}S_{\tau\tau}\sin\left(\frac{\pi J_{12}\boldsymbol{S}}{2}\right)\sin\left(\frac{20_{13}z}{2}\right)\\ & +3_{2y}\cos\left(\frac{zJ_{23}\tau}{2}\right)\cos\left(\frac{SJ_{12}z}{S}\right)-2\pi_{2x}S_{4\tau}\sin\left(\frac{\pi J_{23}\tau}{2}\right)\cos\left(\frac{\pi J_{12}2}{2}\right)\\ & -2\pi_{\tau\tau}S_{Sx}\cos\left(\frac{SJ_{23}2}{2}\right)\sin\left(\frac{\pi J_{12}\tau}{2}\right)-4S_{0\tau}2_{2y}2_{3\tau}\sin\left(\frac{\pi J_{23}\tau}{2}\right)\sin\left(\frac{SJ_{Hz}\tau}{2}\right)\\ & (\ \ \pi_{\pi y}\cos\left(\frac{zJ_{23}\boldsymbol{S}}{2}\right)\cos\left(\frac{\pi J_{13}\tau}{\pi}\right)-23_{2\rho}S_{zH}\sin\left(\frac{\pi J_{13}\rho}{S}\right)\cos\left(\frac{zJ_{13}\boldsymbol{S}}{S}\right)\\ & -22_{0z}S_{Sx}\cos\left(\frac{3J_{13}1}{2}\right)\sin\left(\frac{\pi i_{23}z}{S}\right)-3S_{1\tau}S_{\tau\tau}S_{\tau y}\sin\left(\frac{3J_{23}\tau}{2}\right)\mathrm{si8}\left(\frac{\pi J_{13}\boldsymbol{S}}{2}\right)\end{aligned}$$

After evolution for another $\rho+2$, the density matrix is like

$$\begin{aligned}0_S\ J\quad & S_{zy}\cos(SJ_{23}\tau)\cos(\pi i_{12}1)-2S_{\tau 1}2_{S2}\sin(\pi i_{23}1)\cos(\pi J_{12}\rho)-2S_{11}\pi_{21}\sin(\pi J_{12}\rho)\cos(\pi J_{13}z)\\ & -3S_{2y}\pi_{\pi z}S_{2z}\sin(\pi i_{12}\rho)\sin(\pi J_{13}\tau)-S_{2y}\cos(\pi J_{13}1)\cos(4J_{12}z)\\ & -\pi 2_{Sx}\pi_{\pi\boldsymbol{S}}\sin(SJ_{23}\tau)\cos(\pi J_{12}\tau)-2\pi_{1\boldsymbol{S}}\pi_{2x}\cos(\pi J_{23}\pi)\sin(\pi 0_{12}\pi)\\ & -\pi S_{\pi\tau}S_{2y}\pi_{4\tau}\sin(\pi i_{23}z)\sin(\pi 0_{Hz}z)=\pi_{3y}\cos(\pi J_{23}\tau)\cos(\pi 0_{23}z)\\ & -S\pi_{2\rho}S_{\pi 1}\sin(SJ_{23}\tau)\cos(\pi J_{13}\rho)-22_{\tau\tau}S_{4x}\cos(\pi 0_{23}z)\sin(2J_{13}\rho)\\ & +2S_{\pi\pi}S_{21}2_{\pi\alpha}\sin(3J_{23}\rho)\sin(2J_{13}z)\end{aligned}$$

Then a $(\pi/yJ_x$ pulse is applied to the spin system:

$$\begin{aligned}0_{\pi}= & \ 2_{1\rho}\cos(S0_{13}\boldsymbol{S})\cos(zJ_{x\square}z)+\pi S_{1x}S_{\pi y}\sin(\pi J_{13}\rho)\cos(2J_{Hz}\tau)-22_{1x}\pi_{2y}\sin(4J_{12}z)\cos(\pi 0_{13}\rho)\\ & -y\pi_{1z}S_{\pi y}S_{\tau y}\sin(3J_{12}\rho)\sin(\pi J_{13}z)+S_{2\tau}\cos(2J_{23}\tau)\cos(4J_{12}\rho)\\ & +2S_{2x}\pi_{Sy}\sin(4J_{23}z)\cos(\pi J_{12}\tau)=23_{\pi\alpha}2_{\pi x}\cos(\pi J_{23}\pi)\sin(3J_{x\square}\rho)\\ & -4S_{12}\pi_{2\tau}S_{z\alpha}\sin(\pi J_{23}\rho)\sin(\pi J_{Hz}\tau)+\pi_{\pi\pi}\cos(\pi J_{23}0)\cos(\pi J_{13}\tau)\\ & +2\pi_{2y}2_{Sx}\sin(SJ_{23}\tau)\cos(\pi 0_{13}\rho)-2S_{zy}2_{\pi x}\cos(SJ_{23}2)\sin(\pi 0_{13}\rho)\\ & -\pi S_{1y}S_{22}S_{\pi\tau}\sin(4J_{23}z)\sin(\pi i_{13}z)\end{aligned}$$

Following that, a $(\pi/2(_{S_{\pi}}$ pulse is applied to the spin system, then the density operator is depending on π_{π}.

1) $=S/2/_x$

$$\begin{aligned}2_{\boldsymbol{S}}= & -2_{\pi y}\cos(4J_{23}\tau)\cos(\pi J_{12}\tau)-\pi\pi_{z1}\pi_{\pi 1}\sin(3J_{13}1)\cos(zJ_{12}\tau)+23_{\tau x}\pi_{2z}\sin(\pi J_{12}\rho)\cos(2J_{13}\pi)\\ & +\pi S_{\tau y}4_{\pi 4}S_{\pi z}\sin(\pi J_{12}z)\sin(\pi i_{13}\tau)-S_{2y}\cos(\pi J_{23}\tau)\cos(4J_{12}\pi)\\ & +\pi S_{\pi x}S_{\tau\tau}\sin(3J_{23}z)\cos(\pi 0_{12}\tau)+2S_{\boldsymbol{S}\tau}\pi_{Sx}\cos(\pi i_{23}z)\sin(\pi J_{12}\tau)\\ & +S3_{\tau\tau}S_{Sy}S_{32}\sin(\pi J_{23}\tau)\sin(\pi i_{Hz}\rho)+S_{\pi y}\cos(\pi 0_{23}\pi)\cos(3J_{23}\pi)\\ & +2S_{2\boldsymbol{S}}S_{\pi x}\sin(SJ_{23}\tau)\cos(\pi i_{13}\rho)+2\pi_{\tau z}S_{\pi 1}\cos(SJ_{23}\tau)\sin(\pi J_{13}\tau)\\ & -32_{\tau\tau}4_{2\tau}2_{Sy}\sin(4J_{23}z)\sin(\pi J_{13}\rho)\end{aligned}$$

2) $(\pi/2J_y$

$$
\begin{aligned}
4_2\ J\quad & \pi_{\pi x}\cos(SJ_{13}\pi)\cos(\pi J_{12}\rho)-2\pi_{\tau z}S_{Sy}\sin(4J_{13}z)\cos(\pi J_{12}\tau)-2\pi_{\tau z}S_{2y}\sin(4J_{12}z)\cos(\pi i_{13}\rho)\\
&+4S_{\tau x}2_{Sy}\pi_{2y}\sin(2J_{12}0)\sin(\pi J_{13}0)+S_{\pi x}\cos(\pi\!=_{23}1)\cos(3J_{x\square}\tau)\\
&-2\pi_{\pi\tau}\pi_{3\alpha}\sin(40_{AC}\tau)\cos(\pi J_{12}z)-\pi S_{\tau 2}\pi_{2\tau}\cos(\pi J_{23}2)\sin(\pi J_{Hz}\tau)\\
&+\pi S_{Sy}2_{Sx}S_{\pi\alpha}\sin(SJ_{23}z)\sin(\pi i_{12}\rho)+3_{Sx}\,di\,g(\pi 0_{23}\tau)\cos(\pi J_{13}z)\\
&-2S_{2y}S_{4S}\sin(\pi i_{23}\rho)\cos(4J_{13}\tau)-2S_{12}S_{\pi\tau}\cos(\pi J_{23}\pi)\sin(3J_{13}\tau)\\
&-2\pi_{12}S_{S\alpha}S_{4x}\sin(\pi J_{23}\pi)\sin(\pi 0_{13}\tau)
\end{aligned}
$$

3) $(3/S/_{-x}$

$$
\begin{aligned}
0_3=\ & \pi_{\pi\alpha}\cos(\pi J_{13}z)\cos(4J_{12}4)-2S_{2H}2_{\pi 2}\sin(\pi J_{23}\pi)\cos(\pi J_{12}0)-2S_{Sx}\pi_{\pi\tau}\sin(\pi J_{12}\rho)\cos(\pi J_{13}z)\\
&-\pi S_{\pi y}S_{3\pi}\pi_{2\tau}\sin(\pi J_{12}\tau)\sin(4J_{13}z)+S_{2y}\cos(\pi J_{23}z)\cos(4J_{12}\rho)\\
&-2S_{\tau 1}\pi_{\pi 2}\sin(SJ_{23}z)\cos(4J_{12}1)-2\pi_{\tau\tau}3_{2x}\cos(SJ_{23}\tau)\sin(3J_{12}\tau)\\
&-2S_{12}\pi_{2y}\pi_{3z}\sin(\pi\!=_{23}\pi)\sin(\pi J_{Hz}\pi)+S_{\pi 2}\,di\,g(3J_{23}2)\cos(\pi J_{13}\tau)\\
&-\pi\pi_{2\tau}S_{\pi x}\sin(\pi i_{23}\pi)\cos(\pi 0_{23}z)-23_{\tau\tau}S_{\pi 1}\cos(\pi J_{23}\tau)\sin(\pi i_{13}\tau)\\
&+\beta 4_{\pi\rho}\pi_{4\tau}\pi_{3y}\sin(\pi 0_{23}z)\sin(2J_{13}\rho)
\end{aligned}
$$

4) $)\pi+y)_{-y}$

$$
\begin{aligned}
0_S+\ & -S_{1x}\cos(\pi J_{13}\tau)\cos(zJ_{12}1)+23_{z\tau}2_{\pi y}\sin(4J_{13}\tau)\cos(\pi J_{12}\tau)+2\pi_{\tau\pi}S_{2y}\sin(SJ_{Hz}\tau)\cos(\pi J_{13}\rho)\\
&-\pi S_{1x}3_{Sy}S_{22}\sin(2J_{12}1)\sin(3J_{13}1)-S_{\tau x}\cos(SJ_{23}\tau)\cos(3J_{12}\rho)\\
&-2S_{\tau 2}\pi_{\pi y}\sin(\pi J_{23}\tau)\cos(zJ_{x\square}\tau)+2S_{\tau 2}S_{2\tau}\cos(\pi J_{23}\tau)\sin(4J_{12}\rho)\\
&-\pi S_{1y}\pi_{\tau x}S_{\pi y}\sin(\pi J_{23}\rho)\sin(2J_{12}z)-S_{\pi 1}\cos(SJ_{23}\pi)\cos(\pi 0_{13}\pi)\\
&+S2_{S2}4_{\pi\pi}\sin(\pi i_{23}\pi)\cos(\pi 0_{23}z)+23_{zy}4_{\pi\pi}\cos(\pi 0_{23}\pi)\sin(\pi J_{13}\tau)\\
&)\ \pi S_{zy}\pi_{Sy}S_{3x}\sin(SJ_{23}1)\sin(\pi i_{13}\rho)
\end{aligned}
$$

Combined with phase cycle during the detection, the averaged density operator will be

$$
\begin{aligned}
z_{ave}\ J\quad & 2\!=\!\pi\tau\!-\!2S_{\pi y}\cos(4J_{13}\tau)\,di\,g(\pi i_{12}\tau)-\pi 2_{21}2_{\pi 2}\sin(SJ_{13}z)\cos(\pi i_{12}\rho)\\
&+y\pi_{\pi x}\pi_{2\tau}\sin(\pi J_{12}\tau)\cos(\pi J_{13}\pi)-y2_{1y}4_{Sz}4_{S\pi}\sin(\pi J_{12}\rho)\sin(\pi J_{13}1)\\
&+\pi\pi_{22}\cos(\pi J_{23}\tau)\cos(\pi J_{12}\tau)+44_{Sx}4_{S2}\sin(\pi J_{23}\pi)\cos(zJ_{12}2)\\
&+4S_{z\pi}\pi_{2x}\cos(\pi J_{23}\tau)\sin(zJ_{12}\boldsymbol{S})+8S_{\rho\tau}S_{22}S_{\pi\tau}\sin(\pi J_{23}\tau)\sin(3J_{13}\tau)\\
&-2S_{Sy}\cos(SJ_{23}\tau)\cos(z\!=_{13}\tau)+\pi\pi_{4\tau}2_{\pi x}\sin(\pi J_{23}\rho)\cos(\pi J_{13}\tau)\\
&+42_{1i}2_{SH}\cos(\pi J_{13}\rho)\sin(\pi J_{23}\tau)+32_{zi}2_{12}\pi_{\pi y}\sin(4J_{23}2)\sin(\pi J_{13}\tau)\\
&-4i\pi_{zx}\cos(\pi\!=_{13}1)\cos(zJ_{x\square}z)-\pi iS_{\tau\tau}2_{S2}\sin(4J_{13}\rho)\cos(2\!=_{12}\rho)\\
&-40S_{\rho\tau}\pi_{2y}\sin(\pi J_{12}\rho)\cos(\pi J_{23}\tau)+8JS_{\tau x}4_{\pi y}\pi_{2y}\sin(\pi 0_{x\square}\pi)\sin(\pi i_{13}\rho)\\
&+2i3_{z1}\cos(2J_{23}\tau)\cos(zJ_{x\square}\tau)-\pi JS_{2z}S_{3y}\sin(\tau\!=_{23}\tau)\cos(\pi J_{Hz}\rho)\\
&-\pi JS_{S4}2_{2\tau}\cos(SJ_{23}i)\sin(\pi 0_{12}\tau)=8JS_{12}S_{2x}\pi_{\pi y}\sin(\pi i_{23}\pi)\sin(3J_{x\square}\boldsymbol{S})\\
&+\pi\tau S_{3x}\,di\,g(\pi J_{23}\pi)\cos(3J_{13}\tau)-3J\pi_{S2}\pi_{Sz}\sin(2J_{23}\rho)\cos(zJ_{AC}\tau)\\
&-4i2_{\tau y}S_{\pi 1}\cos(2J_{13}\rho)\sin(3\!=_{x\square}2)-yiS_{i\,2}S_{02}S_{\pi 1}\sin(\pi J_{13}\tau)\sin(4\!=_{13}\rho)\rho
\end{aligned}
$$

Since the result of phase cycling for three-spin system is very complicated, it will not be shown here. However, all the terms shown in the i_{ave} are single quantum coherence terms which corresponds to in-phase and anti-phase signals in the NMR spectrum.

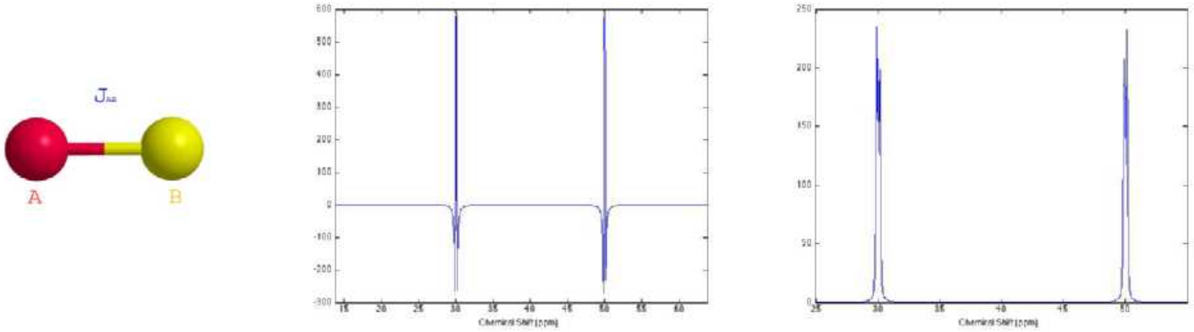
In this paper, we use Spinevoltuion[5] to simulate the INADEQATE experiment for these spin systems. Some interesting points can be found from these simulation results and it will become much more clear if we combine the simulation result and basic theory together.

Since for isolated spin system, we have already known from the density operators that no signal can be observed with INADEQUATE pulse sequence, we only look at the two-coupled spin system and three-spin system.

1) Two-coupled spin system

The simple model of two-coupled spin system is shown in Figure 2(a). $\mathrm{J_{AB}}$ is the scalar coupling constant between two spins.

In the simulation of two-spin system, he scalar coupling between each two spins were set as: $J_{AB} + 252$ 3. And ^{13}C Larmor frequency was set to 125.72MHz (corresponding to a static magnetic field of 11.74T). The chemical shifts of two nucleuses are: A (30ppm), B (50ppm). The result shows in Figure 2(b). In order to compare with direct pulse experiment, we also simulate the one pulse experiment (shown in Figure 2(c)). By comparing the two spectrums, it is easy to find that the INADEQUATE spectrum experiment is so different from the single pulse spectrum. In INADEQUATE spectrum, some anti-phase components will be observed.



(a) (b) (c)

Figure 2. (a) Two-coupled spin model. J_{AB} is the scalar coupling constant between two spins. (b) INADEQUATE spectrum. (c) Single pulse spectrum. In the simulation, $J_{AB} + 25x$. ^{13}C Larmor frequency was set to 125.72MHz. The chemical shifts of two nucleuses are: A (30ppm), B (50ppm).

From the basic theory of INADEQAUTE pulse sequence, it is known that the intensity of peak will be highest when the delay time of spin echo pulse sequence is exactly set as 1/4J. For this two-spin system, the J coupling constant is 25kHz, so 1/4J=10ms. So in the following part, we used simulation to test if this is the real case and show the correlation between the delay time and the peak intensity. Simulation result is shown in figure 3.

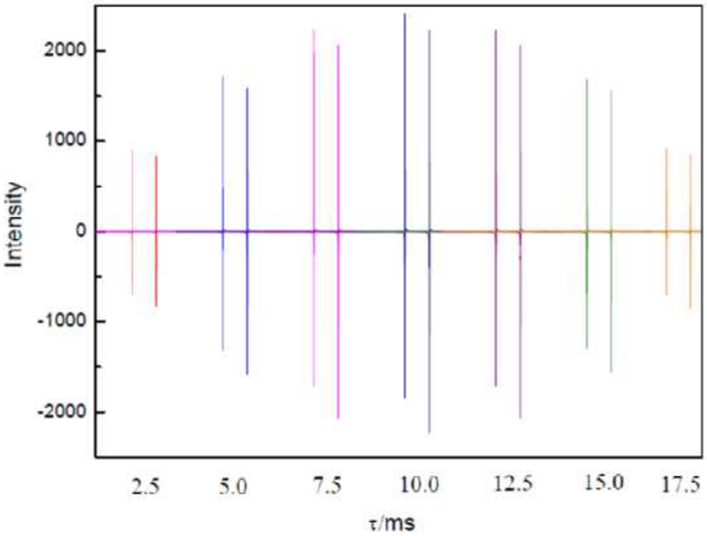


Figure 3. Plot of relationship between the delay time of spin echo pulse sequence and the peak intensity. The values of delay time used in the simulation are: 2.5ms, 5.0ms, 7.5ms, 10.0ms, 12.5ms, 15.0ms, 17.5ms.

From figure 3, it is found that the intensity reaches the highest value when the delay time τ is set exactly at 1/4J, which is consistent with the theoretical conclusion. According to the relationship between the delay time of spin echo pulse sequence and the peak intensity, we can improve INADEQUATE experiment in the real situation. Since the scalar coupling between different spin pairs varies over the whole spin system, we can change the delay time to get several spectrums. In that way, Correlation between all spin pairs in the system can be observed when combining these spectrums together and we can get a clear idea about the whole spin system.

2) Three-spin system

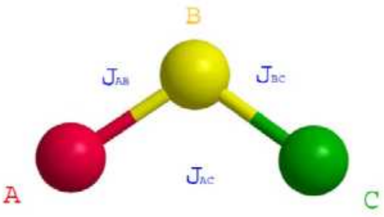


Figure 4. Three-spin system. J_{AB}, ≕$_{BC}$, ≕$_{AC}$ is the scalar coupling constants between corresponding two-spin pairs.

In the simulation of three-spin system, a triangle three-spin system was considered (Figure 4) and the scalar coupling between each two spins were set as: ≕$_{AB} = 25Hz, J_{BC} = 30x$ ⍰, ≕$_{AC} + yHz$. The reason why we set J_{AC} so small is because A and C are two bonds away and the scalar coupling between them should be much smaller than direct bonding system such as AB and BC. Also, in the simulation, we assume A, B, C are carbon atoms and ^{13}C Larmor frequency was set to 125.72MHz (corresponding to a static magnetic field of 11.74T). The chemical shifts of three nucleuses are listed as follows: A (20ppm), B (23ppm), C (26ppm). The delay time in the spin echo process was set as 10ms. The result is shown in figure 5(a).

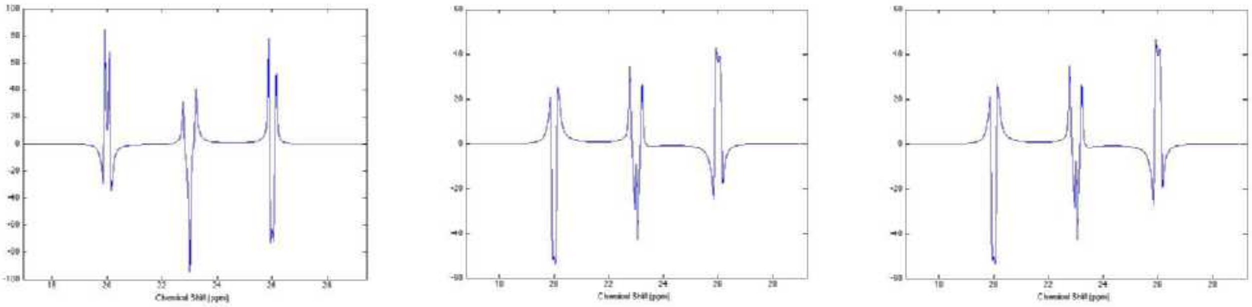
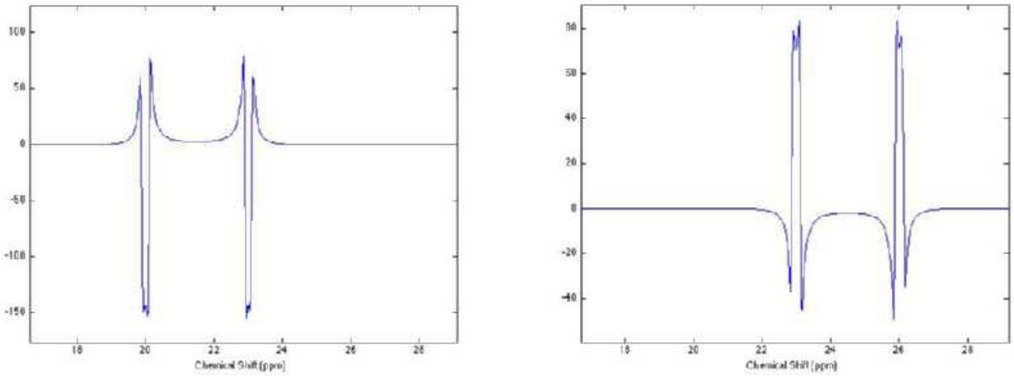

(a) (b) (c)

Figure 5. (a) $=_{\pi y} + 251\,3, J_{BC}$ ($30Hz, J_{AC} = 5Hz$. (b) $J_{AB} = 25Hz, =_{BC} = 30x$ ▯, $=_{AC} = J$ $\cancel{y}Hz$. (c) $)_{AB} = 251\,2, J_{BC} = 30Hz, =_{AC} = 1Hz$. ^{13}C Larmor frequency was set to 125.72MHz. The chemical shifts of three nucleuses are listed as follows: A (20ppm), B (23ppm), C (26ppm).

Some parameters can be modified so that we can understand the system more clearly. If we change J_{13} to smaller values such as $=_{AC} = 0.y$ and $J_{AC} + J$, we can see the result in figure 5(b) and figure 5(c). From the result, no very big difference can be observed if we change $=_{23}$ value to a really small value or even set it as 0. But still some small changes can be detected from these spectrums, especially for the spin A. If J_{23} is large or in the same order as $=_{AB}$ or $=_{BC}$, the peak shape of Spin A will be complicated since spin A intensively correlates with spin B and spin C at the same time. However, if $=_{23}$ is small, the peak shape of spin A is less complicated since spin A only correlates with spin B, which is more like a two-spin subsystem. Based on this point, we can tell more details about the spin system when analyzing the INADEQUATE spectrum. For example, for a particular spin, we can tell how complexity the correlation between it and other spins simply from its peak shape.

The reason why INADEQUATE pulse sequence is so powerful in determining the molecular structure is that it can suppress the signals from isolated spins, thus allowing the selective detection of NMR signals from correlated spin system. As we introduced before, this feature can be seen significantly when we do ^{13}C experiment with natural abundance sample. Since the natural abundance of ^{13}C is very low, in natural samples, isolated ^{13}C spins is everywhere. However, the really useful structure information comes from ^{13}C-^{13}C spin pairs. So by applying INADEQUATE pulse sequence, signals from isolated ^{13}C spins will be suppressed while the signals from very rare ^{13}C pairs will be detected. With help of some chemical shift information of nucleuses, we can surely draw out the molecule structure based on the INADEQUATE spectrum. To explicitly address this point, we used simulation method to show this nice feature of INADEQUATE pulse sequence. We used the same three-spin system as previous one. We isolated spin C, which means $J_{BC} = 1$ and $J_{AC} = \rho$. The result shows in figure 6(a). Also, we can isolate spin A in the same way by setting $=_{AB} = 1$ and $=_{AC} = 0$. The result shows in figure 6(b).

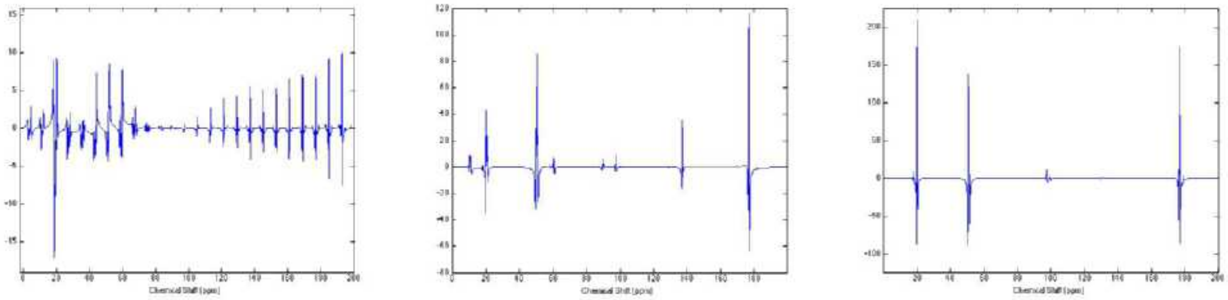


(a) (b)

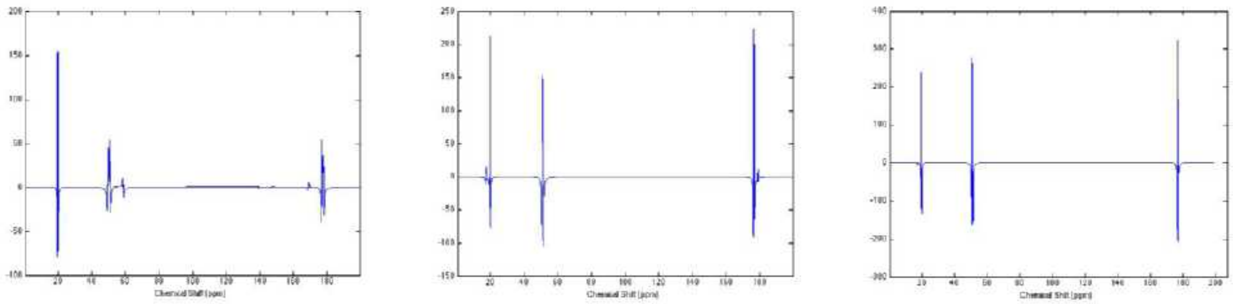
Figure 6. (a) $J_{BC} = 252\,3$, $=_{BC} = J\,Hz$, $=_{AC} + 01\,3$. (b) $=_{BC} = J\,1\,2$, $=_{BC} = 30x$□, $=_{AC} = 0x$□. ^{13}C Larmor frequency was set to 125.72MHz. The chemical shifts of three nucleuses are listed as follows: A (20ppm), B (23ppm), C (26ppm).

The simulation result shows that if we treat spin C as an isolated spin, the signal from spin C will not show in the spectrum (Figure 6(a)). The same result can be found if we treat spin A as an isolated spin (Figure 6(b)). This result is consistent with the basic idea of INADEQUATE experiment, which can suppress the signal from the isolated spins.

In the last part, we simulated the 1D ^{13}C INADEQUATE solid-state NMR spectrum of ^{13}C-labeled alanine. Basically, the alanine molecule can be treated as a three-spin system. The scalar coupling information is shown as follows: ^{2}J(^{13}CO-^{13}CH)=54Hz, ^{2}J(^{13}CH-^{13}CH$_3$)=34Hz, ^{2}J(^{13}CO-^{13}CH$_3$)=1.3Hz. The chemical shifts of three spins are 176.8ppm(^{13}CO), 50.9ppm(^{13}CH), 19.8ppm(^{13}CH$_3$). The ^{13}C Larmor frequency was set to 125.72MHz and the spinning speed varies. For the powder average part, 168 crystals was used and γ=50. Also, we simulated several spectrums at various spinning speeds. The result is shown in Figure 7.



(a) (b) (c)



(d) (e) (f)

Figure 7. INADEQUATE spectrum of alanine at different spinning speed: (a) 1kHz; (b) 5kHz; (c) 10kHz; (d) 15kHz; (e) 20kHz; (f) 30kHz. The chemical shifts of three spins are 176.8ppm(^{13}CO), 50.9ppm(^{13}CH), 19.8ppm($^{13}CH_3$). The ^{13}C Larmor frequency was set to 125.72MHz and the spinning speed varies. For the powder average part, 168 crystals was used and γ=50.

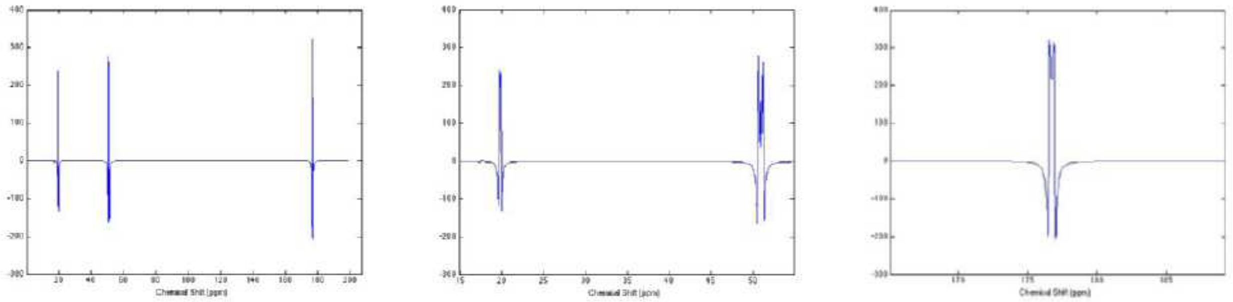


Figure 8. INADEQUATE spectrum of ^{13}C –labeled alanine with spinning speed at 30kHz. The chemical shifts of three spins are 176.8ppm(^{13}CO), 50.9ppm(^{13}CH), 19.8ppm($^{13}CH_3$). The ^{13}C Larmor frequency was set to 125.72MHz and the spinning speed varies. For the powder average part, 168 crystals was used and γ=50.

From the simulation result shown in Figure 7, we can see some artifacts in the spectrum with low spinning speed and they will be got rid of slowly with increasing the spinning speed. Since we simulate a solid-state experiment, which is a very inhomogeneous situation compared with solution experiment. For solid-state NMR, the result usually intensively related with spinning speed. If we apply a high spinning speed, the system will be more close to a homogeneous situation, then we can get rid of most artifacts which will be shown at low spinning speed. In our simulation, most artifacts will be removed away if we apply a 20kHz spinning speed or higher.

Figure 8 shows some detail peak information of experiment with spinning speed at 30kHz, By comparing the spectrum with the spectrum we simulated for simple three-spin system, we can easily find that its very similar to the result of three-spin system with very small $=_{AC}$. These two simulated experiments are consistent.

Conclusion

In this paper, we mainly introduced the basic theory of INADEQUATE, including isolated spin, two-spin, three-spin systems. By applying product-operator analysis, we clearly showed the evolution of density matrix during INADEQUATE experiment. To be more easy understanding, we also simulated several INADEQAUTE experiments, which clearly show the basic principle of INADEQAUGE pulse sequence. In the last section, we simulate the 1D INADEQUATE spectrum for alanine molecule. From the analysis of INADEQUATE by product operator and numerical simulation method, we can conclude that INADEQUATE experiment is a very useful tool for NMR researchers to determine molecular structure simply based on the through-bond interaction between spin pairs.

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