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Diehl P., Sykora S., Vogt J.

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Abstract

The analysis of high resolution NMR spectra of coupled nuclear systems is often a very tedious process. The methods used to solve this task have so far always been based on frequencies (and sometimes also intensities) of selected and well assigned lines. In this paper we present a broad class of methods which not only do not require any interpretation of experimental lines but do not even require any lines should be individually distinguishable. The first experiences with some of these methods are reported. They lead to quite optimistic conclusions regarding the possibility of full automation of the problem in question. The algorithms used consist in (i) converting the spectrum, however complicated, into a limited set of real numbers by means of suitable integral transforms, and (ii) fitting these numbers by means of the usual iterative least-square algorithm. The very simplicity of this idea makes it possible to apply the method with equal ease to the analysis of any complicated function (spectra) defined by a limited number of parameters.

Keywords: NMR spectra, Simulation of spectra, Automatic analysis, Fitting

Automatic Analysis of NMR Spectra: An Alternative Approach

P. DIEHL, S. SÝKORA*, AND J. VOGT

Physikalisches Institut der Universität Basel, Klingelbergstrasse 82, 4056 Basel, Switzerland

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The analysis of high resolution NMR spectra of coupled nuclear systems is often a very tedious process. The methods used to solve this task have so far always been based on the frequencies (and sometimes also intensities) of selected and well assigned lines. In this paper we present a broad class of methods which not only do not require any interpretation of experimental lines but do not even require that any lines should be individually distinguishable. The first experiences with some of these methods are reported. They lead to quite optimistic conclusions regarding the possibility of full automation of the problem in question. The algorithms used consist in (i) converting the spectrum, however complicated, into a limited set of real numbers by means of suitable integral transforms, and (ii) fitting these numbers by means of the usual iterative least-square algorithm. The very simplicity of this idea makes it possible to apply the method with equal ease to the analysis of any complicated functions (spectra) defined by a limited number of parameters.

I. INTRODUCTION

The computer analysis of high-resolution NMR spectra of coupled nuclear systems often represents a tedious and time-consuming problem which has rarely any scientific value in itself. A well established approach to this problem (1) consists in (i) proposing a set of starting spectral parameters (by spectral parameters we will mean chemical shifts, indirect and/or direct coupling constants, etc.), (ii) calculating the theoretical spectrum, (iii) interpreting as many experimental lines as possible in terms of the theoretical transitions, and (iv) adjusting iteratively the starting parameters so as to minimize the quantity

$$\Delta^2 = \sum_{i \in \mathcal{S}} (\omega_i^{\text{ex}} - \omega_i^{\text{th}})^2, \quad [1]$$

where \mathcal{S} is the selected subset of experimental lines, ω_i^{ex} are their experimental frequencies, and ω_i^{th} are the corresponding theoretical frequencies.

Although this method is widely used, it has several practical drawbacks.

(i) The interpretation of experimental lines is time consuming, tedious, and very vulnerable to error, especially if the density of lines exceeds certain limits or when the estimated parameters differ from the true ones so much that there is little similarity between the theoretical and the experimental spectra.

(ii) The initial estimate of the parameters requires usually a great deal of prior knowledge and experience. Sometimes even these are of little help, particularly for

* Present address: Bruker Spectrospin Italiana, Via Pasquale Miglioretti 2, 20161 Milano, Italy.

spectra from oriented species where it is often virtually impossible to make sufficiently precise predictions of the direct coupling constants.

(iii) In many cases, it is difficult to select a sufficient number of experimental lines each of which would correspond to a single transition. This situation arises regularly in large spin systems where any apparently single experimental line is usually composed of a number of closely packed theoretical transitions (consider that there are 792 transitions in a general six-spin-1/2 system and 11,440 transitions in a general eight-spin-1/2 system). The line overlap may also be due to a line-broadening process which is inherent to the particular system and cannot be avoided.

(iv) It is well known (2, 3) that the transition frequencies alone do not always specify the spectral parameters uniquely; however, the solution is unique when both the frequencies and the intensities of the transitions are considered.

(v) In some special cases the spectrum may be insensitive to a certain spectral parameter or to a combination of parameters. Considering the experimental noise, these cases lead to a continuous range of solutions. Situations of this kind are not easy to detect unless a complete error analysis is performed.

The disadvantages listed above prompted many NMR spectroscopists to think about alternative methods of analyzing the spectra. Simple modifications, such as inclusion of transition intensities in the least-squares algorithm (4) may help in special cases but their general value seems rather limited. Other approaches appearing under the general heading "automatic analysis" (5), aim at including in the program as much specific knowledge of the NMR problem as possible; for example, the energy-level diagram, repeated spacing, and intensity flow rules. The general applicability of these methods, however, has not been established and remains somewhat questionable.

In this paper we will sketch an alternative approach to the analysis of NMR (and possibly also EPR) spectra which avoids most of the obstacles listed above. The respective programs as well as the algorithm itself are still in development, but the results we have obtained so far are encouraging enough to warrant a rather detailed report.

Let us consider what are the prerequisites of any iterative analysis of a spectrum. First, a quantity is needed which will summarize within a single number Δ the difference between the experimental spectrum and its theoretical counterpart. Secondly, a local minimum of Δ (or Δ^2) must be found by iterative modification of the initial parameters defining the theoretical spectrum. The adjusted parameters are then tentatively accepted as a solution and a direct comparison of the complete theoretical and experimental spectra is carried out. In the common approach Δ is defined by Eq. [1], but we will see that there is a great freedom in the choice of this quantity and any particular choice amounts to a distinct method with its own advantages and disadvantages.

The most general definition of Δ compatible with the least square algorithm is

$$\Delta^2 = \sum_{k=1}^{ln} [\mathcal{F}_k\{I_{\text{ex}}(\omega)\} - \mathcal{F}_k\{I_{\text{th}}(\omega)\}]^2, \quad [2]$$

where $I_{\text{ex}}(\omega)$ and $I_{\text{th}}(\omega)$ are the experimental and theoretical spectra, respectively, and $\mathcal{F}_k\{I(\omega)\}$ is a functional, i.e., a mapping which assigns a real number $\mathcal{F}_k\{g(\omega)\}$ to any function $g(\omega)$. In other words, the spectrum $I(\omega)$ is characterized by a limited set of n real numbers and the aim is to fit theoretically the set of such numbers derived from the experimental spectrum.

In order to demonstrate the profound relation between the definition of Δ and the basic features of the fitting algorithm, we will describe a method which is itself quite promising, although we have not applied it to any extensive checks as yet. A spectrum may be considered as a continuous and absolutely integrable function $I(\omega)$, where I is the intensity and ω is the frequency. As such, any spectrum belongs to many of the classical functional spaces. One also feels intuitively that the quantity Δ should possess many of the properties of a metric in a functional space including all possible spectra. Vice versa, any metric in such a functional space defines a suitable Δ . Consider, for example, the most common metric function related to the space of quadratically integrable functions, i.e.,

$$\Delta^2 = \int_{-\infty}^{+\infty} [I_{\text{ex}}(\omega) - I_{\text{th}}(\omega)]^2 d\omega. \quad [3]$$

This definition has the advantage that no interpretation of experimental line is necessary in order to evaluate Δ . On the other hand, a new problem arises. Suppose that the spectrum consists of a set of very sharp lines and consider Δ as a function of the spectral parameters. Δ then attains its highest value whenever there is no coincidence at all between the experimental and theoretical lines (i.e., almost everywhere). At those points where any two or more lines coincide, there will be a narrow "hole" in the Δ -surface. Among the large number of such holes, there will also be the one corresponding to the true solution. To find this minimum by any iterative program is evidently nearly impossible because of both the density of the minima and their narrowness.

The above considerations lead to the following requirements for an optimal method. First, Δ should not depend on the details of the computations leading to the theoretical (simulated) spectra. Once this condition is satisfied, the method acquires generality (it can be applied to any spectroscopy, not just NMR) and the bothersome interpretation of the experimental lines is completely eliminated. Second, the definition of Δ must be applicable to the stick spectra as well as to the continuous spectra with extensive transition overlap. Third, Δ should be a very smooth function of the parameters with rather broad minima. This means that it should reflect principally the global features of the spectrum (this is analogous to the way a spectroscopist's eye works when comparing two spectra at a single glance). The smoothness also implies that the local minima of Δ are broad and can be easily located by computer even if the starting parameters are quite wrong.

In order to be sure that Δ of Eq. [2] satisfies the above requirements, it is sufficient to choose the functionals \mathcal{F}_k in such a way that they themselves satisfy the same requirements. In the following sections we will discuss some of the methods which fit into this general category and will demonstrate the applicability of these methods to a broad range of test cases. We will also demonstrate additional problems that may arise, both as mathematical artifacts of the particular choice of the functionals and as a result of real properties of the NMR spectra.

II. SPECIAL CLASSES OF FUNCTIONALS

First, we must determine which sets of functionals are best suited for our purposes. Since any single-number characteristic of a spectrum is a functional, the freedom of choice is quite overwhelming. In principle, the problem could be analyzed by purely

theoretical means. This way, however, was so complex and cumbersome that we abandoned it. A more viable approach was to find a few general criteria limiting the type of the functionals and then to proceed by trial and error selecting each time a different set of functionals and applying it to a number of typical spectra. An important factor in choosing the functional is of course also their mathematical simplicity.

In this study we have limited ourselves to linear functionals. A functional $\mathcal{F}\{\cdot\}$ is linear if it satisfies the relation

$$\mathcal{F}\{af(\omega) + bg(\omega)\} = a\mathcal{F}\{f(\omega)\} + b\mathcal{F}\{g(\omega)\} \quad [4]$$

for any two functions $f(\omega)$ and $g(\omega)$ and for any real numbers a and b .

The linear functionals present some numerical advantages. Thus when a spectrum is composed of a number of transitions, the corresponding functional becomes a sum of terms each of which is related to just one transition. This makes it possible to compute the functionals without actually storing all the transition frequencies and intensities. On the other hand, it is quite possible that suitable nonlinear functionals might lead to a better estimation of the spectral parameters, especially if the experimental spectra are burdened by noise, distorted by saturation, incomplete, etc.

A very general class of linear functionals which are continuous and smooth functions of the spectral parameters are the integral transforms defined as

$$\mathcal{F}_w\{I(\omega)\} = \int_{-\infty}^{+\infty} w(\omega) I(\omega) d\omega, \quad [5]$$

where $w(\omega)$ is a smooth function of ω . A set $B^n \equiv \{W_k(\omega); k = 1, 2, \dots, n\}$ of functions $w_k(\omega)$ then defines the set of functionals $\{\mathcal{F}_k \equiv \mathcal{F}_{w_k}; k = 1, 2, \dots, n\}$. The set B will be called the basis of the integral transforms adopted.

It is evident that the integral transforms still offer an extraordinary freedom of choice since any distinct set of n reasonably smooth functions of ω defines a distinct basis.

Another restriction is that the basis should consist of linearly independent functions. A linear relationship between the functions $w_k(\omega)$ would lead to a linear relationship between the corresponding transforms $\mathcal{F}_k (k = 1, 2, \dots, n)$ which would then contain less information than if the function $w_k(\omega)$ were linearly independent.

The choice of linearly independent functions presents no mathematical problems, but it is well known that for large n it may present considerable numerical problems due to the limited precision of numerical calculations. An elegant way of assuring linear independence consists in choosing a set of mutually orthogonal functions, the orthogonality being defined by the equation

$$\int_{-\infty}^{+\infty} w_i(\omega) w_j(\omega) d\omega = 0 \quad [6]$$

for any $i \neq j$. Although this may be asking too much for some purposes (we will see later that nonorthogonal bases are often quite useful), there are additional reasons for orthogonal bases. As shown in Appendix A, Eq. [A5], the transforms are statistically independent if and only if the basis is orthogonal. Statistical independence simplifies the treatment of error propagation and, on the average, tends to reduce the errors in the final spectral parameters.

It is also useful to have the functions w_k quadratically normalized. The variances V_k of the respective transforms due to the experimental noise are (see Appendix A)

$$V_k = \rho^2 \int_{-\infty}^{+\infty} w_k^2(\omega) d\omega, \quad [7]$$

where ρ is the rms of the noise. When the functions $w_k(\omega)$ are normalized, then all the expected variances are the same. This means that in such a case all the transforms should be given the same weight. Moreover, the residual Δ left after having adjusted the spectral parameters can be compared with the rms of the noise in order to see whether the minimum reached is physically acceptable ($\Delta^2 \simeq n\rho^2$), or not ($\Delta^2 \gg n\rho^2$).

A question concerning the uniqueness of the solution now presents itself. Once again, we can expect only a limited help from the theory since the complexity of the problem makes it nearly intractable. There are two lines of reasoning that may put this aspect of the problem on a somewhat firmer ground. First, suppose that the set $\{w_k; k = 1, 2, \dots\}$ is an orthonormal and *complete* basis in the Hilbert space of the quadratically integrable functions. By the completeness theorem, the deviation Δ defined by Eq. [2] is then identical with that one defined by Eq. [3]. The uniqueness of the latter is assured by the fact that it vanishes only if the experimental and theoretical spectra are exactly identical and by a uniqueness theorem due to Kummer (3). A complete basis necessarily consists of an infinite number of elements. Since, however, the property of uniqueness either is there or not, there must exist a critical number of transforms beyond which the solution is always unique. Second, we can argue that the set of all NMR spectra of a given type (i.e. AB, A_2B_3 , ABC, ABCD₂, etc.) forms a very "thin" finite-dimensional hyper-surface in the infinite-dimensional Hilbert space of all quadratically integrable functions. Choosing a basis of n functions unrelated in any explicit way to the Hamiltonian, it is obvious that when n equals or exceeds the number of spectral parameters, the probability of each of the transforms having the same value for two different sets of the parameters must be very small. In other words, even though no uniqueness theorem is available, we do expect multiplicity of solutions only when there are some physical grounds for it.

Somewhat more bothersome is the existence of spurious local minima of the Δ -function. Here we refer to the local minima associated with the chosen basis rather than those due to the physical similarity of spectra generated from different sets of spectral parameters. In general, the orthogonal bases, which for large n necessarily include rapidly oscillating functions, are more likely to produce false minima than non-orthogonal bases composed of nonoscillatory functions. This is one reason why non-orthogonal bases may be preferable whenever there is a possibility that the starting spectral parameters might be quite wrong.

We considered several bases during different stages of this work and these are briefly discussed below.

(i) *The Fourier Basis Over the Interval $[a - \frac{1}{2}d, a + \frac{1}{2}d]$ (Fig. 1(a))*

Consider the complete, orthonormal set of functions $\{w_k(\omega); k = 1, 2, \dots, \infty\}$ such that

$$\begin{aligned} w_{2l-1}(\omega) &= (d/2)^{-1/2} \cos [(l\pi/d)(\omega - a)] \\ w_{2l}(\omega) &= (d/2)^{-1/2} \sin [(l\pi/d)(\omega - a)]. \end{aligned} \quad [8]$$

Any subset of n such functions may form a basis suitable for computer calculations. In our case we have always used the first n consecutive functions. One might use, however, the set $\{w_K, w_{K+1}, \dots, w_{K+n}\}$, where K is a positive integer. The higher functions, being more oscillatory than the lower ones, are more sensitive to fine details of the spectra and thus better suited for fine refinement of the spectral parameters rather than for a rough initial fit. As far as mathematical simplicity is considered, bases of this type do not lead to any numerical problems (see Appendix B). From the practical point of view, we

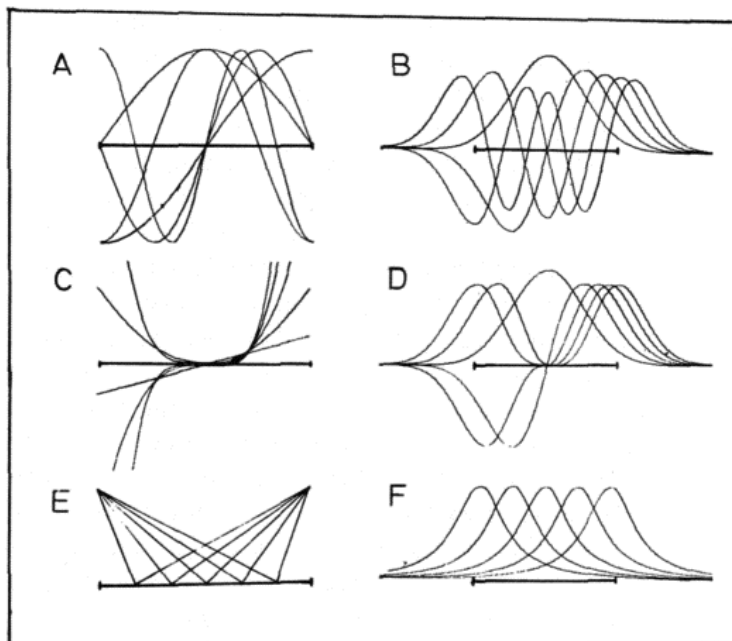


FIG. 1. Some of the bases used (only 5 functions are shown): (A) Fourier trigonometric functions, (B) Hermitian functions, (C) moments, (D) Gaussian functions, (E) broken linear functions, and (F) Lorentzian functions (delocalized basis).

should yet discuss the choice of the center a and the width d of the interval; this we will do in the next section.

(ii) *The Hermitian Basis Over the Interval $(-\infty, \infty)$ (Fig. 1(b))*

In this basis the functions $w_k(\omega)$ are defined as

$$w_k(\omega) = (2^k k! \alpha \pi^{1/2})^{-1/2} \exp[-\frac{1}{2}((\omega - a)/\alpha)^2] H_k((\omega - a)/\alpha), \quad [9]$$

$$k = 0, 1, 2, \dots,$$

where $H_k(x)$ are the Hermitian polynomials, α is a scale parameter, and a is a center. As before, these functions form a complete orthonormal set. We have generally used as a basis the first n of them. They present no numerical problems for stick spectra, although at nonzero linewidths the problems are considerable (Appendix B). As in the previous case, we leave open the choice of the center a and the scale parameter α . It is interesting to notice that for very large k the Hermitian functions look very much like the trigonometric functions (compare Fig. 1(a) and (b)). Although the Hermitian basis was the first

one we adopted, we realized later that, for the reasons just mentioned, the Fourier basis is probably preferable.

(iii) *The Gaussian Basis Over the Interval $(-\infty, \infty)$ (Fig. 1(d))*

The normalized functions

$$w_k(\omega) = (2^{-k}(2k-1)!!\alpha\pi^{1/2})^{-1/2}((\omega-a)/\alpha)^k \exp[-\frac{1}{2}((\omega-a)/\alpha)^2], \quad [10]$$

$$k = 0, 1, 2, \dots,$$

provide a complete set which is closely related to the Hermitian basis, but the functions lack the sometimes undesirable oscillatory character. This was of course achieved only at the price of losing the orthogonality of the functions.

(iv) *The Set of Moments Over the Interval $(a-d/2, a+d/2)$ (Fig. 1(c))*

The functions

$$w_k(\omega) = [(2k+1)/d^{2k+1}]^{1/2}((\omega-a)/d)^k, \quad k = 1, 2, \dots, \quad [11]$$

form a normalized, complete set over the interval considered. They are nonorthogonal but also completely nonoscillatory, and very easy to handle. A further advantage is that they are related to the moments of the spectra which are well-known quantities. Their principle disadvantage is that for high k , $w_k(\omega)$ is negligible anywhere except in the immediate vicinity of the borders of the interval $(a-d/2, a+d/2)$. This is clearly undesirable and so only a limited number of these functions is of any use (we have generally taken the first seven of them).

(v) *The Delocalized Lorentzian Basis Over an Interval (a, b) (Fig. 1(f))*

The definitions of all the above bases refer to a particular point, namely the center, or origin, a . We will call such bases localized. An example of a delocalized basis might be

$$w_k(\omega) = \mathcal{L}(\omega - \omega_k, \delta\nu) = \frac{2}{(\pi\delta\nu)^{1/2}} \left\{ 1 + \left[\frac{2(\omega - \omega_k)}{\delta\nu} \right]^2 \right\}^{-1}, \quad k = 1, 2, \dots, n, \quad [12]$$

where $\mathcal{L}(\omega, \delta\nu)$ is the quadratically normalized Lorentzian line of linewidth $\delta\nu$ and $\omega_k = a + (k-1)(b-a)/(n-1)$. It is actually possible to show that for sufficiently large n and $\delta\nu$ the deviation Δ defined by Eq. [2] using this basis is approximately equal to the deviation Δ defined by Eq. [3].

(vi) *The Broken Linear Functions (Fig. 1(e))*

The nature of this basis is self-explanatory from Fig. 1. We have tried it at one point in an unsuccessful attempt to increase the convergence rate.

III. A BRIEF DESCRIPTION OF THE PROGRAM USED

To carry out the iterative adjustment of the parameters, we used a variant of the conventional least-square algorithm with damping. For each iteration, the transforms and the derivatives of the transforms with respect to all the parameters are evaluated. The derivatives were determined numerically. The transforms themselves were calculated as sums of contributions each arising from a single transition. The transition

frequencies and intensities are read from cards (the experimental spectra) or generated by a conventional program (the theoretical spectra).

Before the iterations are started, the center of gravity of the experimental spectrum is determined and identified with the center parameter a of the localized bases. Somewhat more complicated is the determination of the width d of the basic interval in such bases as Fourier, moments, or broken linear. The criterion is that the interval ($a - d/2$, $a + d/2$) must just about comprise both the experimental and the theoretical spectrum. Whenever this condition is too flagrantly violated (more than 20% discrepancy) during the process of adjustment, the program interrupts the iterations, readjusts d , recalculates the transforms for the experimental spectrum then re-starts the iterations. Similar behavior has been programmed with regard to the scale parameter α in bases such as Hermitian or Gaussian. Here the criterion is that the highest function employed should attain its maximum (in absolute value) at the points which approximately coincide with the points $a - (d/2)$ and $a + (d/2)$ of the previous case.

In the first trials we noticed a general tendency for the coupling constants to go to completely unrealistic regions before the chemical shifts are at least roughly adjusted and only afterwards to return. In order to remedy this undesirable effect, we introduced optional constraints which force any desired parameter to stay within predetermined bounds.

From what has been said in the preceding section, the bases which are suitable for a very fine refinement of the parameters are not optimal for a rough preadjustment. Because of this, we developed a multistep (tandem) algorithm in which the parameters are (i) preadjusted using a basis which includes the first seven moments plus a set of nonoscillatory functions (in our case Gaussian) weighted in such a way as to reduce the runaway tendency of the coupling constants (we also used kw_k instead of the w_k of Eq. [10]), (ii) refined using the Gaussian basis, and (iii) refined still further using the Hermitian bases.

For our trial experiments we have not introduced weak coupling or symmetry factoring of the Hamiltonian.

Since the program is still in development we will not attempt to describe it in more detail. This will be done elsewhere in a specialized report.

IV. PRACTICAL EXAMPLES

The principal aim of this study was to check whether the algorithm described above could be applied with success to cases with quite randomly generated starting parameters. At this stage we were not interested in the much easier problem of the refinement of a set of almost accurate parameters.

We limited ourselves to 2-, 3-, and 4-spin systems. All the localized bases described in Section II were used both separately and in tandem. In the majority of cases, however, the three-step procedure described in Section III was adopted. The number of transforms was arbitrarily set to 10 for the AB cases, 18 for the ABC cases, and 24 for the ABCD cases. A number of trials with different numbers of transforms was also performed. The algorithm did not seem to be very sensitive to this parameter.

Most of the experimental spectra were simulated on the computer. For the purposes of this study we assumed zero linewidth (stick spectra). Given the nature of the method, this can hardly have any bearing on the validity of our results.

The approximate time required by the computer UNIVAC 1108 was 2, 10, and 100 seconds for fitting the AB-, ABC-, and ABCD-spectra, respectively.

(i) *The AB-Type Spectra*

An experimental spectrum was simulated using the parameters $\omega = -30$ Hz, $\omega_B = 30$ Hz, and $J_{AB} = 15$ Hz. For the starting parameters, all possible qualitatively distinct combinations were considered (the ω 's ranged from -2000 to 2000 Hz, the J 's from 0 to 5000 Hz). In all these cases, the correct solution was found. The average number of forward runs needed to find the solution was about 60.

(ii) *The ABC-Type Spectra*

In this case both the starting parameters and the parameters used to simulate the experimental spectra were generated as independent random numbers lying between -100 and $+100$ Hz for the chemical shifts and between -40 and $+40$ Hz for the coupling constants. In 49 test cases, out of a total of 50, the program found a solution. Of the solutions, 24 were identical with the parameter sets used to generate the experimental spectra, and 25 differed from the latter by the sign of one or more coupling constant (this, especially if combined with a small readjustment of the absolute values, often leads to a nearly identical spectrum). The average number of forward runs needed was about 85. In a variation of this test, the starting values of all the coupling constants were put equal to zero. Within the bounds of statistical significance, this did not influence the efficiency of the program.

It turned out, however, that very few of the above cases were really strongly coupled systems. In order to get more experience with strongly coupled systems, we generated two similar sets of test cases but this time the range for the chemical shifts was set equal to -10 to $+10$ Hz while the range for the coupling constants was -10 to $+10$ Hz in the first set and -20 to 20 Hz in the second set. Under these circumstances, the rate of success decreased significantly (only about 70% of the solutions fitted the spectrum either exactly or at least approximately; the percentage of exact solutions was about 40%). Changes in bases, such as substituting the Hermitian basis by the Fourier basis or increasing the number of transforms did not improve the performance.

The program was tentatively applied also to some ABC-spectra of oriented molecules. The ranges of the randomly generated parameters were: -300 to $+300$ Hz for chemical shifts, -1500 to 1500 Hz for direct coupling constants, and -20 to $+20$ Hz for indirect coupling constants. The performance was about as good as in the strongly coupled isotropic cases. Out of 12 examples, 5 were solved exactly, in 3 some of the direct coupling constants switched signs and ended with an approximate fit of the spectrum, and in 4 cases no solution was found (i.e. a local minimum without physical significance was located).

(iii) *The ABCD-Type Spectra*

The tests were carried out in the same way as in the ABC case. The ranges of the randomly generated parameters were -100 to $+100$ Hz for chemical shifts and 0 to 20 Hz for coupling constants. The fit was exact in about one half of the cases studied. All the remaining cases were characterized by at least one very strong coupling leading

to a kind of deceptive simplicity where the solution is not unique. The average number of forward runs needed to find a solution was about 195.

Next we used an experimental proton spectrum of 2-benzoylpyridine (7) and tried to fit it, starting with parameters which were progressively more and more removed from the true values (both positive and negative excursions were adopted at random). A solution was always found even if the starting parameters were wrong by as much as ± 100 Hz for the chemical shifts and ± 10 Hz for the coupling constants. In about two thirds of the cases with very wrong starting parameters, however, the program ended with inverted signs of some of the coupling constants so that the fit was only approximate.

(iv) The Influence of Experimental Noise

As most of our test cases were based on simulated (and therefore noiseless) spectra, we wanted to investigate also the influence of noise on the final fit. From the experimental point of view, the errors in line intensities exceed considerably the errors in line positions. Since our method does take intensities into account, we were somewhat worried that these errors might be reflected in the final parameters.

In order to check this point, we simulated noisy experimental spectra by adding to the intensity of each transition a number produced by a random number generator with a Gaussian distribution characterized by a standard deviation proportional to the intensity of the transition in question. The ratio ρ between the standard deviation and the line intensity (i.e., the relative error) was kept constant for all lines within a spectrum. The resulting set of lines was then fitted exactly as in the noiseless cases.

For the AB-spectra, ρ was increased progressively from 0.05 up to 1.0. Surprisingly, this had little effect on the final parameters. Even an exceedingly large noise ($\rho = 1.0$) led to an error of only about 0.05 Hz (out of 30 Hz) for the chemical shifts and about 0.4 Hz (out of 15 Hz) for the coupling constant.

For the ABC-spectra, the level of the artificial noise was held constant, either at $\rho = 0.01$ or 0.1. In all the cases studied, a solution was still found and the effect of the noise on the final parameters was very small. For some reason, however, the proportion of solutions with inverted signs of coupling constants was substantially higher than in the noiseless cases.

Finally, as a very extreme case, we tried to fit the same ABC spectra with all transition intensities smaller than 0.2 put equal to zero and all intensities bigger than 0.2 put equal to 1. Even these truncated spectra were still fitted with the final chemical shifts within 4 Hz, and the coupling constants within 2 Hz, of the original values.

We also expanded our tests to the noisy experimental spectrum of the ABC system acrylonitrile, using line positions and intensities as given in the literature by Castellano and Waugh (2).

The parameters which we obtained from a conventional fit with line assignment based on preknowledge of parameters are as follows:

$$\begin{aligned}\omega_1 &= 14.35 \pm 0.04 \text{ Hz}, \omega_2 = 19.25 \pm 0.03 \text{ Hz}, \omega_3 = 33.60 \pm 0.04 \text{ Hz} \\ J_{12} &= 1.26 \pm 0.04 \text{ Hz}, J_{13} = 18.07 \pm 0.04 \text{ Hz}, J_{24} = 11.22 \pm 0.07 \text{ Hz}.\end{aligned}$$

In our approach we first assumed that the chemical shifts are approximately known but not the couplings:

$$\omega_1 = 10.0 \text{ Hz}, \omega_2 = 20.0 \text{ Hz}, \omega_3 = 30.0 \text{ Hz}; J_{12} = J_{13} = J_{23} = 0 \text{ Hz}.$$

In a second trial we chose more extreme starting values for the chemical shifts but reasonable couplings:

$$\omega_1 = 5.0 \text{ Hz}, \omega_2 = 15.0 \text{ Hz}, \omega_3 = 40.0 \text{ Hz}, J_{12} = 0.0 \text{ Hz}, J_{13} = J_{23} = 10.0 \text{ Hz}.$$

In both cases line assignment for the conventional methods of analysis was impossible. Our program, however, found the correct solution in the first runs within the errors of the above cited analysis.

V. VARIOUS PROBLEMS ENCOUNTERED

Most of the problems encountered in the iterative fit of an experimental spectrum are usually related to the question whether there exists a one-to-one relation between the parameters of the Hamiltonian and the spectrum or whether the iterative process may lead to a series of equivalent or nearly equivalent solutions. For an iterative fit, it must be kept in mind that not only the exact equivalence but also a simple similarity of spectra may lead to local minima, and consequently, to multiple solutions. The uniqueness problem has several aspects and consequently it has been discussed under many different headings such as permutation of nuclei, inversion of coupling constants, weak coupling, very strong coupling with degeneracy, and deceptive simplicity.

(i) Nuclear Permutation

In systems like ABC, ABCD, etc., the labeling of nuclei is arbitrary (this is not so in systems like AB₂ or AB₂C₃). Consequently, any permutation of the labels does not affect the spectrum (3). The general system composed of n distinct nuclei leads to $n!$ such permutations. In other words, Δ^2 as a function of parameters has at least $n!$ minima which are all equivalent to the exact solution.

(ii) Inversion of the Signs of Coupling Constants

Changing simultaneously the signs of all the internuclear couplings leaves an NMR spectrum intact (3). This doubles the number of physically equivalent solutions.

(iii) Weak Coupling

In weakly coupled systems not only a simultaneous change of all the signs of coupling constants but also the change of any individual sign leaves the spectrum unaltered. Consider the 3 spin system for which there are 8 possible combinations of absolute signs of indirect couplings. As pointed out above, 4 of these are distinguishable for strong coupling. For ABX systems, that is systems with two weak couplings, the relative sign of J_{AB} is lost, so that only 2 combinations of signs remain distinct. Finally in the AMX cases none of the 8 sign combinations is distinct from the others and the computer may find 48 equivalent solutions by inverting the signs of J 's and/or permuting the nuclei. In the intermediate couplings range there must be a continuous transition from 48 (AMX) to 24 (ABX) to 12 (ABC) equivalent solutions. In the latter two regions a switched sign gives only an approximate fit of the spectrum. However, if the fitting program has arrived at such a "wrong" coupling it will probably remain trapped there. To arrive at the correct sign the program would have to proceed through $J = 0$. This step would be unsatisfactory since a larger error will generally be encountered contrary to a least-squares fitting procedure. Consequently, in spectra with any number of weak or moderately weak coupling constants the program may often get trapped in local

minima with switched signs of the coupling constants and slightly altered absolute values of the parameters.

(iv) Very Strong Coupling, Degeneracy

Whereas in the weak coupling case some information about relative signs is lost, very strong coupling leads to a more dramatic loss of information. As an example, we may cite the AB spectrum, the outer lines of which, for strong coupling, disappear with $\delta^2/2J^2$, the separation of the inner transitions being $\delta^2/2J$. Obviously, for sufficiently small $\delta^2/2J$, neither J nor δ can be determined from the experimental spectra.

According to second-order perturbation theory, the line positions in ABC systems which approach the AA'A" limit depend only on 4 quantities, whereas there are 5 parameters to be determined (5). For such systems, we must therefore expect a one-dimensional array of equivalent solutions.

(v) Deceptive Simplicity

Deceptive simplicity may be defined as the degeneracy of one or several subspectra into single transitions (6). Since subspectra usually arise from weak coupling, the phenomenon of deceptive simplicity may be explained as due to the presence of a weak coupling as well as a very strong effective coupling in the same spin system. More exactly, if one internuclear coupling is much stronger than the corresponding chemical shift and the coupling constants between the two nuclei in question and any other nucleus differ less than the strong coupling constant, the sign of the strong coupling may be lost and only absolute values of the sums of some of the other couplings can be determined.

(vi) Special Types of Nonunique Solutions

In cases which do not fall into any of the categories (iii) to (v), the solution should be unique according to the Kummer's theorem (3). It turns out, however, that when allowance for even very small discrepancies is made, the theorem is no longer applicable. In many strongly coupled systems, two (and maybe more) widely different sets of parameters can be found which both reproduce a given spectrum very well. We have actually encountered two kinds of genuine approximate multiplicity. First, (see Fig. 2(a)), there may exist a one-to-one correspondence between transitions, the whole difference between the two spectra being hidden in very small differences in relative intensities of the lines and in the positions of lines with negligible intensities. Second (Fig. 2(b)), one or more single lines of one spectrum may correspond to a multiplet (usually doublet) with very fine splittings in the other spectrum.

The two examples shown in Fig. 2 are much more dramatic than those published earlier (7); nevertheless, we have found a number of such cases and we feel that they are more common than previously thought.

(vii) The Convergence Rate

We have been somewhat disappointed by the slow convergence rate of the proposed algorithm. This has to be attributed to the pronounced nonlinearity of the transforms as functions of the spectral parameters. The nonlinearity might in principle arise either from the nonlinearity of the basis, or it could be directly related to the nonlinear behavior of the transition frequencies and intensities. To find out whether the former effect was

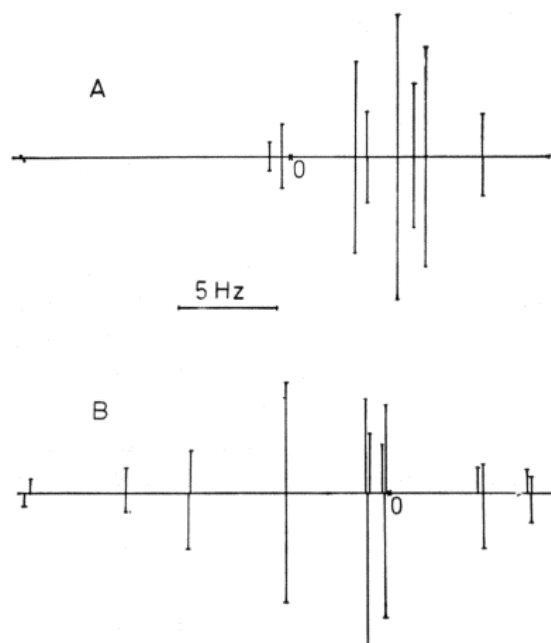


FIG. 2. The two types of multiplicity encountered in ABC systems. (A) One-to-one correspondence between lines, the difference between the two spectra being hidden in very weak outside lines. Upper spectrum: $\omega_A = 8.26$, $\omega_B = 6.13$, $\omega_C = 1.00$, $J_{AB} = 14.23$, $J_{AC} = -9.69$, $J_{BC} = 12.14$ Hz. Lower spectrum: $\omega_A = 9.04$, $\omega_B = 4.89$, $\omega_C = 1.53$, $J_{AB} = 19.15$, $J_{AC} = -3.45$, $J_{BC} = 11.33$ Hz. (B) Singlets corresponding to finely split multiplets of the same total intensity. Upper spectrum: $\omega_A = 1.62$, $\omega_B = 1.68$, $\omega_C = -9.72$, $J_{AB} = 0.49$, $J_{AC} = 8.16$, $J_{BC} = -5.16$ Hz. Lower spectrum: $\omega_A = 2.91$, $\omega_B = 0.47$, $\omega_C = -9.78$, $J_{AB} = 6.55$, $J_{AC} = 7.42$, $J_{BC} = 6.70$ Hz.

not dominant, we have devised a basis composed of broken linear functions (Fig. 1(e)) and checked its efficiency for the rough preadjustment of the parameters. The result was negative—the convergence rate did not improve and the basis was not as successful as the combination of moments with weighted Gaussian functions we described before. Consequently, we believe that the slow convergence rate can be traced directly to the transition frequencies and intensities themselves and that any improvement is possible only by means of some kind of nonlinear transformation of the set of parameters to be adjusted.

CONCLUSIONS AND PERSPECTIVES

Since this report has a preliminary character and further progress is being made continuously, it is perhaps a little bit too early to make any very strict conclusions. What our work does demonstrate quite clearly is the existence of a broad class of algorithms suitable for the analysis of the spectra of coupled nuclear systems. It may not be easy to select the algorithm which is best suited for the particular problem. At the time this paper was being written, we had already realized that our first choices were probably far from optimal since we had put too much emphasis on the mathematical elegance of the adopted basis rather than on its practical efficiency. Even so, however, the method proved to be superior to, and much less tedious than, the conventional approach. We are therefore quite optimistic that a fully automatic and reliable algorithm can be developed.

At present the development concentrates on the delocalized bases and on improving the convergence rate. Attempts are also being made to put the choice of the basis on a more solid theoretical ground, although no success in this direction can be guaranteed. Our most immediate goal is to improve the performance of the method for strongly coupled systems and for oriented molecules. The practical experiences with such systems should, in our opinion, make it possible to find empirically an algorithm which would be sufficiently universal and foolproof.

So far we have concentrated on linear functionals in general and linear integral transforms in particular. The possibility of using nonlinear functionals has been discarded mainly because the freedom of choice of the functionals would then exceed imagination. It is possible to show that any linear functional can be written as an integral transform and thus is defined by a single weight function; this is by no means true for nonlinear functionals. To step into this field without theoretical reasons for doing so, might prove too risky (though this statement is not intended to limit initiative). Careful generalizations of the integral transform method might nevertheless be useful. Suppose, for example, that the functionals are defined by the formula

$$\mathcal{F}_w\{I(\omega)\} = \int_{-\infty}^{+\infty} w(\omega)f(I(\omega))d\omega. \quad [13]$$

For $f(x) = x$ we have our original Eq. [5]. By putting $f(x) = x^\alpha$ we may either decrease ($\alpha < 1$) or increase ($\alpha > 1$) the sensitivity of the transforms to the transition intensities. Similarly a proper choice of $f(x)$ may result in cutting off those parts of the spectrum which are nearly lost in the noise, having only the prominent features.

We want finally to point out that the methods proposed in this paper are especially well suited for the modern NMR spectrometer-computer systems in which the spectra are already stored in a digitized form and the integral transforms can be evaluated straightforwardly without even having seen the spectrum.

APPENDIX A

Error Propagation in the Integral-Transform Method

An experimental spectrum $I(\omega)$ can always be described as a sum of two contributions.

$$I_e(\omega) = I_t(\omega) + r(\omega), \quad [A1]$$

where $I(\omega) = \langle I_e(\omega) \rangle$ is the ensemble average over an infinite sample of such spectra and $r(\omega)$ is the random noise contribution such that $\langle r(\omega) \rangle = 0$. Only the function $I_t(\omega)$ is supposed to be reproducible by theoretical means (in practice, however, such effects as saturation, nonlinearity of the detecting system, form of the magnetic field inhomogeneity within the sample, etc., may affect the form of $I(\omega)$). The noise can be characterized by its correlation function

$$C(\tau) = \langle r(\omega)r(\omega + \tau) \rangle. \quad [A2]$$

For simplicity, we will assume that $C(\tau) = \rho^2 \delta(\tau)$ where ρ is the rms of the noise and $\delta(\tau)$ is the δ -function (this is the so-called white noise). The integral transform $\mathcal{F}_w\{I_e(\omega)\}$ defined by Eq. [5] can be rewritten

$$\mathcal{F}_w\{I_e(\omega)\} = \mathcal{F}_w^t + \phi_w, \quad [A3]$$

where $\mathcal{F}_w^t = \mathcal{F}_w\{I_t(\omega)\}$ and $\phi_w = \mathcal{F}_w\{r(\omega)\}$. By virtue of the linearity of \mathcal{F}_k , $\langle\phi_w\rangle = \mathcal{F}_w\{\langle r(\omega)\rangle\} = 0$ it is therefore evident that

(i) the experimental noise does not lead to any *systematic* errors in the integral transforms and

(ii) the random error due to the experimental noise is given by $\phi(\omega)$.

We can now determine the covariance matrix for the random parts of any set of integral transforms. Let $C_{ij} = \langle\phi_i \phi_j\rangle$, where the indices i and j refer to the basis functions w_i and w_j , respectively. Then

$$\begin{aligned} C_{ij} &= \left\langle \int_{-\infty}^{+\infty} w_i(\omega) r(\omega) d\omega \int_{-\infty}^{+\infty} w_j(\omega') r(\omega') d\omega' \right\rangle \\ &= \left\langle \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} w_i(\omega) w_j(\omega') r(\omega) r(\omega') d\omega d\omega' \right\rangle \\ &= \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} w_i(\omega) w_j(\omega') \langle r(\omega) r(\omega') \rangle d\omega d\omega' \\ &= \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} w_i(\omega) w_j(\omega') C(\omega' - \omega) d\omega d\omega'. \end{aligned} \tag{A4}$$

In particular, for white noise,

$$C_{ij} = \rho^2 \int_{-\infty}^{+\infty} w_i(\omega) w_j(\omega) d\omega, \tag{A5}$$

so that the covariance terms ($i=j$) vanish whenever the basis is orthogonal. The variances V_i of the transforms are of course given by the diagonal terms of the covariance matrix, $V_i = C_{ii}$. Once the covariance matrix for the transforms (i.e., the quantities to be fitted) has been determined, the covariance matrix for the spectral parameters (i.e., the quantities to be adjusted) can be obtained by methods described elsewhere (1).

APPENDIX B

Numerical Problems Connected With the Choice of Bases

For any basis $\{w_k(\omega), k = 1, 2, \dots, \infty\}$, it is always necessary to find a fast numerical algorithm for evaluating the following mathematical quantities:

- (i) $B_k(a, b) = \int_a^b w_k(\omega) d\omega$
- (ii) $C_k(a, b) = \int_a^b \omega w_k(\omega) d\omega$
- (iii) $D_k(\omega_0, \delta\nu) = \int_{+\infty}^{+\infty} w_k(\omega) \mathcal{L}(\omega - \omega_0, \delta\nu) d\omega,$

where

$$\mathcal{L}(\omega, \delta\nu) = \frac{2}{\pi\delta\nu} \left[1 + \left(\frac{2\omega}{\delta\nu} \right)^2 \right]^{-1}$$

is the normalized Lorentzian line. The first two are required for a numerical evaluation of the integral transform $\mathcal{F}_k\{I_{\text{ex}}(\omega)\}$, where $I_{\text{ex}}(\omega)$ is the digitized experimental spectrum; for each interval between two adjacent data points, $I_{\text{ex}}(\omega)$ is approximated by a linear function $(\alpha\omega + \beta)$ so that the contribution of this interval to the transform reduces to the evaluation of the two integrals of the type (i) and (ii). The third integral expresses the contribution of a Lorentzian line of linewidth $\delta\nu$ to the transform; it appears in evaluation of the transforms of the theoretical spectra. For the stick spectra, however, the third integral reduces to $A_k(\omega_0) = w_k(\omega_0)$.

For the Fourier basis defined by Eq. [8], none of the above quantities presents any numerical problems. They can all be calculated either explicitly or, much more efficiently, through simple recurrence formulas. The recurrence process is stable and can be easily started at whatever value of the index k .

The Hermitian basis Eq. [9], is also quite satisfactory as far as quantities A_k , B_k , and C_k are considered. The only disadvantage here is that the recurrence formulas have to be started at $k = 0$ even if the low terms are not required. Otherwise, the recurrence process is also stable. Real problems arise in connection with the quantity D_k . A recurrence formula can be easily found but it does not appear to be stable with respect to the propagation of roundoff errors. Moreover, to start the recurrence, the value of the error function of a complex argument has to be evaluated. For narrow lines, the argument lies in the vicinity of the imaginary axis and it seems that there does not exist any satisfactory numerical approximation in this region.

The Gaussian basis, Eq. [10], is similar to the Hermitian basis in all respects. In both cases, the problems with the quantity D_k would disappear, however, if the lines were assumed Gaussian rather than Lorentzian. It is the mixing of the two types that gives rise to the numerical difficulties. In the case of moments, Eq. [11] presents no problems at all, and the same goes also for the delocalized Lorentzian basis, Eq. [12]. In the latter case, it would not be advisable to assume Gaussian lines; there would of course be no problems with Gaussian lines in connection with a delocalized Gaussian basis.

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