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A Discussion of the Error Analysis in LAOCOON-like Iterative Programs

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This paper is concerned with the iterative refinement of NMR parameters as they are obtained in the LAOCOON program and similar programs.

The error analysis differs from previous subroutines in the introduction of a measure for the variance of the unassigned transitions with respect to the assigned transitions. In terms of this measure a strategy for the gradual enlarging of the set of assigned transitions is presented. The discussion includes several examples from the literature showing that the statistical nature of the errors in the final iterated parameters has often been misinterpreted.

The iterative simulation program LAOCOON [1] has found general acceptance in the interpretation of high resolution NMR spectra. Over the years many modified versions have been published [2–5] each introducing additional facilities. Our Danish descendant from LAOCOON is named MIMER ** and it incorporates most of the facilities published over the years. During its development the program has been used in a number of publications.[6–30] In two respects we have found the published developments less than satisfactory and we have in MIMER tried to remedy these. The error analysis in LAOCOON is frequently misinterpreted or its information disregarded (*vide infra*). This may be explained in part due to the lapidaric coverage in the user manual and partly due to the manner in which the information is presented in the output produced by the computation. In MIMER we therefore have designed a new error analysis, drawing on the experience from other spectroscopic disciplines *in casu* microwave spectroscopy. The procedure of assigning line numbers to observed transitions is the most decisive step in the iteration using LAOCOON. The high degree of subjectiveness involved in this step has been criticized [31,32] with good reason and it is one of the motives for the development of "the automatic method" as described by Diehl *et al.*[31] and Stephenson and Binsch.[32] Progress towards a more objective procedure for line assignment in LAOCOON is intimately connected to the error analysis. In the work of Kirchhoff [33] on assignment of transitions in microwave spectra, we found some suggestions that could be applied to NMR spectra as well. Trial calculations have confirmed that it is possible based on these ideas to define a procedure whereby the step by step enlargement of the set of assigned lines may be performed in an objective fashion. As an example we have included analysis of a four-spin system. A second test is provided by an analysis of the distribution of the normalized errors in the assigned lines. This test may be used to spot incorrectly assigned transitions between trial iterations. When the final iteration is completed it provides a test of the validity of the model Hamiltonian used.

THEORY

Let us consider an experimental spectrum represented by a collection of transition frequencies $\{FR\}$. Through the iterative LAOCOON procedure

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they determine the best values for the parameters appearing in the Hamiltonian of the spin system. Typically the parameters are chemical shifts and spin – spin coupling constants, but occasionally dipole – dipole coupling constants and quadrupole splittings may be present. For each of the observed transitions the experimentalist will provide a qualified estimate of the accuracy of the measurement. The estimate should include the estimate of the performance of the spectrometer affecting all transitions equally, as well as the specific properties of the individual transitions exemplified by variation in linewidth, difference in signal to noise ratio, overlapping band envelopes *etc.* In the initial step the guessed parameters $\{p^\circ\}$ are used to calculate a trial spectrum with transition frequencies $\{FR\}$. To tie an observed and a calculated transition together a line number N_{ij} is introduced, demanding that the transition N_{ij} with the calculated frequency FR_{ij} should approach, under the least squares condition, the observed frequency FR_{ij}. The calculated frequency is related to the eigenvalues E of the Hamiltonian

$$\hat{FR}_{ij} = E_i - E_j \tag{1}$$

In order to keep the risk of divergence during the iterations as low as possible the eigenvalues and eigenfunctions are ordered according to energy after each iteration so that the linenumber relation

$$N_{ij} < N_{ik}$$

always implies

$$E_j > E_k$$

During the process of iteration, a measure of the correspondence between the observed and calculated transitions is provided by the root mean square deviation defined by eqn. 2, where the summation extends over the N assigned transitions. A convergent iteration process is reflected in a sequence of monotonically decreasing values yielding the best solution when no further improvements occur. Since the user should be able to provide an estimate of the accuracy of the experimental data it is appropriate to introduce the function $\hat{\sigma}^2$ defined by eqn. 3,

$$RMS = \sqrt{\frac{1}{N}\sum_{ij}^{N}(FR_{ij} - \hat{FR}_{ij})^2} \tag{2}$$

$$\hat{\sigma}^2 = \frac{1}{N-M}\sum_{ij} W_{ij}(FR_{ij} - \hat{FR}_{ij})^2 \tag{3}$$

$\hat{\sigma}^2$ being an estimate of the variance of the measurement errors. M in eqn. 3 is the number of independent parameters optimized in the iteration and W_{ij} is the weight of the observed transition FR_{ij}. To judge the failure or success of the iteration the standard deviation $\hat{\sigma}$ should be compared to the anticipated accuracy of the experimental data. In a successful calculation the two values should be similar. (In the case of weighted transitions the experimental accuracy should be compared to $\hat{\sigma}/\sqrt{W_{ij}}$). The close similarity of eqns. 2 and 3 makes them equally suitable as a measure of the convergence of the iteration sequence.

If the best solution based on these considerations is found acceptable, the best parameter values should be reported with proper confidence limits. The confidence limits cf_k for the kth parameter can be expressed as

$$cf_k = t\hat{\sigma}V_{kk}^{\frac{1}{2}} \tag{4}$$

The confidence limits are determined by three factors. $\hat{\sigma}$ as defined in eqn. 3 reflects the accuracy of the experimental data. An increase in accuracy of the experimental data will obviously be one way to reduce the confidence limits. V is the dispersion matrix where the elements are defined by eqn. 5.

$$V_{kl}^{-1} = \sum_{ij}^{N} DC_{ij,k}\, W_{ij}\, DC_{ij,l}$$

$$k,l = 1,m. \tag{5}$$

where DC is given by

$$DC_{ij,k} = \frac{\partial \hat{FR}_{ij}}{\partial p_k} = \frac{\partial E_i}{\partial p_k} - \frac{\partial E_j}{\partial p_k} \tag{6}$$

V^{-1} contain a measure of the sensitivity of the assigned transitions to the parameters which the iteration process attempts to determine. V contains the inverse information *i.e.* the sensitivity of the iterated parameters to the calculated and assigned frequencies. At the outset we left open the possibility that only a subset of the observed transitions had been assigned. It is, therefore, conceivable that the assigned lines may be sensitive preferentially to some of the parameters iterated. A low sensitivity of the

assigned lines towards the parameter p_k will be reflected in a large value of V_{kk}. From eqn. 5 it is seen that the diagonal elements in V^{-1} will grow with an increase in the number of assigned transitions and V_{kk} will decrease. The second possibility of reducing the confidence limits in eqn. 4 is therefore to assign additional transitions. A useful device for selecting the proper lines will be provided later in this section.

The factor t in eqn. 4 is the Student factor.[34] It is related to the statistical nature of the results obtained by the least squares procedure. The confidence limits calculated using eqn. 4 will depend on the significance to be associated with the result. If the user is satisfied with a 50 % chance that the "true value" is within the range $p_k \pm \sigma f_k$ a low value of t applies. If, on the other hand, a 95 % chance of bracketing the "true value" is requested a larger value of t must be inserted.

Many results obtained by LAOCOON iterations report the RMS error but omit the confidence limits. From the discussion above it can be deduced that assignment of a small subset of transitions may lead to very favourable RMS values while at the same time V contains large diagonal elements that are reflected in large confidence limits.

The off diagonal elements of V express in a similar manner to which extent the assigned lines characterize the individual parameters rather than linear combinations. Here reference can be made to the properties of the well-known spin systems ABX and AA'XX'. If assignment is made only of the prominent lines spaced by the sum of the coupling constants to the X entity, this will lead to large off diagonal elements between the coupling constants. In order to normalize the values of the elements V_{kl} the covariance coefficient matrix C is introduced

$$C_{k,l} = V_{k,l}/(V_{k,k} * V_{1,l})^{\frac{1}{2}} \tag{7}$$

Using the normalized matrix C the statements can be made more precise. When values of $|C_{k,l}|$ fall in the range 0.9 to 1.0 it is an indication that the assigned transitions determine a linear combination of $p_k \pm p_l$ with better precision than the individual parameters. Let us for a moment abandon the request for best values of the individual parameters and accept best values for linear combinations of parameters p_k^*

$$p_k^* = \sum_j T_{kj} p_j \tag{8}$$

or in matrix notation $p^* = Tp$. In the new parameters the dispersion matrix V would be transformed into

$$V^* = TVT^{-1} \tag{9}$$

Since V is symmetric T may be selected to produce V^* in diagonal form. For this particular choice no covariance exists between the parameters $\{p^*\}$ and the use of eqn. 4 for this case exploits the information present in the assigned transitions as far as possible. This point was fully appreciated by Castellano and Bothner-By [1a] in the error analysis performed in LAOCOON where the diagonalization of the variance covariance matrix F is performed. Since F can be expressed as eqn. 10, the results are the same as indicated above.

$$F = \hat{\sigma}^2 V \tag{10}$$

It is good practice to limit the number of assigned transitions in the first trial iteration to avoid wrong assignments that may derail the iteration from the right track. The penalty for this will be large elements in V. In order to proceed, it is necessary to enlarge the number of assigned transitions including lines that increase the information content in $\{FR\}$. The added lines should fulfil the condition of unique assignment since wrong assignments again may lead the iteration astray. To aid the user in this process we may ask, how accurate are the predictions of unassigned transitions calculated on basis of the previously assigned transitions. The statistical measure of this is the estimated standard deviation of a transition $\delta(\hat{FR}_{kl})$. It can be shown (see appendix A) to depend on three factors, the standard deviation of the assigned transitions found in eqn. 3, the relations of these to the iterated parameters expressed in the dispersion matrix V and the sensitivity of the transition $\hat{FR}_{kl}$ to the parameters $DC_{kl,p}$. This leads to eqn. 11, where $\delta(\hat{FR}_{kl})$ is the desired standard deviation. This expression is valid both for assigned and unassigned transitions, when the proper $DC_{kl,p}$ is inserted.

$$\delta(\hat{FR}_{kl})^2 = \hat{\sigma}^2 \sum_m^M \sum_n^M DC_{kl,m} V_{m,n} DC_{kl,n} \tag{11}$$

Let us consider a transition FR measured but not included in the iteration. If the value FR falls within the limits $\hat{FR}_{ik} \pm \delta(\hat{FR}_{ik})$ then inclusion of the transition $\hat{FR}_{ik} = FR$ will not increase the standard deviation $\hat{\sigma}$ of the iteration. If several observed but not assigned transitions fall within the range $\hat{FR}_{ik} \pm \delta(\hat{FR}_{ik})$ it would be dubious to assign any of these to the calculated linenumber unless additional evidence

for the selection exists. If the list of $\delta(\hat{FR}_{ik})$ is scanned and some of these values are much larger than the measurement error for the observed lines it indicates that some parameter or linear combinations of parameters are poorly determined and assignment of additional lines will be necessary. These lines should be selected among the previously unassigned spectral lines with values of $\delta(\hat{FR})$, large compared to the experimental error yet sufficiently small that an unambiguous assignment can be made. In the next cycle of the iteration the $\delta(\hat{FR})$ values will have changed and some new unassigned lines may be suitable for inclusion in the calculation. As long as the calculation results in $\delta(\hat{FR})^2$ values significantly larger than the estimated variance of the unassigned transitions the process of enlarging the basis of the iteration should be continued. The method is included in the error analysis and its application is illustrated in an example later in this paper.

The fitting of parameters in a large spin system is hardly ever performed using all the chemical shifts and coupling constants as "free parameters". Typically less than 10 parameters are allowed to be free, while the remaining parameters are held at fixed values. Practical considerations justify this strategy, but theoretically it presents problems if the fixed parameter values differ from the "true" but unknown values. In this case the model used in the least squares process is false and accordingly a test for validity of the model would be valuable. Kirchhoff presents in his paper [33] a procedure that might point out an incorrect model.

The test is based on the distribution of normalized errors in the final step of the calculation. The parameter $t(\Delta\nu)$ is defined as eqn. 12.

$$t(\Delta\nu) = \frac{\Delta\nu}{\sigma(\Delta\nu)} \tag{12}$$

For the assigned lines $t(\Delta\nu)_{ij}$ is given as [33]

$$t(\Delta\nu)_{ij} = (FR_{ij} - \hat{FR}_{ij})/(\hat{\sigma}^2 W_{ij}^{-1} - \delta(\hat{FR}_{ij})^2)^{\frac{1}{2}} \tag{13}$$

The values of $t(\Delta\nu)_{ij}$ are listed in the output as shown in Tables 3 and 4. Under the assumption of a correct model and random errors the $t(\Delta\nu)$ values should be distributed around zero with a Student t distribution. The values given in the output are used in a histogram and the distribution is characterized by mean value, variance, third moment and skewness. Significant deviations would indicate that the model used needs reconsideration.

DISCUSSION

The error analysis in LAOCOON [1] uses the Student t factor 0.67 in eqn. 4. This factor determines the magnitude of the probable error often reported in the results of iterative calculations. In the literature much discussion has been devoted to the interpretation of the probable errors produced by the LAOCOON program.

As an example, MacDonald and Schaefer [35] argue against the practice of quoting as error estimates the probable errors produced by LAOCOON.[1] They find that with a standard deviation in experimental frequencies of 0.02 – 0.04 Hz the probable error computed in AB and ABX systems may be as low as 0.002 – 0.004 Hz. This leads them to the conclusion that "the probable errors are not a real measure of the accuracy of the obtained parameters". The probable errors produced by LAOCOON correspond to the 50 % confidence limits for a system with the number of observations N exceeding the number of parameters to be determined M by a large number.[34] The statistical measure of the confidence limits of a parameter determined by the least squares procedure is correctly given by eqn. 4 and choosing for t the $t(N-M,\alpha)$ from the Student t distribution,[34] α being the confidence limit required by the user.

In the case of an AB system there will be three parameters to obtain from the iteration but only four transitions. Using the common 95 % confidence limit $t(1,95\,\%) = 12.7$, this amounts to a whopping factor of 19 as the increase from the LAOCOON probable error to the statistical correct estimate of confidence in the parameter. Similarly an ABX system will in general allow the observation of 12 or occasionally 14 lines while 6 parameters are to be determined. In this case the probable errors produced by LAOCOON should be multiplied by 4 in order to present the statistical correct estimates. The examples discussed by MacDonald and Schaefer, therefore, do not present a criticism of the LAOCOON method and its application but merely emphasize the need for a better and easier interpretable error routine in the simulation programs.

Kostelnik *et al.*[36] have reported a very careful study of α,α,α-trifluorotoluene. They have estimated the standard deviation on the observed lines to be 0.025 Hz. Separation of the spectrum into two subspectra and observation at 100 and 60 MHz permitted four independent iterations to be performed yielding chemical shifts and coupling constants. In all cases and for each parameter they calculated a probable error about 0.010 Hz. Since a

large number of lines were included the $t(N-M, 50\ \%)$ should be 0.67. The confidence limits using $t(N-M, 95\ \%)$: 0.030 Hz and $t(N-M, 99\ \%)$: 0.038 Hz. All the observed values lay within the 99 % confidence range. These results, therefore, seem to support the use of the results of the statistical analysis rather than to discredit it as concluded by MacDonald and Schaefer.[35] In a paper Ewing[37] attempts to estimate the parameter errors in LAOCOON calculations. He states that "the error analysis can only yield information about the internal fit of observed and calculated spectra, and some kind of calibration is required before the error analysis can be really meaningful".

This statement seems rather dubious to us. As discussed in great detail by Albritton[38] it is vital for the outcome of a least squares calculation that the basic assumptions for the use of the method are not invalidated. Among these, two seem to be pertinent to typical NMR calculations. Firstly, the errors in the measured frequencies should be predominantly random. The error distribution is only required to possess a well-defined variance as given by eqn. 3 in order that the least squares method will iterate to the best possible values.[38] For the application of the confidence limits expressed in eqn. 4 the stronger requirement of a normal distribution of the error must be fulfilled. If systematic errors dominate, then the outcome of a least squares fit including the error estimates become meaningless. With modern spectrometers it is entirely possible that systematic errors may dominate. In such cases it is often advocated to enlarge the error estimates by a common factor. This practice may work well for certain classes of problems but being without theoretical justification it should be used with caution. Secondly, the model used may be inaccurate. This may happen in larger spin systems where only some of the parameters are iterated while the remaining parameters are maintained at an inaccurate value. This procedure introduces a bias in the calculation that may produce parameter values and error estimates that do not characterize the unbiased solution. In addition to these factors unequal precision in the determination of different frequencies will be of importance as discussed later.

If we follow the arguments of Ewing[37] and disregard the possibilities above there seems no reason why the statistical method should fail, when properly used. Ewing's test system is a four-spin system with 10 parameters and 35 lines assigned. He then uses the reverse process of calculating the spectrum for small variations in the coupling constants and monitors the RMS error and its relation to the mean change in the coupling constants $\overline{\Delta J}$, eqn. 14.

$$\overline{\Delta J} = \sum(J - J_{\text{real}})/6 \tag{14}$$

In order to interpret the experiments we shall express the confidence limits in terms of the *RMS* error and the dispersion matrix *V*. Inserting eqns. 2 and 3 into eqn. 4 we obtain the relation 15.

$$cf_i = t(N-M,\alpha)\left(\frac{N}{N-M}\right)^{\frac{1}{2}} V_{ii}^{\frac{1}{2}} RMS \tag{15}$$

The elements of the dispersion matrix do not depend on the accuracy of the observed data,[38] but reflect only the structure of calculation, *i.e.* the relation between the selected set of transitions used in the iteration and the parameters to be determined. In the present calculations it means that the use of the same transitions and the same parameters to be fitted assures the unchanged value of *V*. From eqn. 15 it is obvious that in the limit of random errors a linear relation exists between the mean error $\overline{\Delta J}$ and the *RMS* error, the slope of the line being determined by eqn. 16.

$$t(N-M,\alpha)\left(\frac{N}{N-M}\right)^{\frac{1}{2}} \sum\left(\frac{V_{ii}^{\frac{1}{2}}}{6}\right) \tag{16}$$

Ewing did not report the magnitude of the dispersion matrix elements. In a similar four-spin system Laatikainen[5] reports their values to be in the range 0.5 – 0.8. Values in this range would lead to a slope of

$$1.96 \times \left(\frac{25}{35}\right)^{-\frac{1}{2}} 0.65 = 1.5 \tag{17}$$

Using a large number of numerical experiments Ewing finds a slope of 1.21. Ewing also considers the situation where a large error is introduced in one of the coupling constants only. Such a result would only be observed when some sort of systematic error is present in the observed data. As discussed by Albritton,[38] it is common practice to use a larger number in place of $t(N-M,\alpha)$. This reflects, as Ewing suggests, a steeper slope.

It can be concluded that the experiments of Ewing are an example of the use of computers to derive relations that consideration of the theory would have

given naturally. Furthermore the expression given in eqn. 16 shows that the slope in the relation is not a constant of nature but that it depends on the conditions of the iteration. Especially it must be emphasized that when the elements V_{ii} differ significantly in magnitude an average relation with an assumed value of $V_{ii}^{\frac{1}{2}} \sim 0.65$ may be totally misleading. For a five-spin system the number of observable lines increases more rapidly than the number of parameters. This situation leads to a reduction in the value of the elements of the dispersion matrix and accordingly to a relation between $\overline{\Delta J}$ and *RMS* error with a smaller slope. Also this result is established numerically by Ewing. The lack of conception of the statistical nature of the results of the iterative procedure is most clearly phrased in the concluding remarks of Ewing's paper where the "real" error in the iterated parameters is suggested to be 2.5 times the probable errors of LAOCOON. Stated in statistical terms it suggests the use of the 95 % confidence limit instead of the 50 % confidence limit. When choosing the confidence limit the procedure of preference is to use the N and M of the actual calculations and select from a table the corresponding $t(N-M,\alpha)$.

In the present program we have included the weighted least squares method. The technique is described by Albritton [38] and the implications should be discussed in some detail. An unbiased estimate is obtained using weights related to the accuracy of the frequencies determined.

$$W_{ij} = 1/\tilde{\sigma}_{ij}^2 \qquad (18)$$

$\tilde{\sigma}_{ij}$ being the estimate of the standard deviation for the measured frequency of transition N_{ij}. If the spectrum consists of lines of various linewidth due to the presence of scalar broadening [39] or effects of medium slow exchange, it is advisable to introduce the weights according to eqn. 18. Similarly, if groups of lines are not separated and line positions are determined by measurements of local maxima on the band envelope, such lines should carry a reduced weight in the iteration.[40] For quite a different reason weights may be introduced due to the assignment problem. If some spectral lines can be assigned with certainty due to their isolated position in the initial calculation and these lines determine a linear combination of the parameters to be iterated that should be kept almost unchanged, a large weight is introduced. The combination of the last two techniques often permits the untangling of groups of overlapping lines, especially when used in combination with the bootstrap technique depending on $\delta(\hat{FR})$. It is important to note that the probable errors determined in a weighted iteration are the same as in the unity weighted calculation for the same parameter estimate. This is due to the fact that weights enter the dispersion matrix and variance in a compensating manner.[38]

For the iteration procedure it is generally agreed that the four-spin systems, especially the AA'BB' system, are the most troublesome. The reason for this is the low ratio between the number of observable transitions and parameters to be iterated and in the latter case the high degree of correlation among the coupling constants. In a recent paper Manatt [41] has considered the use of LAOCOON and NMRIT [42] for an AA'BB' system. For several of the trial calculations it was found that LAOCOON diverges in the iteration while NMRIT was stable in the iterations over the trials performed. This led to the conclusion that in the case of AA'BB' systems it is advisable not to use LAOCOON but resort to NMRIT. Since our program uses the same basic strategy as LAOCOON we have recalculated the trial systems reported by Manatt using MIMER to examine the performance on the four spin case. The results obtained are reproduced in Table 1 where the data from the work of Manatt have been included.* The overall result is that no divergence can be found using MIMER on the trial cases reported by Manatt. Since we have assigned the same number of lines to the same frequencies the main difference arises in the ordering of energy levels that MIMER performs, while this step is absent in LAOCOON.*

In NMRIT the assignment of energy levels explicitly results in the same ordering. This ordering explains also the large discrepancy between the two methods observed in the initial step. Using many trial parameters further from the real parameters we have obtained convergence to the real parameters in most cases, even when the initial guess is very poor (trial 6). In some cases we have, however, experienced the same convergence to an improper set of parameters that is exemplified by LAOCOON in trial 5. In such cases we have found that the use of the symmetry separation in the assignment results in proper convergence. The problem arising in the AA'BB' case is related to the interchange of symmetric and

* We have obtained the program listing of LAOCOON in the version used by Manatt through the cooperation of Prof. H. Günther.

Table 1. Comparison of the iteration performance between LAOCOON and MIMER. The test example is taken from Ref. 41. For each trial case the interative performance is given. Left column LAOCOON, right column MIMER. Trial 6 only MIMER.

	Real parameters	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
$w(1)$	48.863	48.000	47.90	47.80	47.70	47.825	0.000
$w(2)$	48.863	48.000	47.90	47.80	47.70	47.825	0.000
$w(3)$	61.450	62.000	62.10	62.20	62.30	62.175	1.000
$w(4)$	61.450	62.000	62.10	62.20	62.30	62.175	1.000
$J(12)$	6.200	6.00					
$J(13)$	−16.136	−14.10					
$J(14)$	6.789	6.70					
$J(23)$	6.789	6.70					
$J(24)$	−16.136	−14.10					
$J(34)$	5.166	5.70					
Iteration number 0		1.029 1.202	1.025 1.197	7.771 1.201	9.412 1.212	5.397 1.199	63.284
RMS error 1		0.082 0.096	0.088 0.103	6.445 0.109	7.090 0.116	3.116 0.108	19.282
2		0.000 0.008	0.000 0.008	7.983 0.008	5.496 0.008	2.788 0.008	2.693
3		0.000 0.000	0.000 0.000	0.000	5.361 0.000	2.762 0.000	0.374
4					6.035 0.000		0.020
5							0.000

antisymmetric energy levels in the hierarchy of energy levels. This leads to switching of linenumbers so that the assigned transitions are identified by the calculation as symmetry forbidden transitions. When this happens divergence or oscillatory iterations may be encountered. Laatikainen [5] in his program MAOCOON analyses the symmetry of the eigenstates and avoids interchange of symmetric and antisymmetric states by a modified diagonalization procedure. He refers to the quantum mechanical "non crossing rule" as the origin of the problem in the AA'BB' system. This explanation seems dubious

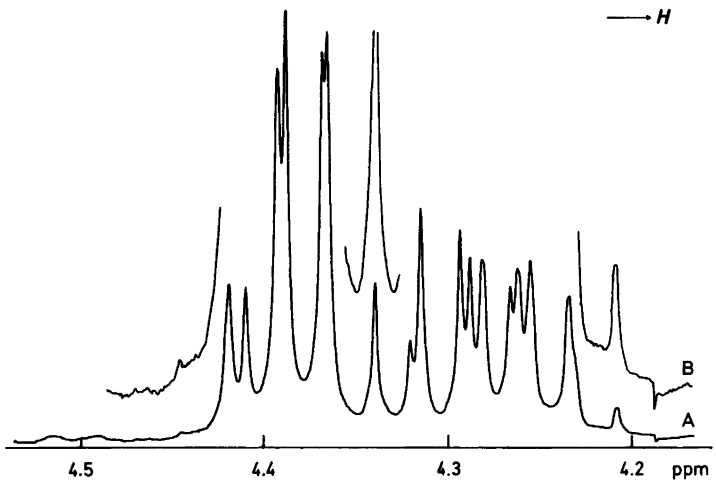
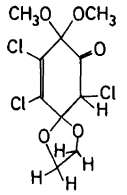


Fig. 1. ^{1}H 270 MHz spectrum of 6,7,10-trichloro-8,8-dimethoxy-1,4-dioxaspiro[4,5]dec-6-en-9-one. Trace A shows the spectrum, while trace B corresponds to increased gain in order to observe the weak lines 22, 29 and 51.

Table 2. The best values for chemical shifts and coupling constants obtained for 6,7,10-trichloro-8,8-dimethoxy-1,4-dioxaspiro[4,5]dec-6-en-9-one using the iterative program MIMER. The parameters are given with the 95 % confidence limits in parentheses. Two iterations are reported. In case I only the strong lines with intensities above 0.4 were assigned. In case II three additional weak lines were included.



Case I				Case II			
H 1	*W*(1)	1147.846	(0.04)	H 1	*W*(1)	1147.853	(0.04)
H 2	*W*(2)	1162.289	(0.04)	H 2	*W*(2)	1162.289	(0.04)
H 3	*W*(3)	1186.033	(0.53)	H 3	*W*(3)	1185.969	(0.12)
H 4	*W*(4)	1183.593	(0.57)	H 4	*W*(4)	1183.677	(0.12)
	J(1,2)	−7.414	(0.06)		*J*(1,2)	−7.390	(0.05)
	J(1,3)	7.189	(0.46)		*J*(1,3)	7.146	(0.15)
	J(1,4)	5.297	(0.43)		*J*(1,4)	5.299	(0.16)
	J(2,3)	6.983	(0.17)		*J*(2,3)	7.017	(0.12)
	J(2,4)	7.119	(0.22)		*J*(2,4)	7.076	(0.16)
	J(3,4)	−7.692	(7.87)		*J*(3,4)	−6.776	(0.07)
	RMS	0.029			RMS	0.035	

Table 3. The iterative results obtained by the program MIMER. The best values of the parameters corresponding to the calculated lines are listed in Table 2 as Case I. The assigned lines all have intensities above 0.4. The result in *t* distribution corresponding to the last column has a mean value of 0.013, a variance 0.989 and a skewness −0.412.

LINE	EXP FREQ	CALC FREQ	INTEN	ERROR	$\delta(FR)$	*t*
51		1136.354	0.351		0.038	
25	1142.390	1142.364	0.468	0.026	0.028	0.927
56	1143.469	1143.462	0.997	0.007	0.026	0.239
18	1143.459	1143.485	0.539	−0.026	0.029	−0.968
46	1148.840	1148.813	1.266	0.026	0.027	0.909
1	1149.230	1149.273	0.793	−0.043	0.024	−1.364
41	1150.890	1150.908	1.462	−0.019	0.026	−0.625
52	1152.106	1152.107	0.978	−0.001	0.028	−0.030
11	1156.066	1156.037	1.946	0.028	0.027	0.989
42		1157.260	0.016		4.297	
27	1158.015	1158.039	1.334	−0.024	0.027	−0.823
55		1159.215	0.147		0.030	
20	1159.431	1159.395	1.444	0.036	0.027	1.242
12		1161.756	0.014		4.292	
38	1164.509	1164.488	0.245	0.020	0.028	0.744
2	1165.243	1165.210	2.072	0.033	0.025	1.078
33	1166.804	1166.818	0.509	−0.014	0.024	−0.456
47		1169.221	0.019		0.036	
13		1170.438	0.110		0.034	
5	1171.931	1171.975	1.369	−0.044	0.024	−1.392
29		1172.537	0.070		4.284	
34		1173.170	0.032		4.289	

Table 3. Continued.

14		1177.666	0.111		4.300	
30	1178.864	1178.889	3.246	−0.025	0.028	−0.912
54	1179.646	1179.622	2.806	0.024	0.027	0.820
3		1183.481	0.032		4.302	
39	1184.919	1184.896	1.755	0.023	0.023	0.716
48	1184.919	1184.974	1.939	−0.055	0.027	−1.901
53		1185.975	0.051		4.288	
15	1186.137	1186.113	1.892	0.024	0.028	0.851
7	1186.337	1186.375	1.421	−0.039	0.028	−1.370
28	1190.919	1190.906	1.289	0.013	0.029	0.482
4	1193.317	1193.284	1.103	0.033	0.027	1.124
44		1193.421	0.080		4.295	
35		1193.578	0.016		4.274	
22		1200.709	0.039		4.314	

Table 4. The iterative results obtained by the program MIMER. The best values of the parameters corresponding to the calculated lines are listed in Table 2 as case II. The assigned lines are as in case I but three weak lines 51, 29 and 22 have been included. The resulting t distribution corresponding to the last column has a mean value of 0.026, a variance 0.990 and a skewness 0.043.

LINE	EXP FREQ	CALC FREQ	INTEN	ERROR	$\delta(FR)$	t
51	1136.486	1136.404	0.351	0.082	0.027	2.262
25	1142.390	1142.386	0.469	0.004	0.026	0.105
56	1143.469	1143.490	0.998	−0.021	0.027	−0.583
18	1143.459	1143.510	0.540	−0.052	0.025	−1.370
46	1148.840	1148.816	1.264	0.024	0.029	0.691
1	1149.230	1149.275	0.794	−0.045	0.027	−1.244
41	1150.890	1150.919	1.461	−0.029	0.029	−0.829
52	1152.106	1152.116	0.978	−0.010	0.030	−0.304
11	1156.066	1156.026	1.946	0.040	0.027	1.091
42		1156.352	0.017		0.047	
27	1158.015	1158.041	1.333	−0.027	0.030	−0.774
55		1159.203	0.147		0.032	
20	1159.431	1159.404	1.443	0.026	0.029	0.755
38	1164.509	1164.471	0.247	0.038	0.027	1.034
2	1165.243	1165.216	2.070	0.027	0.028	0.738
33	1166.804	1166.813	0.514	−0.009	0.027	−0.236
47		1169.258	0.019		0.038	
13		1170.453	0.108		0.033	
5	1171.931	1171.968	1.370	−0.037	0.026	−0.986
34		1172.245	0.024		0.046	
29	1173.491	1173.466	0.072	0.025	0.036	0.924
14		1178.573	0.123		0.044	
30	1178.864	1178.898	3.244	−0.035	0.030	−1.029
54	1179.646	1179.645	2.797	0.001	0.028	0.027
3		1184.385	0.037		0.046	
39	1184.919	1184.913	1.753	0.006	0.025	0.145
48	1184.919	1184.970	1.928	−0.052	0.030	−1.503
53		1185.078	0.057		0.047	
15	1186.137	1186.109	1.882	0.028	0.029	0.793
7	1186.337	1186.395	1.421	−0.058	0.028	−1.610
28	1190.919	1190.896	1.285	0.023	0.031	0.695
44		1192.506	0.092		0.042	
35		1192.688	0.018		0.040	
4	1193.317	1193.284	1.099	0.033	0.028	0.918
22	1199.820	1199.794	0.044	0.025	0.036	0.926

since the noncrossing problem is a one-dimensional phenomenon.[43] In a six-parameter iteration the crossing conditions can be fulfilled in many ways. The one-dimensional problem occurs only when the least squares method used in the iteration prescribe a linear combination of the six parameters as the direction along which the step must be taken. Therefore we find the discussion of the noncrossing problem by Laatikainen unfounded and agree with Castellano and Waugh[44] in their suggestion that noncrossing is introduced by the method of iteration and not by the quantum mechanical nature of the problem itself.

An interesting four-spin system that may serve as an illustration of the ideas in the previous discussion is presented by the ^1H spectrum of 6,7,10-trichloro-8,8-dimethoxy-1,4-dioxaspiro[4,5]dec-6-en-9-one.[45] The CH_2-CH_2 fragment constitutes a tightly coupled ABCD system when a 5 % w/w solution in $CDCl_3$ is used. In Fig. 1 the 270 MHz spectrum is reproduced. The staggered configuration of the CH_2-CH_2 has previously been studied in several dioxolanes.[46] The geminal coupling constants are expected to fall in the range -6 to -8 Hz while the vicinal coupling constants are expected to be about 7 Hz. The data used as starting parameters were

$\delta_1 = 1148.10$ Hz	$\delta_2 = 1162.70$ Hz
$\delta_3 = 1184.20$ Hz	$\delta_4 = 1180.70$ Hz
$J_{12} = J_{34} = -7.5$ Hz	$J_{13} = J_{24} = 7.0$ Hz
$J_{14} = 5.5$ Hz	$J_{23} = 7.3$ Hz

The data simulated the spectrum sufficiently accurately to permit an assignment of all the strong transitions in the spectrum. The subsequent iteration produced a set of best values listed as case I in Table 2. The accuracy of the location of the experimental lines is judged to be from 0.05 to 0.1 Hz. Based on these figures the RMS error of 0.029 obtained (standard deviation 0.04) is acceptable. The insufficiency of the assigned transitions is, however, reflected in the large confidence limits particularly for J_{34}. In Table 3 the deficiency of the provided data is reflected in $\delta(\hat{FR})$ values for some of the unassigned transitions being 40 to 80 times larger than the uncertainty of the input data. To obtain reliable parameters from the iteration it is, therefore, mandatory to reexamine the spectrum to decide if some of the weak transitions previously neglected could be assigned. The wing lines 51 and 22 could be identified uniquely. From the $\delta(\hat{FR})$ values it is anticipated that inclusion of line 51 will have little influence on the results. This is borne out in a subsequent iteration. Line 22 on the contrary has a large $\delta(\hat{FR})$ value and it is expected to influence the results significantly. The same is valid for line 29 that can be identified in the middle of the spectrum.

The best values obtained with this enlarged set of input data is given as case II in Table 2. It is seen that the confidence limits for all parameters become reasonable and in Table 4 the values of $\delta(\hat{FR})$ are all reduced to a uniform size comparable to the measurement accuracy.

Since all parameters are iterated it is expected that the t distributions in Tables 3 and 4 should be good for both iterations, since the model is correct. In calculations where one of the chemical shifts have been fixed on an incorrect value the t distribution showed this in mean value, variance and skewness. Our experience is that imperfect t distributions may arise due to a few outliers or to a small shift of many lines. Only in the latter case will the t values indicate that an incorrect model was used.

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APPENDIX

Consider the observed transitions collected in the column vector $\begin{pmatrix} u \\ y \end{pmatrix}$ where u symbolizes the observed yet unassigned transitions and y the assigned transitions used in the least squares determination of the parameters $\{\hat{p}\}$ according to the expression

$$\hat{p} = (A^{\mathrm{T}} M^{-1} A)^{-1} A^{\mathrm{T}} M^{-1} y \tag{A1}$$

where $\hat{p}$ is the least square solution to the equation

$$y = Ap + \varepsilon \tag{A2}$$

For the unassigned transitions we obtain the predicted values

$$\hat{u} = B\hat{p} \tag{A3}$$

where B is a matrix of known quantities similar to A in the previous equation. The variance covariance matrix for the unassigned transitions is defined by

$$E\{(u-\hat{u})(u-\hat{u})^{\mathrm{T}}\} \tag{A4}$$

where $E\{\ \}$ denotes the expectation values [38] of the quantity in braces.

Using A1 and A2 we obtain

$$
\begin{aligned}
&(u-\hat{u})(u-\hat{u})^{\mathrm{T}}=(u-B\hat{p})(u-B\hat{p})^{\mathrm{T}}= \\
&\{u-B(A^{\mathrm{T}}M^{-1}A)^{-1}A^{\mathrm{T}}M^{-1}[A\hat{p}+\varepsilon]\} \\
&\{u-B(A^{\mathrm{T}}M^{-1}A)^{-1}A^{\mathrm{T}}M^{-1}[A\hat{p}+\varepsilon]\}^{\mathrm{T}}= \\
&\{u-B\hat{p}-B(A^{\mathrm{T}}M^{-1}A)^{-1}A^{\mathrm{T}}M^{-1}\varepsilon\} \\
&\{u-B\hat{p}-B(A^{\mathrm{T}}M^{-1}A)^{-1}A^{\mathrm{T}}M^{-1}\varepsilon\}^{\mathrm{T}}= \\
&(u-B\hat{p})(u-B\hat{p})^{\mathrm{T}}- \\
&(u-B\hat{p})\{B(A^{\mathrm{T}}M^{-1}A)^{-1}A^{\mathrm{T}}M^{-1}\varepsilon\}^{\mathrm{T}} \\
&-\{B(A^{\mathrm{T}}M^{-1}A)^{-1}A^{\mathrm{T}}M^{-1}\varepsilon\}(u-B\hat{p})^{\mathrm{T}} \qquad \text{(A5)} \\
&+\{B(A^{\mathrm{T}}M^{-1}A)^{-1}A^{\mathrm{T}}M^{-1}\varepsilon\}\times \\
&\{B(A^{\mathrm{T}}M^{-1}A)^{-1}A^{\mathrm{T}}M^{-1}\varepsilon\}^{\mathrm{T}}
\end{aligned}
$$

We notice that the second and third term in A5 are vanishing due to

$$E\{\varepsilon\}=0$$

The last term can be rewritten as

$$
\begin{aligned}
&E\{B(A^{\mathrm{T}}M^{-1}A)^{-1}A^{\mathrm{T}}M^{-1}\varepsilon\varepsilon^{\mathrm{T}}M^{-1}A\times \\
&(A^{\mathrm{T}}M^{-1}A)^{-1}B^{\mathrm{T}}\}= \\
&B(A^{\mathrm{T}}M^{-1}A)^{-1}A^{\mathrm{T}}M^{-1}E\{\varepsilon\varepsilon^{\mathrm{T}}\}M^{-1}A\times \qquad \text{(A6)} \\
&(A^{\mathrm{T}}M^{-1}A)^{-1}B^{\mathrm{T}}
\end{aligned}
$$

The variance covariance matrix of the errors is given as

$$E\{\varepsilon\varepsilon^{\mathrm{T}}\}=\sigma^2M \qquad \text{(A7)}$$

Inserting A7 in A6 we find

$$
\begin{aligned}
&E\{(u-\hat{u})(u-\hat{u})^{\mathrm{T}}\}= \\
&B(A^{\mathrm{T}}M^{-1}A)^{-1}B^{\mathrm{T}}=BVB^{\mathrm{T}} \qquad \text{(A8)}
\end{aligned}
$$

In A8 we have neglected the first term in A5. This term we have no means of calculating based on the previously assigned transitions. For the present purpose only the variance *i.e.* the diagonal elements in A4, is of interest. The term neglected is expected to be approximately the size of the variance of the measurement error for the unassigned lines.

Using the subscripted notation $\delta(\widehat{FR}_{\mathrm{ln}})^2$ for the left hand side of A8 and $DC_{\mathrm{ln,k}}$ for the elements of B the expression in eqn. 11 is obtained.