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Precision in Estimating the Frequency Separation between Spectral Lines

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It is common to estimate the frequency separation between peaks in a digitized frequency-domain spectrum by fitting an appropriate function to the experimental spectrum using least-squares procedures. In this paper, we assess from first principles the precision associated with such measurements of frequency separation. In addition to the frequency separation between the peaks, other parameters involved in fitting the spectrum are the peak widths, the lineshape functions (Gaussian, Lorentzian, etc.) for the peaks, and the peak amplitudes. The precision also depends on the signal-to-noise ratio and the spacing between adjacent data points in the digitized spectrum. It is assumed that the residuals considered in the least-squares fitting procedure are the differences between the intensities of corresponding digitized data points in the experimental and fitted spectra. Under these conditions, analytical expressions for the precision in peak separation are derived for the following cases: (i) when the amplitudes of two peaks are known and the two peaks have known equal widths; (ii) when the ratio of the amplitudes of two peaks is known, and the widths of the two peaks are known to be equal, but the actual value of the peak width is not known. In each case, the situation with two Gaussian peaks and the situation with two Lorentzian peaks are considered. In all cases, the absolute precision $P(\eta)$ in the estimated frequency separation η between the two peaks is approximated by an equation of the type $P(\eta) \simeq F(\eta/\Delta, \alpha)S\sqrt{K}$, where Δ is the peak width, α is the ratio A_2/A_1 of amplitudes of the two peaks, S is the signal-to-noise ratio, and K is the density of data points in the frequency-domain spectrum. The form of the function $F(\eta/\Delta, \alpha)$ depends on the type of lineshape (Gaussian or Lorentzian), and depends on which of the parameters A_1, A_2, and Δ are known independently of the fitting procedure. Attempts to extend our first-principles approach to assess the precision in least-squares estimates of frequency separation between peaks in more complex situations than those discussed above generally lead to analytical expressions that are formidably complicated. In such cases, numerical approaches based on the theoretical framework developed here may be employed to assess the precision in estimating the frequency separation. © 1998 Academic Press

Key Words: **peak separation; peak shape fitting; precision; Gaussian peak shape; Lorentzian peak shape.**

1. INTRODUCTION

In many applications of NMR spectroscopy, and other spectroscopic techniques, it is necessary to measure the separation between two or more spectral lines (corresponding to peaks in the spectral curve), and an understanding of the precision in such measurements is of general interest and importance. In this paper, we focus on this question within the context of NMR spectroscopy, although the results are generally applicable to other types of spectroscopy.

In modern NMR spectroscopy, the spectrum is generally recorded as a digitized signal in the time domain, with subsequent Fourier transformation giving a digitized spectrum in the frequency domain (i.e., a plot of signal intensity *versus* frequency). A curve with a single peak in the frequency-domain spectrum is characterized by its lineshape function (usually Gaussian or Lorentzian) and by various parameters, which include the peak centre frequency, the peak width, and the peak amplitude; experimental factors such as the signal-to-noise ratio and the separation in frequency between adjacent digitized data points are also important considerations.

In this paper, we assess the separation between the peak centre frequencies of *two peaks* in a digitized frequency-domain spectrum, and we focus on the precision in estimating this frequency separation. Clearly, the question of precision becomes particularly important when the two peaks of interest overlap substantially.

In general, any accurate determination of fundamental information from an NMR spectrum involves fitting an appropriate function (as justified on theoretical and/or empirical grounds) to the measured (experimental) spectrum. We shall refer to the fitted function as the "calculated spectrum." In general, the aim of fitting a calculated spectrum to the experimental spectrum is that it may be possible to assign physical interpretations to the values of the fitted parameters that define the calculated spectrum. Commonly, the calculated spectrum is fitted to the experimental spectrum using least-squares procedures, in which the residuals are the differences between the

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intensity values of corresponding digitized data points in the experimental and calculated spectra. Thus, it is common practice to assume that errors arise only in the intensity of each data point, with no error in the frequency of each data point. In certain cases, some of the parameters used to define the calculated spectrum may be known independently of the fitting procedure, although in general most (or all) of these parameters will be handled as variables in the fitting procedure. The precision in estimating the value of a particular parameter, such as frequency separation between two peaks, will depend on any assumptions that can be made about the other parameters that define the calculated spectrum. In the case of the frequency separation between two peaks, the other parameters that characterize the calculated spectrum are the widths of the peaks, the lineshape functions (Gaussian, Lorentzian, etc.) for the peaks, and the amplitudes of the peaks. The precision in fitting the calculated spectrum to the experimental spectrum is also dependent on factors such as the signal-to-noise ratio and the frequency separation between adjacent data points in the digitized spectrum. The actual method adopted for the fitting procedure may also influence the precision in the estimate of the frequency separation between the peaks.

Within the context of NMR spectroscopy, a number of studies (*1–5*) have sought to derive general mathematical descriptions of the precision in estimating the peak centre frequency of a *single peak* (where relevant, passing reference is made in the present paper to this previous work). However, the question of the frequency separation between two peaks has essentially been ignored. Of the papers referenced above, only (*2*) touches upon this issue, and makes only the qualitative suggestion that "if the only source of error is attributable to random Gaussian noise, the error in measuring the separation between two peaks will always be larger than the error in measuring the position of one isolated peak."

In the present paper, we obtain *from first principles* expressions for the precision in estimating the frequency separation between two peaks in a digitized frequency-domain spectrum. It is important to emphasize that we are interested in the case in which only a single spectrum is available, rather than assessing the precision by measuring the frequency separation between the two peaks in independent, repeated measurements of the same spectrum (i.e., for the same sample under the same conditions). Using the first-principles approach developed here, we find that a straightforward analytical description of precision is obtained only in certain specific situations, as follows:

(i) the amplitudes of the two peaks are known and the two peaks have known equal widths

(ii) the *ratio* of the amplitudes of the two peaks is known, and the widths of the two peaks are known to be equal, but the actual value of the peak width is not known. (Subsequently, we consider separately the situation in which the ratio of the amplitudes of the two peaks is known to be unity, and the situation in which the ratio of the amplitudes of the two peaks is known to have some other value.)

In each situation, we consider both the case with two Gaussian peaks and the case with two Lorentzian peaks. Clearly, situation (ii) finds widespread application in many aspects of NMR spectroscopy, in which well-defined splitting mechanisms give rise to known amplitude ratios of *two peaks* in a spectrum (with the widths of the two peaks equal, but the value of peak width not known *a priori*). Examples include (*6–8*) J-coupling due to a single spin-$\frac{1}{2}$ nucleus (1:1 amplitude ratio), isotope effects on chemical shifts when two different isotopes are present (amplitude ratio dependent on the relative abundances of the two isotopes), isotropic peaks for chemically or crystallographically inequivalent nuclei (amplitude ratio dependent on the relative numbers of the different nuclei), dipolar interaction between two spin-$\frac{1}{2}$ nuclei in single crystal NMR (1:1 amplitude ratio), quadrupolar interaction for a spin-1 nucleus in a single crystal (1:1 amplitude ratio), and so on.

2. THEORY

For a spectral curve comprising two spectral lines, each of a given lineshape, the frequency-domain signal has the form

$$f(\nu) = A_1 g(u_1) + A_2 g(u_2), \qquad [1]$$

where the reduced frequencies u_1 and u_2 are defined by

$$u_1 = \frac{\lambda}{\Delta_1}(\nu - [\nu_0 - \eta/2])$$

$$u_2 = \frac{\lambda}{\Delta_2}(\nu - [\nu_0 + \eta/2])$$

for a suitable constant λ. Here A_i is the peak amplitude and Δ_i is the width at half the maximum height of the peak (for $i = 1, 2$), while ν_0 is the mean of the peak centre frequencies of the two peaks and η is the separation between the peak centre frequencies (see Fig. 1). Thus curves of the form given by Eq. [1] can be described by the 6 parameters (A_1, A_2, Δ_1, Δ_2, ν_0, η). In the case of Gaussian lineshape

$$g(u) = \exp(-u^2) \qquad \text{and} \qquad \lambda = \lambda_G = 2\sqrt{\ln 2},$$

whereas in the case of Lorentzian lineshape

$$g(u) = \frac{1}{1 + u^2} \qquad \text{and} \qquad \lambda = \lambda_L = 2.$$

We shall consider just the case $\Delta_1 = \Delta_2 = \Delta$. Note that for spectral curves of the form given in Eq. [1] with $\Delta_1 = \Delta_2$ the lineshapes form a 2-parameter family classified by (A_2/A_1, η/Δ), where A_2/A_1 is the ratio of peak amplitudes.

Our interest is in the peak separation η. It is appropriate to

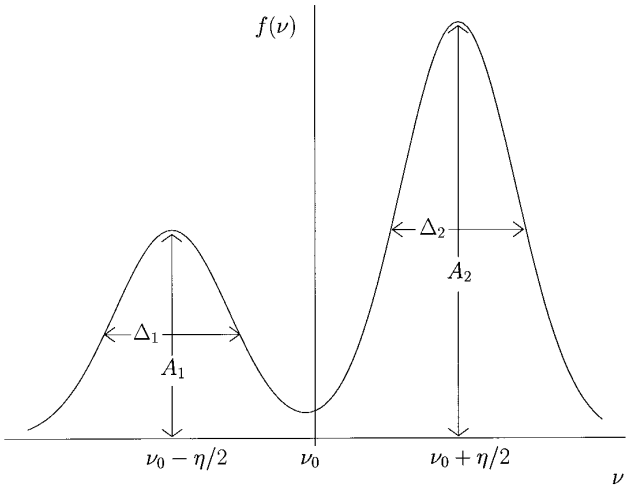

FIG. 1. Typical spectral curve containing two peaks, illustrating the peak amplitudes A_1 and A_2, peak widths Δ_1 and Δ_2, mean peak centre frequency ν_0, and peak separation η.

measure the precision in the estimate of the peak separation by the *absolute precision* $P(\eta)$ of η, defined by

$$P(\eta) = \frac{\Delta}{\sigma_{\hat{\eta}}},$$

where $\sigma_{\hat{\eta}}$ denotes the standard deviation of the least-squares estimate $\hat{\eta}$ of η.

We note in passing that an alternative way of measuring the precision in estimating η is by the *relative precision* $R(\eta)$, defined by

$$R(\eta) = \frac{\eta}{\sigma_{\hat{\eta}}}.$$

One advantage of the absolute precision over the relative precision is that $P(\eta)$ tends to a finite limit as η tends to infinity, whereas $R(\eta)$ tends to infinity as η tends to infinity.

In order to derive formulae for $P(\eta)$, we make the following assumptions:

(i) the noise occurs only in the amplitude (i.e., the observed digitized frequencies are known exactly);

(ii) the noise is independent of the signal and its standard deviation does not depend on the frequency;

(iii) the type of lineshape (e.g., Gaussian or Lorentzian) is known (although the values of the parameters are not necessarily known);

(iv) the calculated spectrum is fitted to the experimental spectrum by least squares;

(v) the number of data points in the frequency range $(\nu_0 - \eta/2 - 2\Delta,\ \nu_0 + \eta/2 + 2\Delta)$ is large and these points are evenly spaced in this interval.

We use K to denote the number of data points per peak width, i.e.,

$$K = \frac{\Delta}{d},$$

where d is the frequency-separation between adjacent data points. (We assume that d is a constant and known without error.) Because we are considering two peaks, care is needed in defining the signal-to-noise ratio. It is convenient to define the signal-to-noise ratio S as

$$S = \frac{\sqrt{A_1 A_2}}{\sigma},$$

where σ denotes the root mean square spectral (vertical) noise.

In order to obtain a formula for $P(\eta)$, we use the general theory of least-squares estimation. Consider a general spectral curve with amplitude $f(\nu; \theta_1, \ldots, \theta_k)$, where $\theta_1, \ldots, \theta_k$ are parameters. Then least-squares estimation of $\theta_1, \ldots, \theta_k$ is equivalent to maximum likelihood estimation in non-linear normal regression. Applying the general asymptotic theory of maximum likelihood estimation (*9, 10*) shows that the standard error $\sigma_{\hat{\theta}_i}$ of the maximum likelihood estimate $\hat{\theta}_i$ of θ_i is given by

$$\sigma_{\hat{\theta}_i} \simeq \sigma \sqrt{(H^{-1})_{ii}},$$

where H is the $k \times k$ matrix with elements

$$H_{ij} = \sum_{m=1}^{n} \frac{\partial f(\nu_m)}{\partial \theta_i} \frac{\partial f(\nu_m)}{\partial \theta_j}, \qquad [2]$$

and $\nu_1, \ldots, \nu_n$ are the observed digitized frequencies. Let G be the $k \times k$ matrix with elements

$$G_{ij} = \frac{\lambda}{\Delta} \int_{-\infty}^{\infty} \frac{\partial f(\nu)}{\partial \theta_i} \frac{\partial f(\nu)}{\partial \theta_j}\, d\nu$$

$$= \int_{-\infty}^{\infty} \frac{\partial h(u)}{\partial \theta_i} \frac{\partial h(u)}{\partial \theta_j}\, du,$$

where

$$h(u) = f\left(\nu_0 + \frac{\Delta}{\lambda} u\right)$$

and

$$u = \frac{\lambda}{\Delta}(\nu - \nu_0)$$

is a reduced frequency using an appropriate constant λ. Then using the approximation

$$H \simeq \frac{K}{\lambda} G,$$

as in (*3*), we obtain

$$\sigma_{\hat{\theta}_i} \simeq \sigma \sqrt{\frac{\lambda}{K}} \sqrt{(G^{-1})_{ii}}.$$

Note that, in general, $\hat{\theta}_1, \ldots, \hat{\theta}_k$ are dependent, leading to the important consequence that the precision of (say) $\hat{\theta}_1$ when $\theta_2, \ldots, \theta_k$ are known may be different from the precision of $\hat{\theta}_1$ when $\theta_2, \ldots, \theta_k$ are unknown.

3. RESULTS

Applying the above general theory to spectral curves of the form given in Eq. [1] and performing the necessary algebra with the assistance of the symbolic computation package Maple yield approximations

$$P(\eta) \simeq Q(\eta) \qquad [3]$$

to the absolute precision $P(\eta)$. These approximate precisions $Q(\eta)$ have the form

$$Q(\eta) = F(\rho, \alpha) S \sqrt{K}, \qquad [4]$$

where

$$\rho = \frac{\eta}{\Delta} \qquad \text{and} \qquad \alpha = \frac{A_2}{A_1}.$$

It is convenient to write Eq. [4] in the form

$$Q(\eta) = \sqrt{w(\rho, \alpha)}\, S \sqrt{K}.$$

Table 1 gives analytical expressions for the functions $w(x, \alpha)$ in the situations

(I) the amplitudes A_1, A_2 of the two peaks are known and the two peaks have known equal widths Δ;

(II) the amplitudes A_1, A_2 of the two peaks are known to be equal, but the actual value of the amplitude is not known *a priori*, and the widths Δ_1, Δ_2 of the two peaks are known to be equal, but the actual value Δ of the peak width is not known *a priori;*

(III) the *ratio* A_2/A_1 of the amplitudes of the two peaks is known, and the widths of the two peaks are known to be equal, but the actual value Δ of the peak width is not known *a priori*.

In each situation, we consider both the case with two Gaussian peaks and the case with two Lorentzian peaks.

The important features of these formulae for the approximations $Q(\eta)$ to $P(\eta)$ are that for all values of the known parameters

(a) $Q(\eta)$ tends to a non-zero finite limit as η tends to infinity;

(b) $Q(\eta)$ tends to 0 as η tends to 0;

(c) there is an upper bound on $Q(\eta)$.

An intuitive explanation of property (a) is that when the peaks are very far apart, the error in estimating the separation η is almost independent of η. An explanation of property (b) is that when the peaks are very close together the matrix G is almost singular. Since Q is a continuous function, property (c) follows from properties (a) and (b).

The fact that $Q(\eta)$ tends to 0 as η tends to 0 does not mean that $P(\eta)$ has the same behaviour. Indeed, the approximations in Eq. [3] become poor as η tends to 0. This is not a great drawback of these approximations, since the region near $\eta = 0$ is the region in which the normal equations for the least squares estimates of the unknown parameters are ill-conditioned (so that in this region calculation of these estimates by any Newton–Raphson type algorithm will encounter problems). The intuitive explanation of this ill-conditioning is that if $\eta \simeq 0$, then small changes in ν_0 have almost the same effect as small changes in η.

Some convenient upper bounds (which are not necessarily attained) on $F(x, \alpha)$ are given in Table 1.

Graphs of $F(x, \alpha)$ for $\alpha = 1$ and 10 when only A_2/A_1 is known are given in Fig. 2 for the Gaussian case and in Fig. 3 for the Lorentzian case.

4. DISCUSSION

In summary, in all cases considered, the precision $P(\eta)$ of the frequency separation between two peaks is given by an approximation of the type

$$P(\eta) \simeq F(\rho, \alpha) S \sqrt{K}, \qquad [5]$$

where ρ is the ratio η/Δ and α is the ratio A_2/A_1. The form of the function F depends on the type of lineshape (Gaussian or Lorentzian), and depends on which of the parameters A_1, A_2, or Δ are known independently of the fitting procedure. The general approximation in Eq. [5] for $P(\eta)$ is directly analogous to the approximation of the form

$$P_{\text{centre}}(\nu) \simeq F_{\text{centre}} S \sqrt{K} \qquad [6]$$

given in Ref. (*3*) for the precision $P_{\text{centre}}(\nu)$ in determining the peak centre frequency ν of a single peak in a spectrum. Thus, these approximations to both $P(\eta)$ and $P_{\text{centre}}(\nu)$ increase linearly with the signal/noise ratio and increase linearly with the square root of the density of data points in the digitized spectrum. However, the function $F(\rho, \alpha)$ encountered in the

TABLE 1

Approximate Precision $Q(\eta) = F(\eta/\Delta, A_2/A_1)S\sqrt{K}$ of Peak Separation η for the Three Situations I, II, III Defined in the Text

Lineshape	$w(x, \alpha) = F(x, \alpha)^2$	$\lim_{x\to\infty} F(x, \alpha)$	Upper bound
I. A_1, A_2, and Δ known			
Gaussian	$(2\pi \ln 2)^{1/2} \dfrac{1-\xi^2(1-x^2)^2}{\kappa + 2\xi(1-x^2)}$	$1.445/\sqrt{\kappa}$	$1.445/\sqrt{\kappa - 0.893}$[a]
Lorentzian	$\dfrac{\pi}{2} \dfrac{x^2(96 + 12x^2 + x^4)(128 + 12x^4 + x^6)}{(4 + x^2)^3(64[\kappa + 2] + 48[\kappa - 2]x^2 + 12\kappa x^4 + \kappa x^6)}$	$1.253/\sqrt{\kappa}$	$1.618/\sqrt{\kappa}$[a]
II. $A_2 = A_1$			
Gaussian	$\left(\dfrac{\pi \ln 2}{2}\right)^{1/2} \dfrac{2(1+\xi)^2(1-\xi) - 2\xi(1+\xi)x^2 + \xi(1-\xi)x^4}{2(1+\xi)^2 - 4\xi(1+\xi)x^2 + \xi x^4}$	1.021	1.071 at $x = 2.765$[b]
Lorentzian	$\dfrac{\pi}{2} \dfrac{x^2(256 + 160x^2 + 32x^4 + x^6)}{2(1024 + 112x^4 + 32x^6 + x^8)}$	0.886	0.960 at $x = 2.290$[c]
III. A_2/A_1 known			
Gaussian	$\left(\dfrac{\pi \ln 2}{2}\right)^{1/2} \dfrac{A_0 + A_2x^2 + A_4x^4 + A_6x^6}{(\kappa + 2\xi[1-x^2])(B_0 + B_2x^2 + B_4x^4)}$	$1.445/\sqrt{\kappa}$	—[d]
	$A_0 = 2(1-\xi^2)(\kappa + 2\xi)^2$ $\quad B_0 = (\kappa + 2\xi)^2$		
	$A_2 = -2\xi(\kappa + 2\xi)(4 + \kappa\xi - 2\xi^2)$ $\quad B_2 = -4\xi(\kappa + 2\xi)$		
	$A_4 = 2\kappa\xi(1 + \kappa\xi + \xi^2)$ $\quad B_4 = \kappa\xi$		
	$A_6 = -\kappa(\kappa - 2\xi)\xi^2$		
Lorentzian	$\dfrac{\pi}{2} \dfrac{x^2(C_0 + C_2x^2 + C_4x^4 + C_6x^6 + C_8x^8 + C_{10}x^{10} + C_{12}x^{12})}{(D_0 + D_2x^2 + D_4x^4 + \kappa x^6)(E_0 + E_2x^2 + E_4x^4 + E_6x^6 + \kappa^2x^8)}$	$1.253/\sqrt{\kappa}$	$1.568/\sqrt{\kappa}$[a]
	$C_0 = 8192(\kappa + 2)^2$ $\quad D_0 = 64(\kappa + 2)$		
	$C_2 = 20480(\kappa - 1)(\kappa + 2)$ $\quad D_2 = 48(\kappa - 2)$		
	$C_4 = 7168(\kappa - 1)(\kappa + 2)$ $\quad D_4 = 12\kappa$		
	$C_6 = 64(\kappa + 2)(25\kappa - 14)$ $\quad E_0 = 256(\kappa + 2)^2$		
	$C_8 = 16(21\kappa^2 + 28\kappa - 4)$ $\quad E_2 = 256(\kappa^2 - 4)$		
	$C_{10} = 4\kappa(7\kappa + 8)$ $\quad E_4 = 32(3\kappa^2 + 2\kappa - 2)$		
	$C_{12} = \kappa^2$ $\quad E_6 = 16\kappa(\kappa + 2)$		

Note. In all cases, the linewidths of the two peaks are equal: $\Delta_1 = \Delta_2 = \Delta$. *Note* that $\kappa = \alpha + \alpha^{-1}$ and $\xi = \exp(-x^2/2)$.

[a] Not necessarily attained.

[b] $x \geq 1.374 \Rightarrow F(x, 1) \geq 1.021$.

[c] $x \geq 1.633 \Rightarrow F(x, 1) \geq 0.886$.

[d] No useful bound known.

expression in Eq. [5] for $P(\eta)$ reduces to a constant F_{centre} in the expression in Eq. [6] for $P_{\text{centre}}(v)$. (Note that the value of F_{centre} depends on the type of lineshape.)

When the two peaks are well separated, ρ is large, whereas at the other extreme in which the peaks merge into one, the value of ρ becomes zero. As can be seen from Table 1, for large ρ, the approximate precision $Q(\eta)$ is almost constant, as is physically reasonable given that, when the peak separation is large compared with the peak width (i.e., no significant overlap of the peaks), the ability to fit the two peaks should not depend on the actual value of the peak separation. However, it is found that the maximum of the approximate precision $Q(\eta)$ is not necessarily equal to the limit of $Q(\eta)$ for large ρ. Indeed, in several of the situations considered (see Section 3), the maximum approximate precision is achieved in the region of $\rho = 1$ to $\rho = 2$ (see Figs. 2a, 3a, and 3b). However, it is clear from the present work that no generalizations can be made regarding the conditions for maximum precision, and any particular case must be considered on its own merits by applying the full equations developed here. In all cases, as ρ tends towards zero, the approximate precision $Q(\eta)$ approaches zero. However, this does not mean that the true precision $P(\eta)$ has the same behaviour. As ρ approaches zero, the approximations of $P(\eta)$ by $Q(\eta)$ become poor. This is not a great disadvantage, since when ρ is small, least-squares estimates cannot be calculated reliably.

In principle, it is possible to extend the work reported here to assess the precision in least-squares estimates of the frequency separation between peaks in situations that are more complex than those considered here. For example, we have carried out preliminary research to derive analytical expressions for precision in peak separation for the following situations:

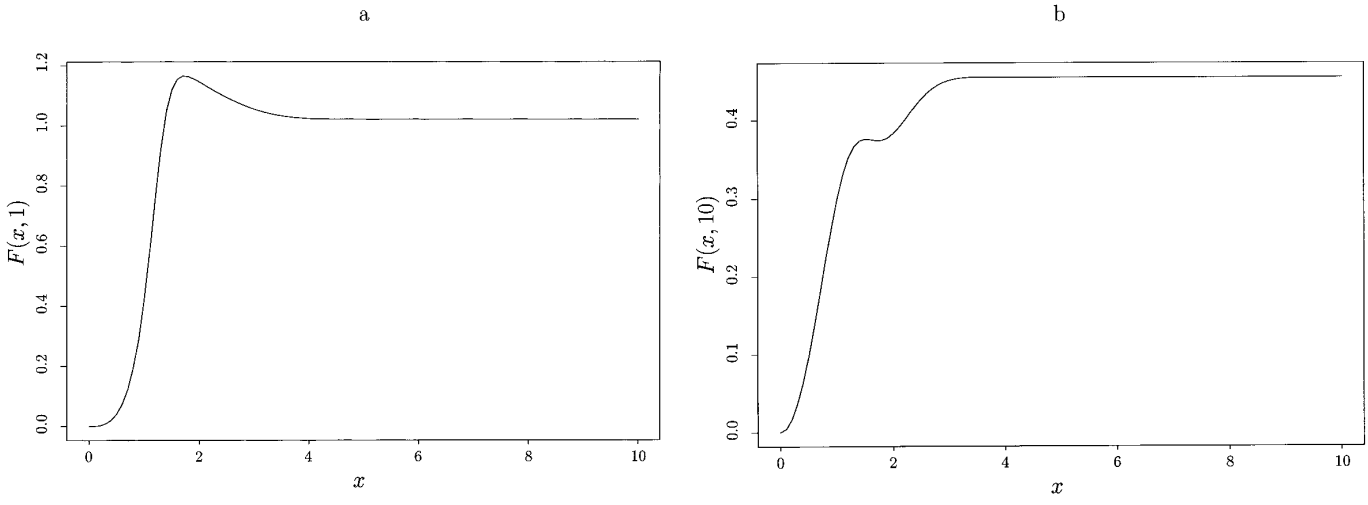

FIG. 2. $F(x, \alpha)$ for the Gaussian case with only the ratio $A_2/A_1 = \alpha$ known: (a) $\alpha = 1$, (b) $\alpha = 10$.

(i) two peaks, as in Section 3 above, but with unequal widths ($\Delta_1 \neq \Delta_2$)

(ii) three peaks (either Gaussian or Lorentzian), for which the Fourier-transformed signal has the form

$$f(\nu) = A_1 g\left(\frac{\lambda}{\Delta_1}(\nu - \nu_0)\right) + A_2 g\left(\frac{\lambda}{\Delta_2}(\nu - [\nu_0 - \eta_2])\right) + A_3 g\left(\frac{\lambda}{\Delta_3}(\nu - [\nu_0 + \eta_3])\right),$$

with η_2 and η_3 denoting the separations between the central peak and the two outer peaks.

However, the analytical expressions obtained for $P(\eta)$ in these cases are formidably complicated, and unlikely to be of any real practical usefulness. This is true even for the apparently straightforward situations comprising

(i) two peaks with known amplitude ratio A_2/A_1 and known ratio Δ_2/Δ_1 of widths, but with Δ_2/Δ_1 not necessarily equal to 1 (recall that Section 3 considered the case of $\Delta_2/\Delta_1 = 1$),

(ii) three peaks with known amplitude ratios A_2/A_1 and A_3/A_1, a known common width $\Delta = \Delta_1 = \Delta_2 = \Delta_3$, and equal separations $\eta_2 = \eta_3 = \eta/2$ between the central peak and the two outer peaks.

In spite of the fact that this complexity restricts the generalization of the present work, the situations considered in Section 3 nevertheless occur widely in different types of spectroscopy, particularly for the situation with two peaks of known amplitude ratio (A_2/A_1) and equal (but unknown) widths. These results are generally applicable to different types of spectroscopic data, although our original motivation in the present case was to consider the precision in peak separation in Fourier transform NMR spectra. The physical situation covered by situations II and III of Subsection 3.2 is indeed often encoun-

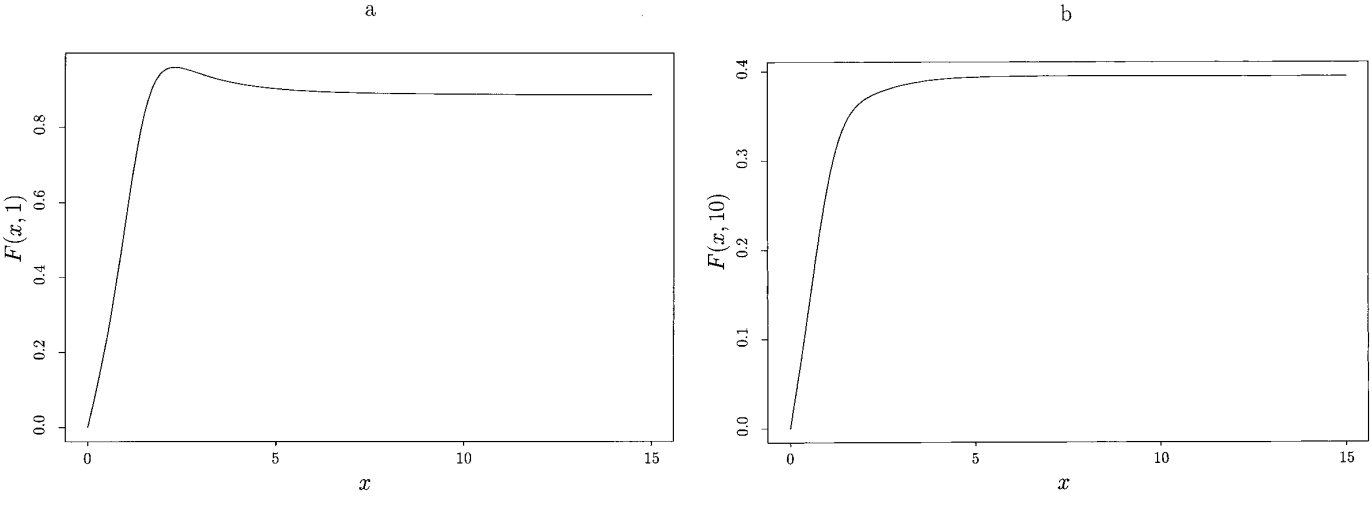

FIG. 3. $F(x, \alpha)$ for the Lorentzian case with only the ratio $A_2/A_1 = \alpha$ known: (a) $\alpha = 1$, (b) $\alpha = 10$.

tered in different aspects of liquid state and solid state NMR spectroscopy—in many cases, two peaks are generated by some splitting phenomenon such that the widths of the two peaks are equal and their amplitudes are governed by a well-defined ratio (controlled by the physics underlying the particular situation). Clearly, however, the case of "accidental" overlap of peaks would need to be considered with more caution, as (depending on the physical origin of the two peaks) there need not necessarily be well-defined and known relationships between the amplitudes and/or the widths of the two peaks.

There is an important distinction between general formulae of the form given in Eq. [5] (and analogous formulae in the case of 3 peaks) and numerical expressions for approximations to $P(\eta)$. The general formulae hold for *all* values of the parameters, whereas the numerical expressions hold only for the *specific* (estimated) values of the parameters which are used in the calculated spectrum. The complexity of the general formulae arises mainly from the inversion of a moderately large matrix containing fairly complicated formulae, whereas the numerical expressions are obtained from the inversion of matrices of numbers (analogous to numerical forms of H in Eq. [2]), and are supplied almost automatically by many statistical computer packages. Clearly, numerical methods provide a viable approach for estimating precision in the more complicated situations discussed above.

Finally, we emphasize that the present work has focused on the precision associated with the fitting procedure. Other sources of error may be present in the experimental spectrum, and would complicate further the assessment of precision. Thus, various sources of experimental or instrumental error may be significant, such as (in the case of NMR spectroscopy) magnetic field instability or inhomogeneity, or temperature instability or inhomogeneity. Further, it often happens that closely spaced or overlapping peaks are strongly coupled, leading to distortion of the lineshape. Such factors, which are not considered in the present analysis, may lead to displacements and/or broadening of the peaks in the spectrum, and in particular may lead to the experimental lineshape being non-Gaussian or non-Lorentzian. Clearly, there are intrinsic difficulties in achieving a good fit between experimental and calculated spectra for such cases in which the experimental lineshape may be poorly defined.

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