CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 36, No. 3 March 1988

Regular Articles

[Chem. Pharm. Bull. 36(3) 849—856 (1988)]

Characteristics of a Lorentzian to Gaussian Transformation Function as a Weighting Function in Processing the Two-Dimensional Nuclear Magnetic Resonance Spectrum of a Drug–Phosphatidylcholine Vesicles Solution

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(Received June 23, 1987)

Advantages and disadvantages of a Lorentzian to Gaussian transformation function, which has been commonly employed in enhancing the resolution of two-dimensional nuclear magnetic resonance (2D NMR) spectra, were investigated by applying this function to the 2D nuclear Overhauser effect (NOE) NMR spectrum of a drug–phosphatidylcholine vesicles solution. It was found that, although this function is known to be useful in enhancing the resolution of an NMR spectrum of a low-molecular-weight molecule which can afford relatively narrow NMR resonance peaks, the function often fails to yield a good line shape or contour map in the case where there exist broad resonance peaks having various line widths as in the presently tested sample solution. A careful choice of the parameters relevant to the function is required. In conclusion, the Lorentzian to Gaussian transformation function is less superior to a sine-bell function which has also been employed as an alternative function to process 2D NMR spectra. Some examples are presented in this paper.

Keywords——Lorentzian–Gaussian transformation function; weighting function; two-dimensional NMR; phosphatidylcholine vesicle

Introduction

Undoubtedly one of the most important advantages in using a pulse Fourier transform method in nuclear magnetic resonance (NMR) spectroscopy is that it can easily perform a deconvolution (or convolution) process against the overall NMR signals in a spectrum by simply multiplying the corresponding free induction decays (FID) by a relevant mathematical function to increase the resolution (or to improve the signal-to-noise (S/N) ratio) of the NMR spectrum.[1)] This advantage is extremely useful for processing a two-dimensional (2D) NMR spectrum which is observed in the absolute value mode, since in this mode each NMR peak is accompanied with long tails on both sides because of a dispersion component in the NMR

signal, and thus these should be eliminated by an appropriate weighting function. Among the various mathematical functions, Lorentzian to Gaussian transformation function (LG function) (Eq. 1)[2] and sine-bell functions (Eq. 2)[3]

$$g(t)=\exp(t/C1)\exp(-t^2/C2^2) \quad (1)$$

$$g(t)=\sin^n(\pi t/ACQ+\pi\phi/180) \quad (2)$$

which include shifted sine-bell[4] ($\phi \neq 0$) and shifted squared sine-bell ($n=2$)[5] functions have been widely used.[5,6] Here, C1 and C2 in Eq. 1 and ϕ in Eq. 2 denote parameters which should be arbitrarily determined and ACQ means the acquisition time employed for the measurement. For the best results, all these functions should be tried. Unfortunately, however, commercially available spectrometers are not always equipped with the two types of functions simultaneously. In the present work, the applicability of the LG function to the 2D nuclear Overhauser effect (NOE) NMR spectrum (NOESY)[7] of a drug–phosphatidylcholine vesicles solution has been tested. This sample solution is a good one for testing, since it produces fairly broad NMR peaks. Moreover, the broad peaks have various line widths and thus it is very difficult to make allowances for the extent of applying the weighting function properly. The NOEs of the solution employed in the present work have already been investigated by conventional 1D NOE difference spectroscopy and discussed elsewhere.[8]

Experimental

Materials——L-α-Egg yolk phosphatidylcholine (egg PC) and dibucaine hydrochloride were purchased from Sigma and used without further purification. Single bilayer vesicles were prepared by a conventional method as in our previous work, using a Nihon Seiki US-150 ultrasonic sonicator.[8,9]

Measurements——^{1}H-NMR spectra were measured on a Varian XL-300 (300 MHz) NMR spectrometer. 2D NMR spectra treated by sine-bell functions were obtained on a Bruker AM-400 (400 MHz) spectrometer. Chemical shifts were referenced to the signal from the terminal methyl protons of the acyl chains of egg PC.

Results and Discussion

As shown in Eq. 1, the LG function depends on the two parameters C1 and C2, where C1 characterizes the increasing (positive) exponential and C2 the decreasing (negative) squared exponential functions. Figures 1 and 2 show graphic representation of the LG function with various combinations of C1 and C2 for ACQ of 0.19 s. This acquisition time was adjusted so as to give a digital resolution that can distinguish the adjacent choline methyl signals from egg PC's located at the inner and outer halves of the bilayer vesicles.[8] A shifted sine-bell function with $\phi=5°$ and a squared sine-bell function are also shown in Fig. 2 (curves i and j). In curve d of Fig. 1, C1 and C2 were so determined as to give an envelope which decays in a symmetrical fashion on each side of the midpoint of the time axis. This is a so-called pseudo-echo function[10] and is expected to yield a symmetrical FID with respect to the midpoint of the acquisition time. Curves a, b, and c in Fig. 1 were drawn, respectively, by giving C1 and C2 the values which are 1/4, 1/2, and 3/4 of the corresponding magnitudes of C1 and C2 in curve d. In curve e of Fig. 2, C1 and C2 were so determined as to mimic the envelope of the sine-bell functions i and j. Here, it should be noted that since the magnitude of the LG function varies to a great extent with different choices of C1 and C2 (*i.e.*, it is not normalized to 1 as in the sine-bell functions), curves a—d in Fig. 1 and curve e in Fig. 2 were drawn by reducing their absolute magnitudes by 1/10. Thus, in the case of curve d, if we similarly shrink its absolute magnitude by *ca.* 1/55 (not by 1/10), we could also obtain a sine-bell like envelope which is slimmer than that of curve j (squared sine-bell function) shown in Fig. 2. Curves f and g are

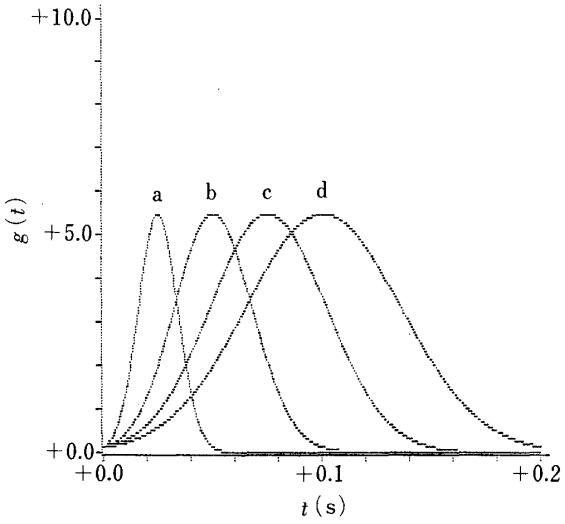

Fig. 1. The Form of the Various Lorentzian to Gaussian Transformation Function within an Acquisition Time of 0.19 s

The parameters C1 and C2 in Eq. 1 were as follows: a, (0.003, 0.012); b, (0.006, 0.024); c, (0.009, 0.036); d, (0.012, 0.048). The absolute magnitudes of these functions are reduced by 1/10.

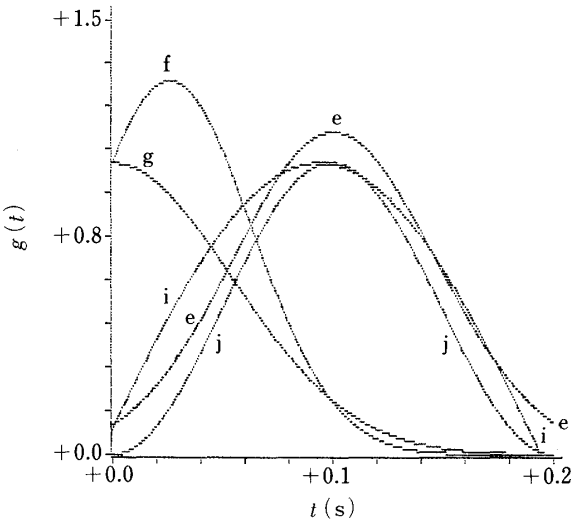

Fig. 2. The Form of the Various Lorentzian to Gaussian Transformation Functions (e, f, g), a Shifted Sine-Bell Function with $\phi = 5°$ (i), and a Squared Sine-Bell Function (j) for an Acquisition Time of 0.19 s

The parameters C1 and C2 in Eq. 1 were as follows: e, (0.02, 0.062); f, (0.05, 0.05); g, (20, 0.073). The absolute magnitude for curve e is reduced by 1/10.

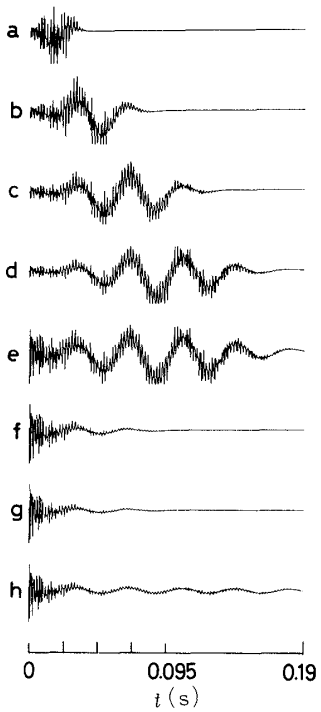

Fig. 3. a—g: FID Signals Processed by the Weighting Functions Shown, Respectively, in Figs. 1a—d and Figs. 2e—g
h: An Unweighted Original FID

The acquisition time was 0.19 s. Vertical scales are arbitrarily changed among the FIDs shown in a—h.

other typical shapes of weighting functions that can be produced by the LG function; they do not cut the initial stage of an FID as markedly as in the cases of curves a—e.

Figure 3 shows the effects of these weighting functions on an actual FID signal ($ACQ =$ 0.19 s) obtained from a dibucaine–egg PC vesicles solution; the FID was taken from the first block of accumulation with respect to the t_1 increment in a 2D NOESY experiment.[7] Relative vertical scales among Figs. 3a—h were also arbitrarily changed for the reason mentioned above. Figures 3a—g, respectively, show the FIDs weighted by the functions which are drawn

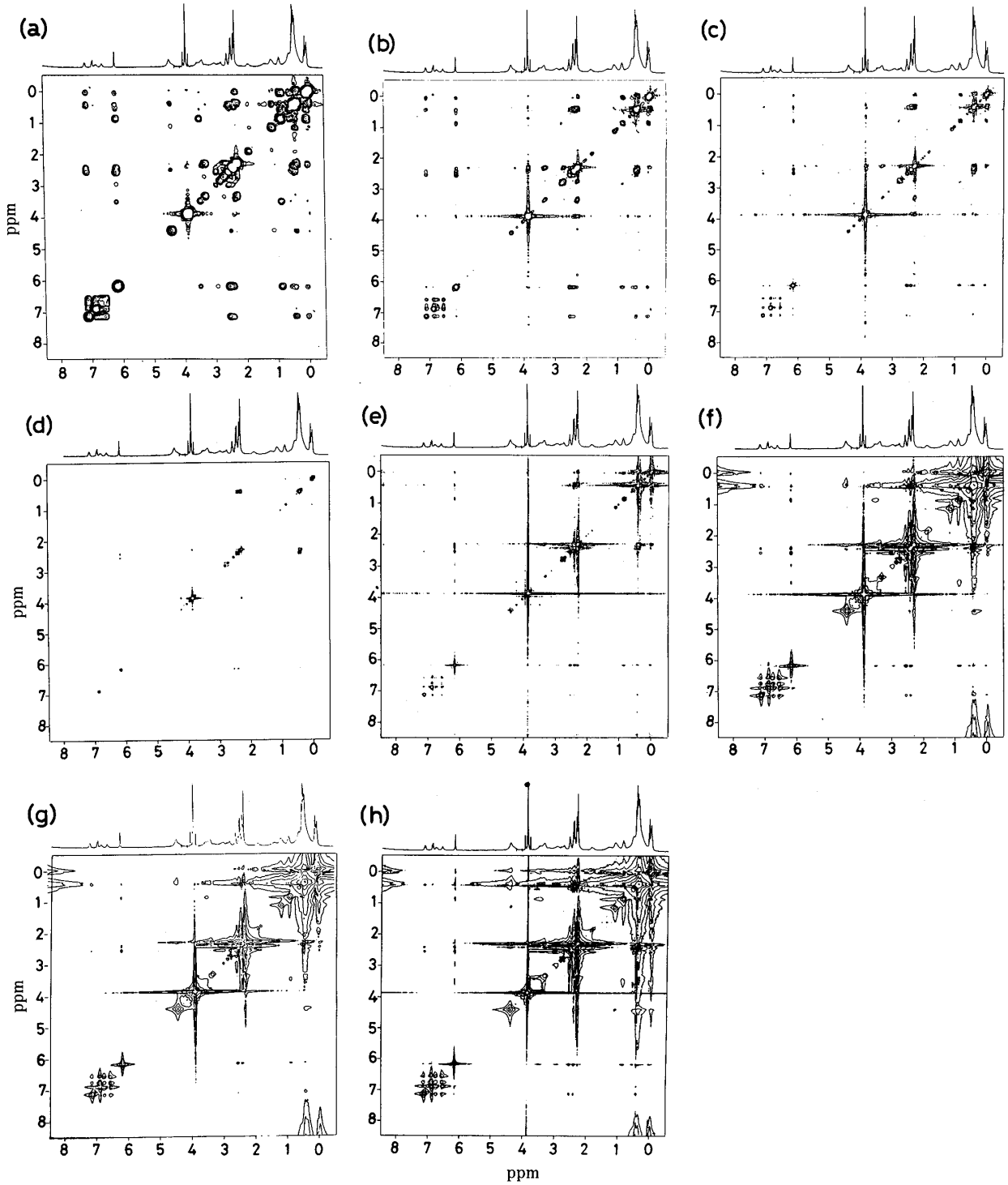

Fig. 4. Contour Plots of Symmetrized, Absolute-Value NOESY Spectra of Dibucaine Hydrochloride (42 mM)–Egg PC Vesicles (42 mM) in D_2O Solution (pH 5.3) Observed at 300 MHz

a—g: NOESY spectra processed by the Lorentzian to Gaussian transformation functions shown, respectively, in Figs. 1a—d and Figs. 2e—g.

h: A NOESY spectrum from the unweighted FIDs. In each contour plot, the vertical scale was arbitrarily adjusted so as to give the contour map with a moderate S/N ratio. A spectral width of 2700 Hz was used, collecting 512 t_1 experiments, each in 1024 points; the relaxation delay between measurements was 2.0 s and four dummy cycles were inserted at the beginning of each acquisition block in order to attain steady-state magnetizations in the NOESY pulse sequence. The mixing time to grow the NOE cross-peak was 0.2 s with $\pm 10\%$ random variation. The digital resolution for the final spectrum was 5.3 Hz/point.

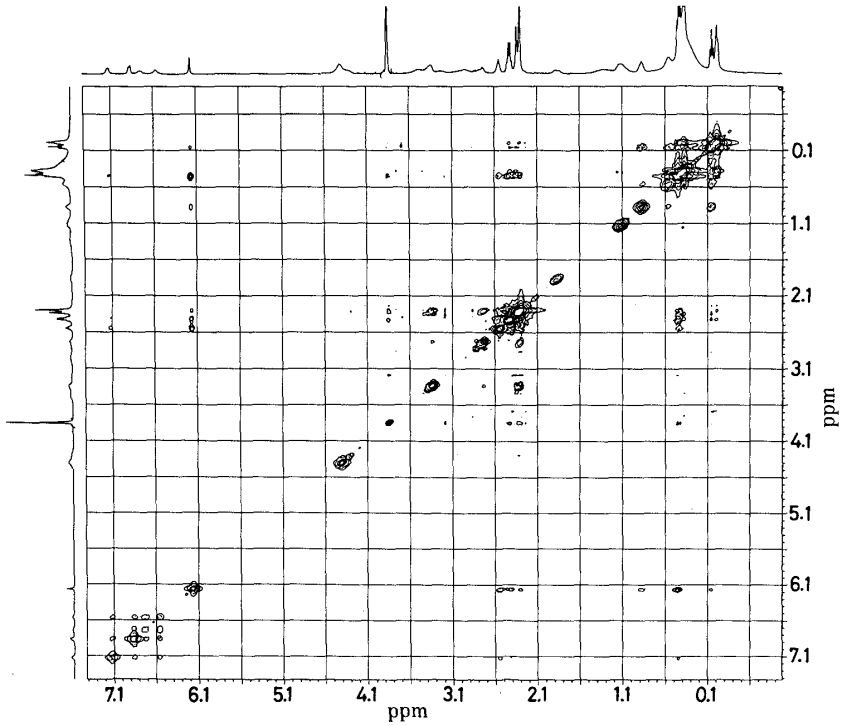

Fig. 5. Contour Plot of Symmetrized, Absolute-Value NOESY Spectrum of Dibucaine Hydrochloride (45 mM)–Egg PC Vesicles (45 mM) in D_2O Solution (pH 5.1) Observed at 400 MHz, Showing an Example Processed by a Sine-Bell Function

A spectral width of 3290 Hz was used, collecting 256 t_1 experiments, each in 1024 points; the relaxation delay between measurements was 2.0 s and two dummy cycles were inserted at the beginning of each acquisition block. The mixing time was 0.12 s with $\pm 14\%$ random variation. The digital resolution for the final spectrum was 6.4 Hz/point. This spectrum was observed by applying a continuous, selective radio frequency field to the residual HDO resonance at about 3.9 ppm at all times except during the periods t_1 and t_2.

as curves a—g in Figs. 1 and 2; the corresponding NOESY spectra are shown in Figs. 4a—g, respectively. Figure 3h is the original unweighted FID; the corresponding NOESY spectrum is shown in Fig. 4h. Again, the relative magnitudes of the contour maps shown in Fig. 4 are not always plotted with the same vertical scale, but are plotted to give an appropriate S/N ratio for an overall contour map in each NOESY spectrum treated by the different weighting functions. This was inevitable, since the differences in the weighting functions among a—g not only caused differences in the vertical scale as mentioned above but also caused differences in the S/N ratio of the resulting NMR spectrum. Figure 5 shows a typical 2D NOE contour plot treated by a sine-bell function. Here, the spectrum was obtained with an acquisition time of 0.16 s and by applying a homogated decoupling power to the residual HDO resonance at about 3.9 ppm.

As can be seen from Fig. 4h, the 2D contour plot calculated with no weighting function has long tails and broad pedestals, especially on resonances having a high peak height. It is desirable to eliminate these extraneous lines. Among the presently tested LG functions, the pseudo-echo filter (curve d of Fig. 1) which gives a symmetrical envelope of an FID (Fig. 3d) is expected to cut those lines most effectively, since in addition to the line narrowing effect owing to cutting the FID signals at a shorter acquisition time, a dispersive component in the NMR signal can be eliminated in principle.[10] However, as shown in Fig. 4d, this filter not only cuts those obstacles in the contour map, but it also cuts the broad resonance peaks themselves too much to afford a moderately good contour map for an NMR spectrum of the presently employed solution. Of course, if we use the weighting functions f and g (Fig. 2), such an

excessive filtering can be avoided at the expense of line narrowing efficiency (Figs. 4f and 4g). The weighting function e in Fig. 2 represents a compromise between the pseudo-echo filter and the functions f and g. Therefore, it seems to be one of the best LG functions tested presently (Fig. 4e). Another means of overcoming the excessive filtering encountered in the pseudo-echo function is to shift the top of the function to a shorter time of acquisition, as in the curves a—c in Fig. 1. This should be reasonable, since, as can be understood from the original FID (Fig. 3h), the free induction signals from various frequency components have mostly decayed out at about 50 ms. Thus, in order to leave the major parts in the FID, we should displace the top of the weighting function to the left. Inspection of the resulting 2D contour maps shown in Figs. 4a—c informs us, however, that to shift the top of the filtering function causes a decrease in the resolution (sharpness) of the contour lines (see Fig. 4a). This is due to the decreased C2 value in Eq. 1, since line width at half height of a Gaussian line shape is proportional to 1/C2.[6a] Apparently reduced acquisition time which omits a still remaining FID component also leads to vague outlines. This can be seen from inspection of the shapes of an envelope on the right side of the functions a—c (Fig. 1).[11] In spite of the reduced resolution in the contour map, however, we can find a good compromise in Fig. 4b or 4c. Interestingly, these contour plots, especially those shown in Fig. 4b, closely resemble Fig. 5 in appearance.[12] Figure 5 was processed by a sine-bell function. This similarity in appearance may be because the slopes of the envelope of the left side in these functions resemble each other. However, when we compare these contour plots (in Figs. 4b, 4c, and 5) with those calculated using no weighting function (Fig. 4h), we notice that all these functions failed to give an NOE cross peak involving a very broad resonance. For example, the NOEs between the resonance at about 6.2 ppm and the very broad resonances at about 3 and 3.5 ppm are missing in Figs. 4b, 4c, and 5. In this case, we should use such an LG function as shown in Fig. 1a. This function does an excellent job of omitting long tails and broad pedestals, on the one hand, but can incorporate broad diagonal peaks and the NOE cross-peaks mentioned above into the contour map, on the other (see Fig. 4a). In a sine-bell function (Eq. 2), incorporation of the broad resonances (and NOE cross-peaks) into a 2D NMR contour map can be dealt with by shifting the phase ϕ at the expense of the efficiency of cutting the long tails and broad pedestals. However, as shown in Fig. 6, where we showed a NOESY spectrum treated with a shifted sine-bell function of $\phi = 5°$ for an FID accumulated with an acquisition time of 0.13 s,

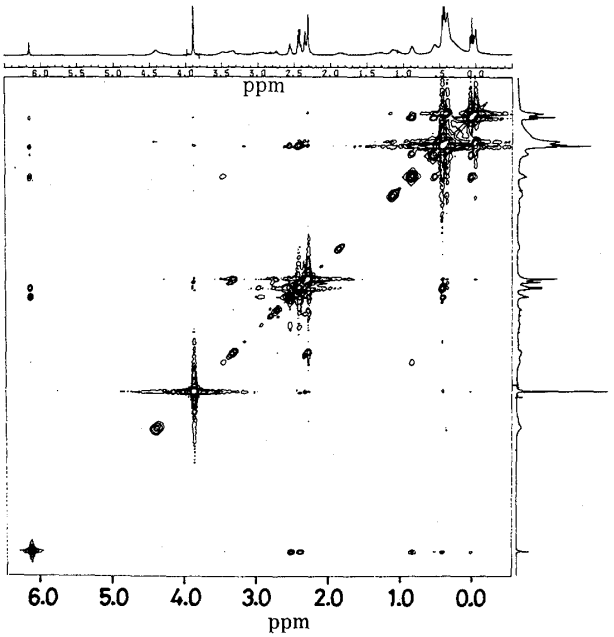

Fig. 6. Contour Plot of Symmetrized, Absolute-Value NOESY Spectrum of Dibucaine Hydrochloride (42 mM)–Egg PC Vesicles (42 mM) in D_2O Solution (pH 5.3) Observed at 400 MHz, Showing an Example Processed by a Shifted Sine-Bell Function ($\phi = 5°$)

A spectral width of 4000 Hz was used, collecting 256 t_1 experiments, each in 1024 points; the relaxation delay between measurements was 1.0 s and two dummy cycles were inserted at the beginning of each acquisition block. The mixing time was 0.2 s with $\pm 11\%$ random variation. The digital resolution for the final spectrum was 7.8 Hz/point.

shifting the phase ϕ in order to recover the NOE cross-peaks involving the broad resonances is not as effective as in the case shown in Fig. 4a. In Fig. 6, long tails on each resonance have already begun to extend, whereas the NOE cross-peaks between the resonance at about 6.2 ppm and the resonances at about 3 and 3.5 ppm have not yet been recovered. Again, the different effects between the weighting function a and the shifted sine-bell one on the resulting contour maps are understandable when we consider the differences in the slope on the left side of the envelope in these functions. The shifted sine-bell function cuts the FID signals at the acquisition times of *ca.* 13—32 ms more effectively than does the LG function a, while it cuts those shorter than 13 ms, which may be responsible for long tails of a contour map, less effectively.[13] In contrast, the LG function a can incorporate a large portion of the FID components into the signal energy (compare Fig. 3a with 3h), producing an NMR spectrum having a better S/N ratio than in the case of the shifted sine-bell function (or any other LG function tested presently). This good S/N ratio of an NMR spectrum facilitates plotting of the NOE cross-peaks involving the broad resonances at about 3 and 3.5 ppm. As can be appreciated from Eq. 2, the slope of the envelope of the sine-bell function on both sides of its maximum point depends on the acquisition time (ACQ). Thus if we want to handle the broad NOE peaks as above with this sine-bell function, we should decrease the number of points to be Fourier transformed in order to shift the top of the sine-bell function to the left as in the curve a in Fig. 1.

Concluding Remarks

The ^{1}H-NMR spectrum of a phosphatidylcholine (PC) vesicles solution consists of signals having various line widths. This is a consequence of the different mobilities of the constituents of the PC in the vesicles, *i.e.*, choline head group, glycerol moiety, and acyl chains including olefinic and terminal methyl groups. The chemical shift difference of the same proton(s) which arises from different sizes and shapes of the PC vesicles can also be responsible for the apparent differences in the line widths. Similarly, a drug which is interacting with the PC in the solution also shows NMR signals with various line widths depending on the degree and the site of the interaction, and on the location in the PC vesicles if it was incorporated into the vesicles. In applying an LG function to the FIDs of these samples in order to enhance the appearance of the resulting 2D NMR contour maps, we should exercise special care to avoid such an excessive weighting as shown in Fig. 4d. We should also take care not to lose cross-peaks actually existing in an unweighted contour plot. To this end it is necessary to employ at least two kinds of LG function as illustrated above. However, in practice the LG function is highly dependent on the magnitudes of C1 and C2, and/or on the relative magnitudes of these parameters, producing a very different envelope shape. In this sense, although the LG function can be considered more versatile than a sine-bell function on the one hand, it is also true that the LG function is harder to deal with than the sine-bell one. The recently developed phase-sensitive mode of operation in 2D NMR spectroscopy can give pure absorption phase spectra[14] and thus does not always require such vigorous reshaping of the NMR signals as in the absolute-value 2D experiments. However, the phase-sensitive method cannot be applied to such variations in 2D NMR pulse sequences as in homonuclear 2D J-spectroscopy and in correlation spectroscopy with radio frequency pulses not equal to 90° for the second (detection) pulse.[15] In these cases, again, careful consideration of an appropriate weighting function as stated above is still required.

Acknowledgement We thank Professor M. Okamoto of Kyoto Pharmaceutical University for providing facilities to prepare the egg PC films. We also thank Dr. M. R. Wälchli of Bruker Japan Co., Ltd. for the 2D NMR measurements (Fig. 5).

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11) For this reason, in Fig. 4a, even if the number of points to be Fourier transformed was reduced by 1/2 as compared to the other cases, the decrease in the resolution of the contour map which is attributable to this reduction in the data points is negligible in appearance.
12) On account of the difference in the mixing time employed, Fig. 5 gives smaller magnitudes of NOE cross-peaks than those shown in Fig. 4b.
13) The origin of the long tail in a contour map is not always due solely to the dispersion component in the NMR signal which is contained in an absolute-value mode of display. Another possible cause is attributable to the incorrectly sampled FID signals at their first points of every FID and every interferogram [G. Otting, H. Widmer, G. Wagner, and K. Wüthrich, *J. Magn. Reson.*, **66**, 187 (1986)].
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