MAGNETIC RESONANCE IN CHEMISTRY, VOL. 35, 379–383 (1997)

Measurements of Long-Range Homonuclear Coupling Constants

L. Mahi and J. C. Duplan*
Université Claude Bernard Lyon I, CPE, Laboratoire de Résonance Magnétique Nucléaire, CNRS UPRESA 5012, 43
Boulevard du 11 novembre 1918, bâtiment 308, 69622 Villeurbanne, France

Coupling constants in proton systems provide access to useful structural information. Several methods have been proposed to measure these constants in high-resolution spectra, but many of them are not well suited when the coupling constants are comparable to the spectral linewidth. In such a case the measurement of the apparent splitting, obtained from conventional NMR or from two-dimensional correlation spectroscopy (COSY), can cause a miscalculation of the true coupling constant value. In this work, data processing for extracting small coupling constants is described. Signals are obtained from spin-echo experiments and analysed in the time domain in such a way that couplings are apparently multiplied by $n + 1$, where n is positive. Small coupling constants in 4-methyl-1,3-dioxane were obtained by this method. © 1997 by John Wiley & Sons, Ltd.

Magn. Reson. Chem. 35, 379–383 (1997) No. of Figures: 2 No. of Tables: 0 No. of References: 21

Keywords: coupling constants; data processing; *J* spectroscopy; measurement of couplings

Received 6 May 1996; revised December 1996; accepted 11 December 1996

INTRODUCTION

The measurement of scalar coupling constants gives valuable information in structural conformational analysis.[1,2] Various methods have been developed for evaluating coupling constants: two dimensional J,δ spectroscopy,[3,4] E-COSY,[5] Z-filtered COSY[6] and COSY.[7] The last approach has proved to be an efficient and powerful technique for extracting coupling constants in complex molecules. It is especially powerful for analysing proton spin systems. The DISCO technique[8,9] can also be used when in-phase and anti-phase structures of a given spin multiplet are available from the COSY spectrum. It simplifies the fine structure of the multiplet and improves the accuracy of the measurement. The coupling constant is particularly difficult to measure when it is of the order of the linewidth. In the COSY spectrum, for example, the overlap of anti-phase absorption signals (or partially resolved in-phase absorption signals) leads to interference and cancellation effects[10] and causes an erroneous evaluation of coupling constants. In the technique developed by Titman and Keeler,[11] two different homonuclear spectra, a double quantum-filtered COSY[12,13] and a TOCSY[14–16] or HOHAHA[17] were compared. The spectra contained cross peaks at an identical frequency but having different internal multiplet structures. Recently, other methods have also been proposed for evaluating coupling constants when the linewidth becomes comparable to J. In the so-called J-doubling[18] method, the time-domain signal corresponding to the multiplet is isolated and multiplied by $\sin(\pi J^* t)$, where J^* is a trial coupling constant. An automated search program detects the case where $J^* = J$. The coupling constant J is then doubled. In the J-deconvolution[19] method, the corresponding time-domain signal is divided by $\sin(\pi J^* t)$, and a search algorithm finds the condition where $J^* = J$.

We suggest here a simple method which simultaneously increases all coupling constants in the spectrum and hence provides accurate estimates. This method also allows the complete suppression of the J-modulation[20] effect. The conventional spin-echo sequence, 90°–$\tau/2$–180°–$\tau/2$–Acq(t), described by Aue *et al.*[4] is used for different values of τ and the analysis is performed in the time domain.

THEORY

For two weakly coupled spin-1/2 systems, the observed magnetization, corresponding to one resonance line, is given by[4]

$$M(t, \tau) = M_0 \exp(-\tau/T_2)\exp(-t/T_2^+) \times \exp[i\pi J(t + \tau)]\exp(i\delta t) \qquad (1)$$

where

$$\frac{1}{T_2^+} = \frac{1}{T_2} + \frac{1}{T_2^*}$$

T_2 is the transverse relaxation time, T_2^* describes the damping due to the magnetic field inhomogeneity and δ is the chemical shift of the considered line.

The aim of the work is to evaluate the integral:

$$M'(t) = \int_{-\infty}^{\infty}\int_0^{\infty} M(t, \tau)\exp[i\pi\nu(\tau - nt)]\, d\tau\, d\nu \qquad (2)$$

Correspondence to: J. C. Duplan

CCC 0749–1581/97/060379–05 $17.50

where n is a positive real and v is a variable measured in Hz.

Equation (2) can be approximately written as

$$M'(t) = \int_{-\infty}^{\infty} \int_{0}^{T_m} M(t, \tau)\exp[i\pi v(\tau - nt)]\ d\tau\ dv \quad (3)$$

assuming that $M(t, \tau) = 0$ for $t > T_m$, then

$$M'(t) = g(t) \int_{-\infty}^{\infty} \int_{0}^{T_m} \exp(-\tau/T_2)\exp[i\pi J(t + \tau)] \times \exp[i\pi v(\tau - nt)]\ d\tau\ dv \quad (4)$$

where $g(t) = M_0 \exp(-t/T_2^+)\exp(i\delta t)$. (5)

$M'(t)$ is equivalent to

$$M'(t) = g(t) \int_{-\infty}^{\infty} \int_{0}^{T_m} \exp(-\tau/T_2)\exp\{i\pi J[(1 - n)t + (\tau - nt)]\}\exp[i\pi v(\tau - nt)]\ d\tau\ dv \quad (6)$$

$M'(t)$ can be written as

$$M'(t) = g(t)\exp[i\pi J(1 + n)t]\int_{-\infty}^{\infty} \int_{0}^{T_m} \exp(-\tau/T_2) \times \exp[i\pi J(\tau - nt)]\exp[i\pi v(\tau - nt)]\ d\tau\ dv \quad (7)$$

Now, by substituting $f = J + v$, $M'(t)$ becomes

$$M'(t) = g(t)\exp[i\pi J(1 + n)t]\int_{-\infty}^{+\infty} \int_{0}^{T_m} \exp(-\tau/T_2) \times \exp[i\pi f(\tau - nt)]\ d\tau\ df \quad (8)$$

The integration of $M'(t)$ [Eqn (8)], with respect to τ gives

$$M'(t) = -T_2\, g(t)\exp[i\pi J(1 + n)t] \times \int_{-\infty}^{\infty} \frac{\{\exp(-T_m/T_2)\exp[i\pi f(T_m - nt)] - \exp(-i\pi fnt)\}(1 + i\pi f T_2)}{1 + (\pi f T_2)^2}\ df \quad (9)$$

As the sine function is odd, Eqn (9) reduces to

$$M'(t) = 2T_2\, g(t)\exp[i\pi J(1 + n)t] \times \int_{0}^{\infty} \left\{ \frac{\exp(-T_m/T_2)\cos[\pi f(T_m - nt)] - \pi f T_2 \sin[\pi f(T_m - nt)]}{1 + (\pi f T_2)^2} - \frac{[\cos(\pi fnt) - \pi f T_2 \sin(\pi fnt)]}{1 + (\pi f T_2)^2} \right\} df \quad (10)$$

Finally, one obtains

$$M'(t) = -T_2\, g(t)\exp[i\pi J(1 + n)t] \times \{\exp(-T_m/T_2)[A(t) + B(t)] + C(t) + D(t)\} \quad (11)$$

where the four terms $A(t)$, $B(t)$, $C(t)$ and $D(t)$ are calculated using the property

$$\int_{0}^{\infty} \frac{\cos(mx)\ dx}{x^2 + a^2} = \frac{\pi}{2a} \exp(-|ma|) \quad (12)$$

Then $A(t)$ becomes

$$A(t) = \int_{0}^{\infty} \frac{\cos[\pi f(T_m - nt)]}{1 + (\pi f T_2)^2}\ df = \frac{1}{2T_2} \exp\left(-\left|\frac{T_m - nt}{T_2}\right|\right) \quad (13)$$

The calculation of $B(t)$ can be deduced from Eqn (13) by noting that

$$B(t) = -\int_{0}^{\infty} \frac{\pi f T_2 \sin[\pi f(T_m - nt)]}{1 + (\pi f T_2)^2}\ df = -\frac{T_2}{n}\frac{d[A(t)]}{dt} \quad (14)$$

and consequently

$$B(t) = -\frac{1}{2n}\frac{d}{dt}\left[\exp\left(-\left|\frac{T_m - nt}{T_2}\right|\right)\right] \quad (15)$$

The terms $C(t)$ and $D(t)$ are evaluated likewise:

$$C(t) = -\int_{0}^{\infty} \frac{\cos(\pi fnt)}{1 + (\pi f T_2)^2}\ df = \frac{-1}{2T_2} \exp(-nt/T_2) \quad (16)$$

and

$$D(t) = -\int_{0}^{\infty} \frac{\pi f T_2 \sin(\pi fnt)}{1 + (\pi f T_2)^2}\ df = \frac{T_2}{n}\frac{d}{dt}[C(t)] \quad (17)$$

which gives

$$D(t) = \frac{-1}{2T_2} \exp(-nt/T_2) = C(t) \quad (18)$$

Two cases have to be considered:

(i) If $T_m < nt$:

$$A(t) = B(t) = \frac{1}{2T_2} \exp[-(nt - T_m)]$$

and

$$C(t) = D(t) = \frac{-1}{2T_2} \exp\left(-\frac{nt}{T_2}\right)$$

then $M'(t)$ cancels;

(ii) If $T_m > nt$

$$C(t) = D(t) = \frac{-1}{2T_2} \exp\left(-\frac{nt}{T_2}\right),$$

and

$$A(t) + B(t) = 0$$

then the integral $M'(t)$ is given by

$$M(t) = 2g(t)\exp(-nt/T_2)\exp[i\pi J(1 + n)t] \quad (19)$$

After substituting $g(t)$ from Eqn (5) in Eqn (19), one obtains the final expression for $M(t)'$:

$$M'(t) = 2M_0 \exp(-t/T_2^*)\exp[-t(n + 1)/T_2] \times \exp[i\pi J(1 + n)t]\exp(i\delta t) \quad (20)$$

These results show that $M'(t)$ leads to the conventional spectrum with couplings multiplied by $n + 1$. If the apparent coupling constant, J_{appt}, is defined by $J_{appt} =$

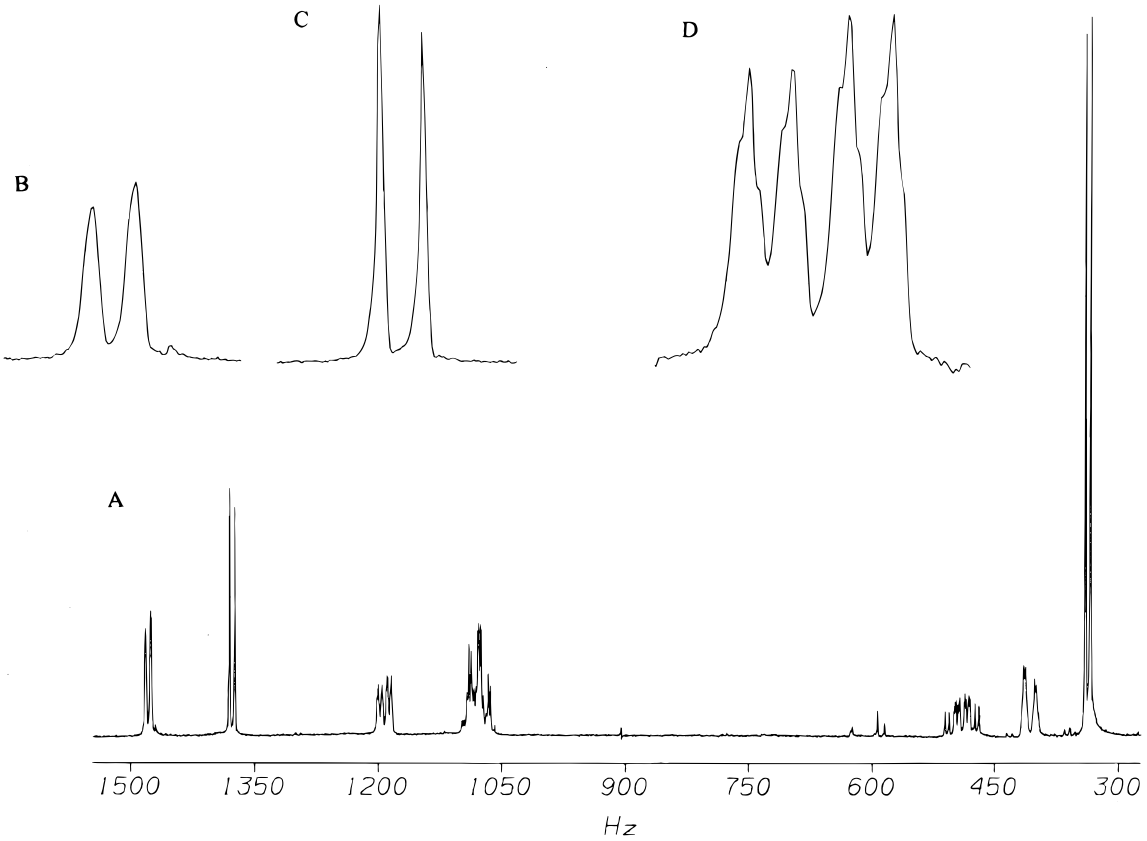

Figure 1. 300 MHz proton spectra of 4-methyl-1,3-dioxane in chloroform-*d*. (A) Normal spectrum acquired for $T_{\mathrm{acq}} = 1.8$ s. Spectral width, 1400 Hz; relaxation delay, 3 s; eight scans were used for each echo. (B), (C) and (D) show the expansion of multiplets at 1470.63, 1350.58 and 1170.50 Hz, respectively.

$(n+1)J$, $M'(t)$ can be written as

$$M'(t) = 2M_0 \exp(-t/T_2^*)\exp[-t(n+1)/T_2] \times \exp(i\pi J_{\mathrm{appt}} t)\exp(i\delta t) \qquad (21)$$

The Fourier transformation of $M'(t)$ gives a pure absorption signal, where splittings are multiplied by $n - 1$, and where the natural linewidths are increased and given by $(n+1)/\pi T_2$.

APPLICATION OF THE METHOD

In practical situations, one may replace Eqn (3) by

$$M'(t) = \int_{-F}^{F} \int_{0}^{T_m} M(t, \tau) \times \exp[i\pi\nu(\tau - nt)]\, \mathrm{d}\tau\, \mathrm{d}\nu \qquad \text{if } FT_2 \gg 1 \quad (22)$$

When $F \gg J$, Eqns (22) and (9) lead to the same expression of $M'(t)$.

As the variables τ and ν ae independent, we can intervert the order of integration in Eqn (22)

$$M'(t) = \int_{0}^{T_m} \int_{-F}^{F} M(t, \tau)\exp[i\pi\nu(\tau - nt)]\, \mathrm{d}\nu\, \mathrm{d}t \qquad (23)$$

After integration with respect to ν, Eqn (23) becomes

$$M'(t) = 2\int_{0}^{T_m} M(t, \tau)\, \frac{\sin[F\pi(\tau - nt)]}{\pi(\tau - nt)}\, \mathrm{d}\tau \qquad (24)$$

If a set of spin-echoes is recorded by incrementing τ, T_m must be chosen such as $T_m > nT_{\mathrm{acq}}$, where T_{acq} is the signal acquisition time. Each echo is then multiplied, in the time-domain, by $h(t, \tau) = 2\sin[\pi F(\tau - nt)]/\pi(\tau - nt)$. The new set of signals is used to perform calculation in Eqn (24) using the Romberg integration method.[21]

Note that in Eqn (21), the lineshape distortions due to J modulation are fully suppressed. After Fourier transformation of $M'(t)$, the spectrum can be phased and it is not necessary to use the absolute value mode, as is usually done in spin-echo experiments. If n is large, the term $\exp(-nt/T_2)$ can be compensated by multiplying $M'(t)$ by $\exp(nct)$, where $c \approx 1/(T_2)_{\mathrm{average}}$. Line broadening caused by the term $\exp(-nt/T_2)$ is then limited.

PRACTICAL EXAMPLE

The method was applied to analyse the proton spectrum of 4-methyl-1,3-dioxane observed at 300 MHz [Fig. 1(A)]. A set of 100 spin-echoes was recorded using $T_{\mathrm{acq}} = 1.8$ s and an increment of τ equal to 19 ms. Eight scans were used for each echo. The echoes were processed by taking $F = 40$ Hz and $T_m = 1.9$ s. The aim was to measure coupling constants in the AX system between 1350.58 and 1530.66 Hz. By setting $n = 3$, the coupling constants were multiplied by 4 (Fig. 2) and easily measured. For the doublet at 1470.63 Hz, J was

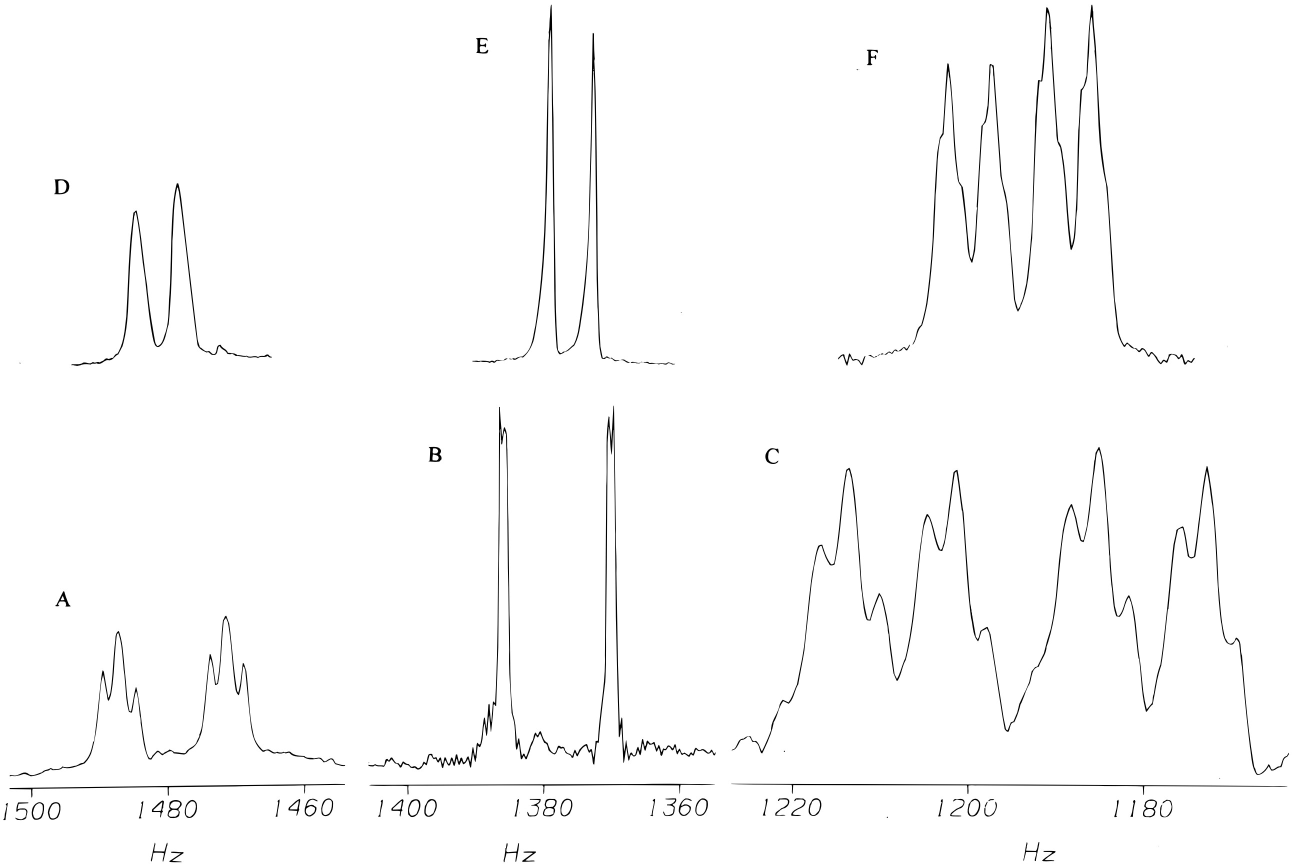

Figure 2. ^{1}H 300 MHz spectra of 4-methyl-1,3-dioxane. (A), (B) and (C) show the expansion of multiplets at 1470.63, 1350.58 and 1170.50 Hz, respectively, obtained from 100 processed half spin echoes. Acquisition time, 1.8 s; spectral width, 1400 Hz; increment of the delay, $\tau = 19$ ms; relaxation delay, 3 s, eight scans were used for each echo. F and T_m are equal to 40 Hz and 1.9 s, respectively. Coupling constants are multiplied by 4, by taking $n = 3$, which allows small couplings to be measured. The total acquisition time of echoes is 1.46 h. The data processing needs only 1 mm in (D), (E) and (F) are the expansion of the same multiplets as in (A), (B) and (C) obtained from the normal spectrum.

found to be 0.62 ± 0.05 Hz and for the doublet at 1350.58 Hz, the coupling constants $J_1 = 0.1 \pm 0.05$ *Hz and* $J_2 = 0.2 \pm 0.05$ Hz were evaluated. Couplings in the multiplet at 1170.50 hz were also measured and were between 0.68 and 0.92 Hz.

DISCUSSION

The proposed method is closely similar to conventional J-spectroscopy. Indeed, the projection of the J-spectrum on to the F_2 axis (by using the usual tilt, for example) gives a spectrum where the sizes of splittings are enhanced. This increase depends on the tilt angle. Nevertheless, the spectrum obtained exhibits lineshape distortions because the signal is made of phase-twist peaks. The advantage of the F_2 projection of J-spectroscopy is that the apparent increase of splittings does not entail any treatment. The proposed method produces absorption lines and it can be used with a small number of echoes (usually less than 100).

CONCLUSION

The proposed method is simple and does not entail isolation of the multiplet of interest for measuring coupling constants. The modulation of the signal by the scalar coupling is suppressed and the resulting spectrum is shown in the absorption mode. Nevertheless the method causes an increase in the linewidth. For large values of n, the linewidth can be improved by using an apodization technique. Since the chemical shift δ is not multiplied by $n - 1$, the number of lines in the spectrum must be restricted to a low value in order to overlap in the processed spectrum. When a large number of lines is present, one can isolate the multiplet (as in J-doubling or J-deconvolution methods) prior to data processing. The method is especially useful in the presence of inhomogeneously broadened lines ($T_2^* \ll T_2$).

Acknowledgement

We thank Professor A. Briguet for help and useful suggestions during the preparation of the manuscript.

REFERENCES

1. M. J. Karplus, *Chem. Phys.* **11**, 110 (1959).
2. V. F. Bystrov, *Prog. Nucl. Magn. Reson. Spectrosc.* **10**, 41 (1976).
3. W. P. Aue, J. Karhan and R. R. Ernst, *J. Magn. Reson.* **70**, 106 (1986).
4. W. P. Aue, J. Karhan and R. R. Ernst, *J. Chem. Phys.* **64**, 4226 (1976); G. Bodenhausen, R. Freeman, G. A. Morris and D. L. Turner, *J. Magn. Reson.* **31**, 75 (1978).
5. C. Griesinger, O. W. Sorensen and R. R. Ernst, *J. Am. Chem. Soc.* **107**, 6394 (1985).
6. H. Oschkinat, A. Pastore, A. P. Pfändler and G. Bodenhausen, *J. Magn. Reson.* **69**, 559 (1986).
7. W. P. Aue, E. Bartholdi and R. R. Ernst, *J. Chem. Phys.* **64**, 2229 (1976).
8. H. Oschkinat and R. Freeman, *J. Magn. Reson.* **60**, 164 (1984).
9. H. Kessler, A. Müller and H. Oschkinat, *Magn. Reson. Chem.* **23**, 844 (1985).
10. Y. Kim, J. H. Prestegard, *J. Magn. Reson.* **84**, 9 (1989).
11. J. J. Titman and J. Keeler, *J. Magn. Reson.* **89**, 640 (1990).
12. U. Piantini, O. W. Sorensen and r. R. Ernst, *J. Am. Chem. Soc.* **104**, 6800 (1982).
13. A. Shaka and R. Freeman, *J. Magn. Reson.* **51**, 169 (1983).
14. M. Rance, *J. Magn. Reson.* **74**, 557 (1987).
15. L. Braunschweiller and R. R. Ernst, *J. Magn. Reson.* **53**, 521 (1983).
16. S. P. Rucker and A. J. Shaka, *Mol. Phys.* **68**, 509 (1989).
17. D. G. Davis and A. Bax, *J. Magn. Reson.* **65**, 355 (1985).
18. L. Mcintyre and R. Freeman, *J. Magn. Reson.* **96**, 425 (1992).
19. J.-M. Leparco, L. McIntyre and R. Freeman, *J. Magn. Reson.* **97**, 553 (1992).
20. L. Mahi, J. C. Duplan and B. Fenet, *Chem. Phys. Lett.* **211**, 27 (1993).
21. W. H. Press, S. A. Teukolsky and W. T. Vetterling, *Numerical Recipes, the Art of Scientific Computing*. Cambridge University Press, Cambridge (1986).