

A new program based on stochastic Liouville equation for the analysis of superhyperfine interaction in CW-ESR spectroscopy

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Received 3 February 2003; revised 27 May 2003

Abstract

In the slow-motion region, ESR spectra cannot be expressed as a sum of simple Lorentzian lines. Studies of Freed and co-workers, on nitroxides in liquids gained information on the microscopic models of rotational dynamics, relying much on computer programs for simulation of ESR spectra based on the stochastic Liouville equation (SLE). However, application of Freed's method to copper system of biological interest has been for a long time precluded by lack of a full program able to simulate ESR spectra containing more than one hyperfine interaction. Direct extension of the Freed's approach in order to include superhyperfine interaction is not difficult from a theoretical point of view but the resulting algorithm is problematical because it leads to substantial increase in the dimensions of the matrix related to the spin-hamiltonian operator. In this paper preliminary results of a new program, written in C, which includes the superhyperfine interactions are presented. This preliminary version of the program does not take into account a restoring potential, so it can be used only in isotropic diffusion conditions. A comparison with an approximate method previously developed in our laboratory, based on a post-convolution approach, is discussed.

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Keywords: CW-ESR spectroscopy; Copper complexes; ESR spectra simulation

1. Introduction

ESR spectroscopy has been widely used to study complexes formed in solution among copper(II) ion and various ligands. ESR spectroscopy allows to study the nature of the Cu(II) coordination with nitrogen donor atoms present in imidazole, pyridine, glycine, ethylenediamine among the others. Besides ENDOR technique, which allows a precise determination of coordination properties, a number of different methods have been developed in order to analyze nitrogen coordination in copper complexes directly from the CW-ESR spectra at room temperature [1]. However, all these methods are strongly based on the availability of computer simulation of the ESR spectra in different motional conditions. As far as copper biomolecules are concerned, perform-

ing ESR experiments in solution at room temperature is crucial for maintaining the biological significance of the model because only in this case sample conditions match the physiological reaction conditions. Furthermore, undesirable effects due to changes in physical state at low temperature (like changes in coordination of ligand to metal, changes in local pH, induction of artificial aggregation and changes in temperature dependent equilibria) can be avoided [2–5]. Nevertheless retrieving information from ESR spectra of copper complexes in solution may be cumbersome. In fact, even at room temperature with an isotropic solvent of membrane-like viscosity, ESR spectra have to be analyzed by the stochastic Liouville equation (SLE) based theory [6–8]. This is due to the usually large anisotropy of Cu(II) hyperfine interaction (typical values for $\Delta g = g_{\parallel} - g_{\perp}$ and $\Delta A = A_{\parallel} - A_{\perp}$ are, respectively, 0.15 and 150 G). For most square planar copper complexes dissolved in an isotropic solvent, when the probe fulfills the fast motion conditions, the rotational correlation time, τ_R , is

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less than 2×10^{-9} s, but for viscous solvents like paraffin oil, even at 45 °C, τ_R is more than 2×10^{-9} s. Furthermore in anisotropic solvents (membranes or liquid crystals) Cu(II) spectra should be analyzed by the SLE theory at any temperature [9–11]. The SLE theory for an ESR spectrum was applied to paramagnetic metal ions in solution only in a limited number of cases [12]. The application of Freed's method to copper system of biological interest has been for a long time precluded by lack of a full program able to simulate ESR spectra containing more than one hyperfine interaction. For this reason a simplified approach based on post-convolution techniques was developed independently by our group [13] and another group at the National Biomedical ESR Center (Milwaukee, USA) [10]. Direct extension of the Freed's approach to include superhyperfine interaction is not difficult from a theoretical point of view but the resulting algorithm is problematical because it leads to a substantial increase in the matrix dimension related to the spin-hamiltonian operator. The computer program needs a huge amount of memory and only recently, thanks to the striking improvement in personal computer performance, a preliminary solution of this problem has been addressed.

The aim of this work is twofold: (1) to describe a computer program, based on the original Freed's work, which includes superhyperfine interactions expanding the starting vector; (2) to test the applicability of the post-convolution procedure on different motional conditions. In the next sections a short review of the stochastic Liouville theory together with a description of the computer program is reported. Preliminary results for the simulation of a copper complex of chemical interest and a comparison between the two simulation procedures are presented. Eventually perspectives and future developments of the program together with a critical analysis of the present version are addressed.

2. The stochastic Liouville approach to simulation

The slow motion theory for an ESR spectrum, based on the stochastic Liouville equation, was developed by Freed and co-workers [6,7,14–16] in an impressive series of work to which the reader is referred. In this approach, the absorption of energy from the microwave field by a spin system far from saturation can be written as [8,17]

$$I(\omega - \omega_0) = \frac{1}{\pi} \text{Re}[\langle \langle v | [(\Gamma - iL) + (\omega - \omega_0)I]^{-1} | v \rangle \rangle], \quad (1)$$

where Γ is the symmetrized diffusion operator, L is the Liouville operator associated with the hamiltonian of the magnetic interactions, ω is the frequency of the microwave field and $|v\rangle$ is the so-called *starting vector*

constructed from the spin transition moment averaged over the equilibrium ensemble. As we have previously stated, in this paper, we assume the following hamiltonian

$$\mathbf{H} = \mu \vec{S} \cdot \tilde{g} \cdot \vec{H} + \vec{I} \cdot \tilde{A} \cdot \vec{S} + \sum_{i=1}^N \vec{I}_i \cdot \tilde{A}_i \cdot \vec{S}, \quad (2)$$

where \tilde{A}_i are the tensors describing the superhyperfine coupling of the electronic spin with a set of completely equivalent N nuclei [18]. The Liouville operators Γ and L are defined in the Liouville space of the direct product of the normalized Wigner rotation matrices and the spin transitions. Using Dirac notation the basis vectors of this space in our case are

$$\begin{aligned} & \left\langle \sqrt{\frac{2L+1}{8\pi^2}} D_{M,K}^L(\Omega) \left| p^S, q^S; p', q'; \prod_i (p^N, q^N)_i \right. \right\rangle \\ &= \sqrt{\frac{2L+1}{8\pi^2}} D_{M,K}^L(\Omega) |S, m_S\rangle \langle S, m_S| \otimes |I, m_I\rangle \langle I, m_I| \\ & \otimes \prod_i (|N, m_N\rangle \langle N, m_N|), \end{aligned} \quad (3)$$

where m_S and m_I are the eigenstates of the z components of the electronic spin operator S and of the nuclear spin operator I , respectively and m_N are the eigenstates of the z component of the nuclear spin operator of the completely equivalent nuclei. It is worthwhile to mention that, in order to discuss the types of terms which are included in the Liouville and diffusion superoperators, several coordinate system needs to be introduced [19,20]. For this reason it is convenient to write the hamiltonian in the spherical tensor notation

$$\mathbf{H}_0 = \sum_{\mu} F_{\mu,L}^{0,0} A_{\mu,L}^{0,0}, \quad (4)$$

$$\mathbf{H}_1(\Omega) = \sum_{\mu, m', m'', m'''} d_{mm'}^2(\Psi) D_{m'm''}^2(\Omega) d_{m''m'''}^2(\Phi) F_{\mu,L}^{2,m''} A_{\mu,L}^{2,m}, \quad (5)$$

where μ specifies the kind of interaction (Zeeman, Hyperfine, Superhyperfine), $F_{\mu,L}^{l,m}$ are proportional to the standard irreducible spherical tensor operator (ISTO) components of the magnetic tensor of type μ in the reference frame L , $A_{\mu,L}^{l,m}$ are the ISTO components of the tensors which arise from the coupling of spin and/or magnetic field operators. Since L is the superoperator associated to the hamiltonian we can write

$$L = \sum_{\mu, m', m'', m'''} d_{mm'}^l(\Psi) D_{m'm''}^l(\Omega) d_{m''m'''}^l(\Phi) F_{\mu,m}^{l,m''} A_{\mu,m}^{l,m}. \quad (6)$$

The Freed's approach to the solution of the SLE is based on the Lanczos algorithm which can be applied only when the matrix associated with $\Gamma - iL$ is complex symmetric. In order to symmetrize the matrix a proper basis set must be introduced defined as [8,17]

$$\begin{aligned}
|\sigma_n\rangle = & [2(1 + \delta_{K_n,0})]^{-1/2} e^{i(\pi/4)(1-j^{K_n})} \\
& \times \left| p_n^S, q_n^S, p_n^I, q_n^I, \prod_i (p_n^N, q_n^N)_i \right\rangle \\
& \times \{ |L_n, M_n, K_n\rangle + j^{K_n} (-)^{L+K} |L_n, M_n, -K_n\rangle \} \quad (7)
\end{aligned}$$

using this basis, the matrix elements of the Liouville superoperator can be written as

$$\begin{aligned}
\langle \sigma_1 | \Gamma_{\text{iso}} | \sigma_2 \rangle = & \left\langle L_1, M_1, K_1, j^{K_1}, p_1^S, q_1^S, p_1^I, q_1^I, \prod_i (p_1^N, q_1^N)_i \left| L \right. \right. \\
& \times \left. \left. L_2, M_2, K_2, j^{K_2}, p_2^S, q_2^S, p_2^I, q_2^I, \prod_i (p_2^N, q_2^N)_i \right\rangle \right. \\
= & \sum_{l,\mu,m',m''} d_{mm'}^l(\Psi) d_{m''m'''}^l(\Phi) F_{\mu,m}^{l,m'''} \\
& \times \sqrt{(2L_1+1)(2L_2+1)} \\
& \times \frac{1}{8\pi^2} \int d\Omega D_{M_1,K_1}^{L_1*}(\Omega) D_{m',m''}^l(\Omega) D_{M_2,K_2}^{L_2}(\Omega) \\
& \times \left\langle p_1^S, q_1^S, p_1^I, q_1^I, \prod_i (p_1^N, q_1^N)_i \left| A_{v,m}^{\times(l,m)} \Gamma_{\text{iso}} \right| p_2^S, \right. \\
& \times \left. q_2^S, p_2^I, q_2^I, \prod_i (p_2^N, q_2^N)_i \right\rangle. \quad (8)
\end{aligned}$$

As far as the diffusion operator is concerned, in our approach we assume an isotropic rotational diffusion operator. The corresponding matrix elements are

$$\begin{aligned}
\langle \sigma_1 | \Gamma_{\text{iso}} | \sigma_2 \rangle = & \left\langle L_1, M_1, K_1, j^{K_1}, p_1^S, q_1^S, p_1^I, q_1^I, \prod_i (p_1^N, q_1^N)_i \right. \\
& \times \left. \left| \Gamma_{\text{iso}} \right| L_2, M_2, K_2, j^{K_2}, p_2^S, q_2^S, p_2^I, q_2^I, \right. \\
& \left. \prod_i (p_2^N, q_2^N)_i \right\rangle. \quad (9)
\end{aligned}$$

At the moment the complete treatment of the diffusion component of the Liouville matrix has been omitted. The inclusion of the restoring potential is under study and will be implemented in a parallel version of our program currently under development. This program version is therefore unable to simulate the dynamics of complexes in anisotropic liquids.

The continued fraction representation of the spectral function holds a central position in the Freed's approach since it represents an efficient mean of obtaining an entire sequence of approximations to the spectral function directly from the tridiagonal matrix obtained by applying the Lanczos algorithm [21] to the matrix described by Eqs. (8) and (9). The sequence of continued fraction approximants to the spectral function are defined in terms of the elements of this tridiagonal matrix. The sequence is then truncated at an appropriated value depending on the motional condition of the complex.

The results of numerical calculations of ESR spectra by this procedure depend strongly on the choice of the so-called *minimum truncation scheme* (MTS) that determines the dimension M of the matrix representation of the super-operators L and (Γ) and the number of recursive steps in the Lanczos algorithm that tridiagonalizes the matrix A . The minimum truncation scheme consists of the indices, L , K and M of the Wigner rotation matrices. General requirements are, $L \geq 2$, $L \leq K$, $M \geq 0$ and K is even. K is zero for a complex with axial symmetry. In the case when the complex is dissolved in an isotropic solvent (this is the case addressed in the present study) we have $M \geq 2I$ where I is the nuclear spin of the paramagnetic element ($I = 3/2$ for copper).

The computer program CoSMoS 2.0 (Copper Slow Motion Simulation) has been written in standard ANSI C language in order to guarantee the maximum portability on different operational system. The program is based on a Fortran version for nitroxide spin label written by Schneider and Freed [19]. The original version (EPRL) is not suitable to perform simulation of ESR spectra of copper complexes due to lack of treatment of the non secular terms in the spin hamiltonian. Complete treatment of the nonsecular terms was introduced later (EPRLF) leading to a better agreement with experimental spectrum [17]. Our program is based on the last version (EPRLF) which contains the complete treatment of the secular and nonsecular terms. Although those terms are less relevant in X band experiments, nevertheless they cannot be neglected when transition metal ions are present. The use of C language has the advantage of dynamical memory allocation which permits a more efficient use of the computer memory (though this characteristic is shared with more recent versions of Fortran, e.g., 90 and 95). The program can be used either to run a single ESR spectrum simulation or a best fit procedure. Data input is realized with Excel spreadsheets.

3. Experimentals

Isotopically enriched ^{63}CuO was obtained from Oak Ridge National Laboratory, Oak Ridge, TN. The aminoacid glycine was purchased from Sigma and used without further purification. The Cu(II)bis(glycinato) solution for ESR experiments was prepared in water with a final concentration of 12 mM. The metal to ligand concentration ratio was 1:4 in order to assure complete copper ligation and the formation of the bis-coordination. The pH was adjusted by adding NaOH to give the final value of 10. Finally the solution was evaporated and dissolved in 50% v/v glycerol mixture with water. X-band ESR spectra were obtained with a Bruker 200D SRC X-band spectrometer. Microwave frequencies were measured with an XL Microwave Model 3120 counter

(Jagmar, Krakow, Poland). The spectrometer was interfaced with a PS/2 Technical Instruments Hardware computer and the data acquired using the ESR data system CS-ESR produced by Stelar, Mede, Italy. The temperature control apparatus is a Bruker ER 411VT variable temperature unit.

4. Results and discussion

The current version of the program has been tested on a Pentium4 Personal Computer with a 2GHz CPU and 1.5Gbyte RAM. In Tables 1 and 2 computational data for the simulation of CW-ESR spectra of a copper complex in different motion condition has been reported (magnetic and dynamic parameters values are reported in Table 3). As it is evident the increase of the computational resources (allocated memory and elapsed time) raising the nitrogen number is always relevant. Although it is possible to run a single simulation with four coordinated nitrogens, in this condition, the relevant computational time prevent us to successfully integrate the simulation algorithm with an automatic best fit procedure. A copper spectrum with two bound nitrogens has been used to test the effect of the anisotropic superhyperfine coupling contribution (see Table 4). The nitrogen coupling constant values has been chosen in order to maintain the isotropic value fixed. In Figs. 1 and 2 fast motion ($D_{xy} = 4.0E8 s^{-1}$) and slow motion ($D_{xy} = 5.5E7 s^{-1}$) spectra are reported. For simplicity the simulations are performed considering axial symmetry, so spectra are independent from the z component of the diffusion tensor. As it was expected, the introduction of the anisotropic superhyperfine coupling result in a broadening effect on the low field lines. However, as can be inferred from Figs. 1 and 2, this

Table 1
CPU time and memory allocation vs Nr of coordinated nitrogens

Nr of nitrogen atoms	Liouville matrix dimension	Nr of nonzero elements	Elapsed time (s)
1	3144	33,960	11
2	38,300	514,412	486
3	414,384	7,154,638	40,490

Table 2
CPU time, matrix dimension (N dim) and number of nonzero elements of Liouville matrix calculated for isotropic and anisotropic nitrogen coupling constant (A_N) for fast and slow motional condition

Motion	A_N	N Dim	Nr el. nonzero	CPU time (s)
Fast	iso	29030	219318	316
	not iso	29030	367250	335
Slow	iso	93920	818980	1342
	not iso	93920	1397384	1485

Table 3
Magnetic and dynamic parameters for test run

<i>Spin Hamiltonian parameters</i>	
Frequency	9.5
g_{xx}	2.05
g_{yy}	2.05
g_{zz}	2.2
A_{xx}	30
A_{yy}	30
A_{zz}	170
$A_{N_{xx}}$	9
$A_{N_{yy}}$	9
$A_{N_{zz}}$	18
<i>Diffusion tensor parameters</i>	
D_{xy}	1.67E + 09
<i>MTS</i>	
Lemx	8
Lomy	1
Kmx	0
Mmx	3
Ipmtx	3
Lanczos step	200

With the MTS notation the minimum truncation scheme is indicated. All the values of coupling constant are in Gauss, the dynamics parameters (D_{xy} , D_{zz} are in s^{-1}) and the frequency is in GHz.

effect is always very small, except in those cases which correspond to ΔA_N values never observed in real copper complexes. This fact, a posteriori confirms the applicability of the post-convolution approach previously developed and briefly discussed in the following.

Whenever it is appropriate to assume that the superhyperfine constant of equivalent bound nuclei is isotropic, it is possible to approach the problem of spectra simulation using a simplified algorithm. To be more specific, in the context of the SLE approach, the simplified solution requires to simulate the copper spectrum without nitrogens and then performing a convolution between the spectrum obtained and the pattern function of the nitrogens under the desired conditions [13]. The simplified procedure can be summarized as follows:

1. a solution of the SLE for the copper only (without ligands) is obtained;
2. FTs of the copper spectrum and nitrogen stick diagram are performed;
3. the two FTs are multiplied and the reverse FT of the results is performed in order to obtain the final spectrum.

Since the two programs are based on different computational approaches, various tests have been performed to verify the convergence of the two procedures. The ESR spectra simulations have been run using the same magnetic and dynamic parameters and two bound nitrogen atoms with isotropic superhyperfine coupling constant. The resulting spectra originated by the two programs are perfectly overlapping.

Table 4
Magnetic and dynamic parameters for the simulations showed in Figs 1 and 2

Spin Hamiltonian parameters								
Frequency					9.5			
g_{xx}	2.05				2.05			
g_{yy}	2.05				2.05			
g_{zz}	2.2				2.2			
A_{xx}	30				30			
A_{yy}	30				30			
A_{zz}	170				170			
Nr of N atoms	2				2			
$A_{N_{xx}}$	1	5	9	12	1	5	9	12
$A_{N_{yy}}$	1	5	9	12	1	5	9	12
$A_{N_{zz}}$	34	26	18	12	34	26	18	12
Diffusion tensor parameters								
D_{xy}	4.000E+08				5.556E+07			
MTS								
Lemx	6				20			
Lomy	3				3			
Kmx	0				0			
Mmx	3				3			

All the values of coupling constant are in Gauss, the dynamics parameters (D_{xy} , D_{zz} are in s^{-1}) and the frequency is in GHz.

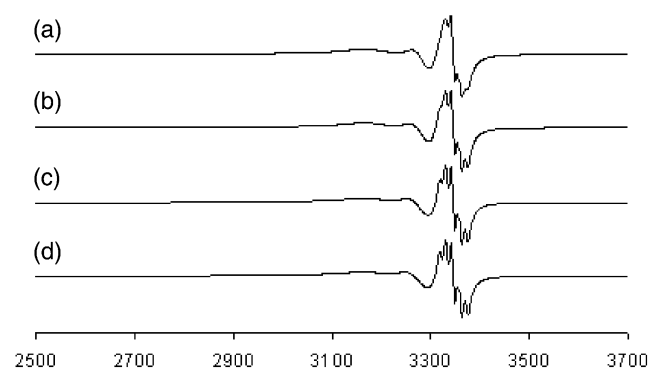


Fig. 1. Anisotropic effects of the superhyperfine coupling constant. These simulations have been run on a hypothetical copper complex with the same set of magnetic parameters (see Table 4) and $D_{xy} = 4.000E+08 s^{-1}$. Different values of anisotropic superhyperfine coupling constant (a) $A_{N_{xx}} = 1$, $A_{N_{yy}} = 1$, $A_{N_{zz}} = 34$; (b) $A_{N_{xx}} = 5$, $A_{N_{yy}} = 5$, $A_{N_{zz}} = 26$; (c) $A_{N_{xx}} = 9$, $A_{N_{yy}} = 9$, $A_{N_{zz}} = 18$; (d) $A_{N_{xx}} = 12$, $A_{N_{yy}} = 12$, $A_{N_{zz}} = 12$ have been used. All the values are in Gauss.

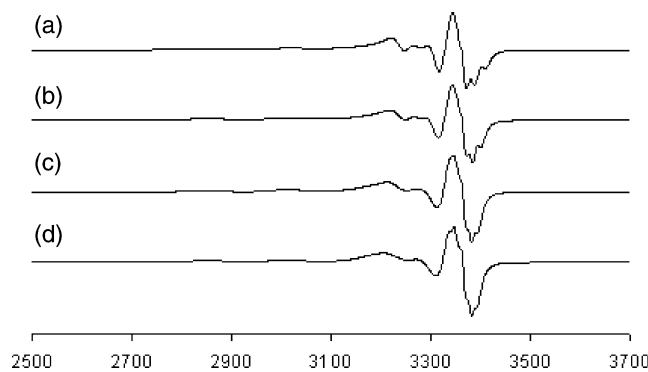


Fig. 2. These simulations have been run with the same set of input parameters of spectra reported in Fig. 1 but with $D_{xy} = 5.566E+07 s^{-1}$.

The new program has been tested for the simulation of experimental spectra combining it with best fit procedures. Since the complete simulation of a copper complex with four nitrogens would still require a prohibitive amount of computational time, the procedure has been applied to a simplified system with N_2O_2 coordination, Cu(II)bis(glycinato) complex. In Figs. 3–5 experimental and simulated spectra at $T = 213$ K, $T = 253$ K and $T = 313$ K are reported. The values for the minimum truncation scheme and the Lanczos steps used in our simulations were established by a trial-and-error calculation of spectra with different basis sets in order to specify which types of basis vectors are important. In Table 5 magnetic and dynamic parameters, for the simulations reported in Figs. 3–5, are reported. The starting values of magnetic parameters for the simulations, reported in Table 5, have been extracted directly from the experimental spectra at low temperature (120 K) at X and S bands (data not shown). All the values reported in Table 5 have been calculated using an automatic best fit procedure which searches for an optimal set of parameters varying their values within a user defined range. This range is of the same order of magnitude of the experimental error for the particular parameter under consideration. The estimated error on the parameters are: ± 0.001 for g , ± 1 G for A_{xx} , A_{yy} , $A_{N_{xx}}$, $A_{N_{yy}}$ and $A_{N_{zz}}$, ± 3 G for A_{zz} . As far as D tensors are concerned, the error estimation is more difficult. In the framework of the *Sensitivity Analysis* introduced by Hyde et al. [22,23] it is possible to find an estimate for the error of the correlation time, τ (which is related to the tensors values by the relation $\tau = 1/6\sqrt{D_{xy}D_{zz}}$) of 10–15 ps. The agreement among the ESR parameters set at different temperatures appears to be in the range of

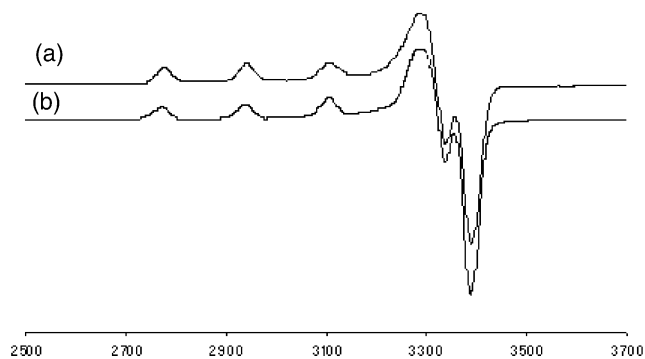


Fig. 3. X band experimental (a) and simulated spectra (b) for Cu(II)bis(glycinato) complex in 50% v/v glycerol/water at $T = 213$ K. ESR parameters for the simulated spectrum are given in Table 5.

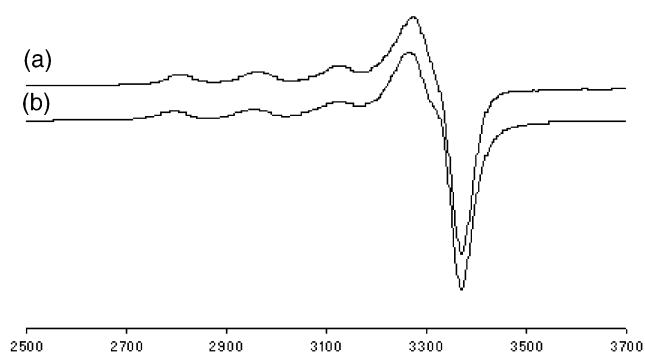


Fig. 4. X band experimental (a) and simulated spectra (b) for Cu(II)bis(glycinato) at $T = 253$ K. ESR parameters for the simulated spectrum are given in Table 5.

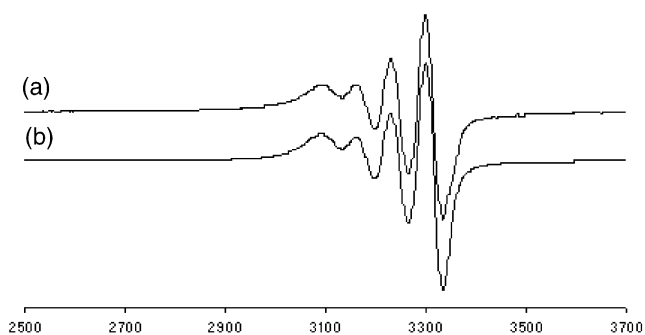


Fig. 5. X band experimental (a) and simulated spectra (b) for Cu(II)bis(glycinato) at $T = 313$ K. ESR parameters for the simulated spectrum are given in Table 5.

the experimental errors even if an equilibrium between trans and cis isomers cannot be ruled out and it deserves a further insight.

5. Conclusion and perspectives

Only few years ago Pasenkiewicz-Gierula et al. [10] correctly wrote: “including superhyperfine interactions

Table 5
ESR parameters for simulated spectra given in Figs. 3–5

Temperature (K)	213	253	313
Frequency	9.5793	9.5789	9.576
g_{xx}	2.068	2.072	2.074
g_{yy}	2.033	2.042	2.040
g_{zz}	2.259	2.258	2.260
A_{xx}	9.0	8.5	10.4
A_{yy}	14.8	15.1	15.2
A_{zz}	167.7	170.7	169.6
$A_{N_{xx}}$	12.3	13.0	12.2
$A_{N_{yy}}$	9.1	10.9	9.0
$A_{N_{zz}}$	10.6	11.2	10.4
$A_{N_{iso}}$	10.6	11.7	10.5
<i>Diffusion tensor parameters</i>			
D_{xy}	4.3E+06	5.2E+07	2.4E+09
D_{zz}	5.9E+07	5.1E+11	8.8E+09
Residual linewidth	3.0	0.6	0.6
<i>MTS</i>			
Lemx	24	26	26
Lomx	9	5	5
Kmx	9	5	5
Mmx	9	3	3
Ipnmx	3	3	3
Lanczos step	100	200	200

All the values of coupling constants are in Gauss, the dynamic parameters (D_{xy} , D_{zz} are in s^{-1}) and the frequency is in GHz.

directly in the hamiltonian would result in a significant increase in the dimension of the starting vector making it practically impossible to calculate an ESR spectrum even for moderately slow motion.” In this paper the extension of the spin hamiltonian to include spin interaction has been shown and a comparison with post-convolution procedure has been discussed. This procedure seems to be sufficiently precise in all those cases which are currently found in practice. However, the current version of the program is usable in a limited number of cases (coordinated nitrogens minor or equal to 3) and therefore further developments are required to use it on routinary base. At the moment a Fortran 90/95 version is under implementation in order to use automatic best fit methods also in the presence of a number of bound nitrogens greater than two and to integrate the restoring potential. This new version will include also a procedure based on conjugate gradient method to ascertain in a precise manner the parameters set for the Liouville matrix truncation (MTS).

Acknowledgments

This work was funded by PAR research project, University of Siena, and by Cineca (Centro di Calcolo Interuniversitario per l'Italia del Nord Est) for the grant project and the use of the SGI Origin 3800 computer.

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