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ESR Spectral Simulations of 4,4-dimethyl-3-oxazolydinyloxy Substituted Stearic Acid Spin Labels in Dry and Aqueous Dimyristoyl and Diphytanoyl Phosphatidylcholine

S. MASUMURA* AND M. D. PACE

Polymeric Materials Branch
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By using a FORTRAN program written for the Compaq 286 personal computer, ESR (electron spin resonance) spectra were simulated for the compounds dimyristoyl phosphatidylcholine (DMPC) and diphytanoyl phosphatidylcholine (DPhPC) containing 4,4-dimethyl-3-oxazolidinyloxy substituted stearic acid spin labels in both the dry phase and aqueous phase to determine the correlation times of these labels. The anisotropic motion of these labels indicates the order and fluidity of the DPhPC and DMPC bilayers.

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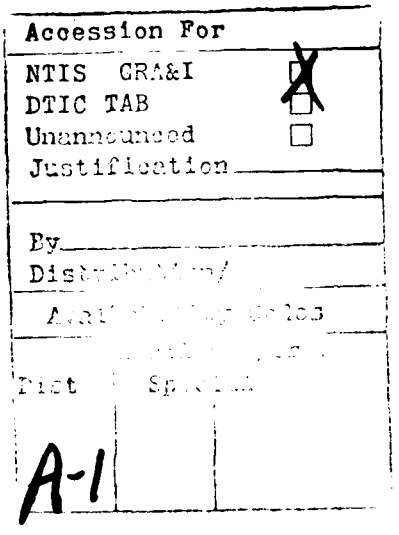
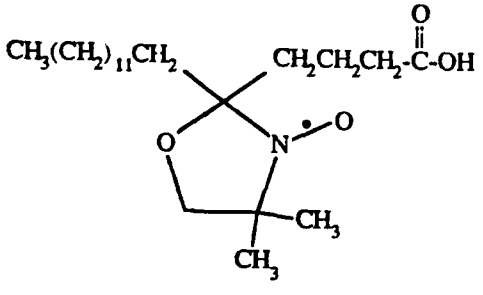


ESR SPECTRAL SIMULATIONS OF 4,4-DIMETHYL-3-OXAZOLYDINYLOXY SUBSTITUTED STEARIC ACID SPIN LABELS IN DRY AND AQUEOUS DIMYRISTOYL AND DIPHYTANOYL PHOSPHATIDYLCHOLINE

INTRODUCTION

The compounds dimyristoyl phosphatidylcholine (DMPC) and diphytanoyl phosphatidylcholine (DPhPC) form unique membranes having structures that are not well characterized. One way to investigate the dynamic properties of these membranes is by using spin labels. We have used in this study 4,4-dimethyl-3-oxazolidinyloxy substituted spin labels including 2-tridecyl-2-(3-carboxypropyl)-4,4-dimethyl-3-oxazolidinyloxy (I), 2-hexyl-2-(10-carboxydecyl)-4,4-dimethyl-3-oxazolidinyloxy (II), 2-ethyl-2-(14-carboxytetradecyl)-4,4-dimethyl-3-oxazolidinyloxy (III) (See Fig. 1). These labels self-organize into membranes and their anisotropic motion, which depends upon the membrane's structure, is detected and analyzed by electron spin resonance spectroscopy.



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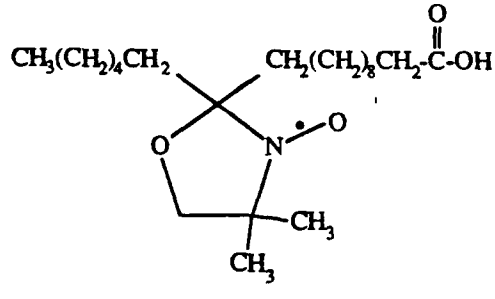

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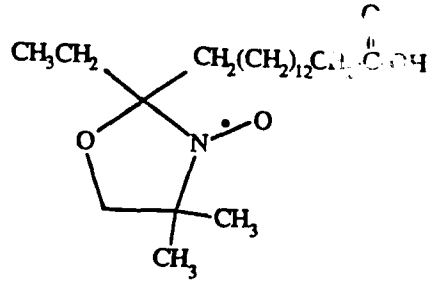

(III)

Figure 1

Structures of Stearic Acid Spin Labels Used in this Study

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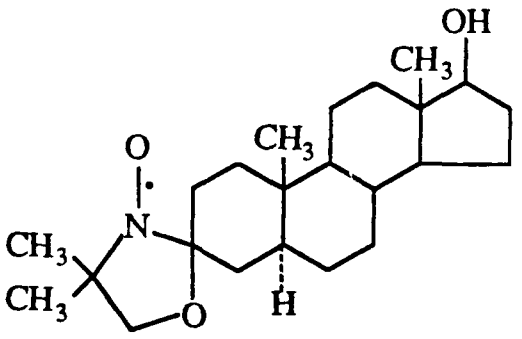
As a result, the spectra that are produced have lineshapes which are influenced by the degrees of motional freedom of these compounds. If membrane structure does not allow for a wide range of movement, then linebroadening will be noticeable.

A drawback with the spin labels shown in Fig. 1 is that the 4,4-dimethyl-3-oxazolidinyloxy group may indicate local methylene chain motion of the label rather than motion associated with the lipid. A label such as 17ß-hydroxy-4',4'-dimethylspiro(5α-androstane-3,2'-oxazolidin)-3'-yloxy spin label (Fig. 2) is rigid and motion of the 4,4-dimethyl-3-oxazolidinyloxy group indicates motion of the entire molecule. By using information from both types of labels measurement of membrane order , fluidity, or permeability is possible.



IV

Figure 2

Structure of the Spin Label 17ß-hydroxy-4',4'-dimethylspiro-(5α-androstane-3,2'-oxazolidin)-3'-yloxy

One such example is that of a vesicle, or microscopic bubble, capable of holding medication or other drugs. We can investigate porosity by placing the spin labels inside the vesicle and then filtering the vesicles from the solution which contains excess labels. With only the labels in the vesicle, one signal should appear. As time passes, the labels will begin to pass through the porous vesicle surface. Either that, or the vesicle itself may start to deteriorate, releasing the label. As this happens, a second signal of the spin label in aqueous solution will start to appear. Hence, by keeping track of the time lapse the porosity of the vesicle can be monitored.

This has been done with glycerol dialkyl-nonitol tetraether (GDNT), an asymmetric lipid (Fig. 3). Over a period of 12 hours, the label's aqueous phase ESR spectrum of three sharp peaks can be seen to appear (Figure 4) indicating diffusion

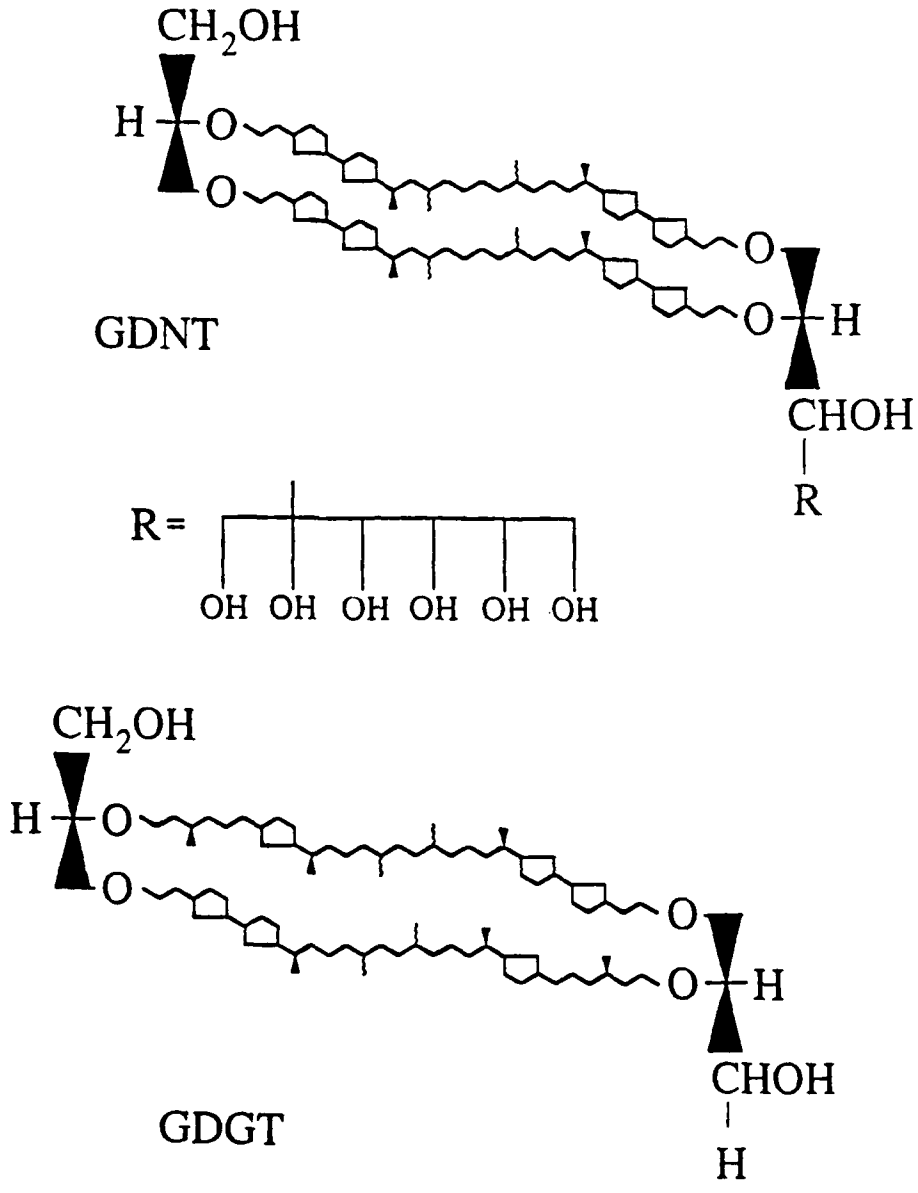


Figure 3. Structures of archaebacteria glycerol-dialkyl-glycerol-tetraether (GDGT) and glycerol-dialkyl-nonitol-tetraether (GDNT).

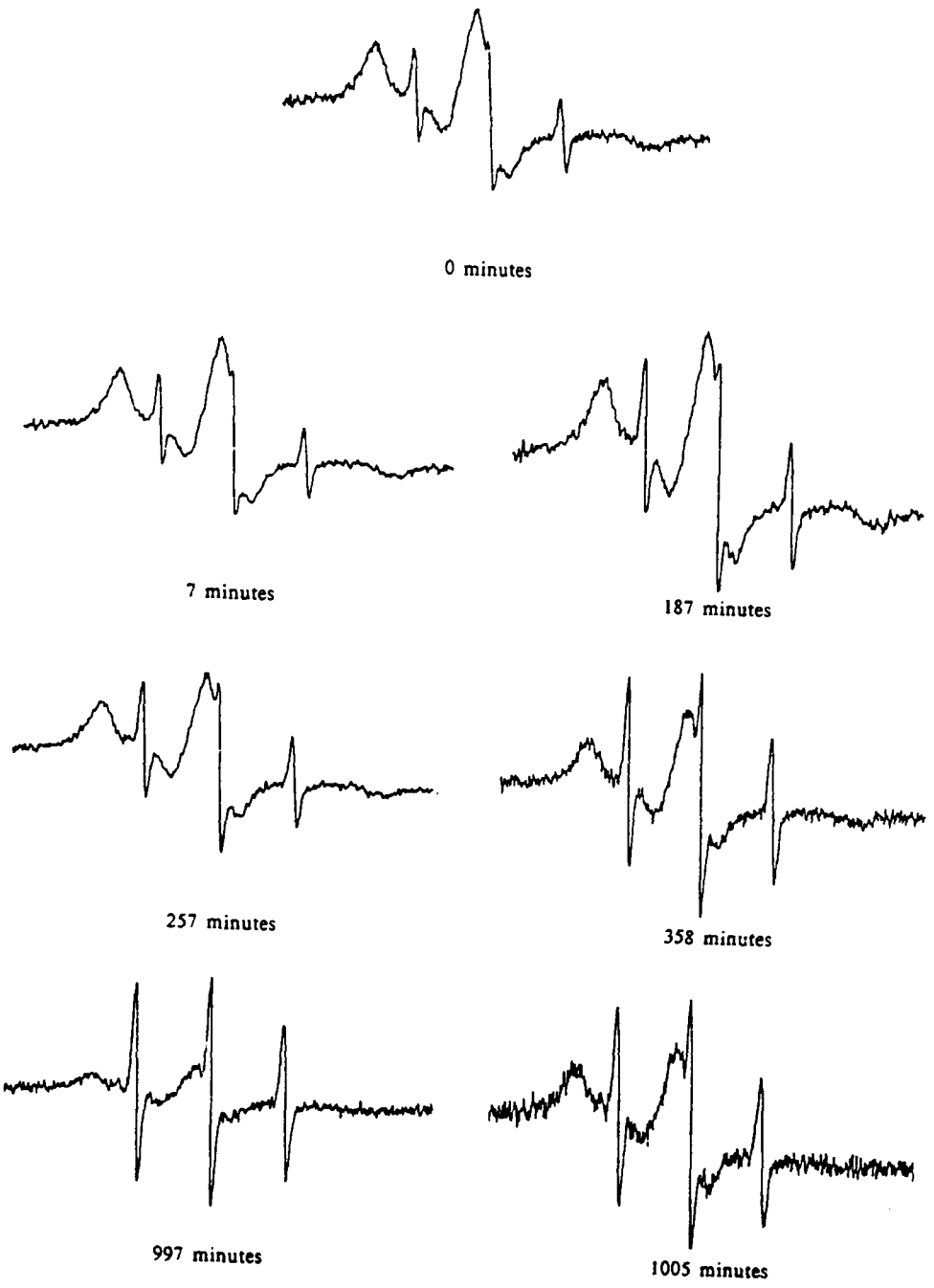


Figure 4. Time lapse ESR Spectra of spin label II in vesicles of GDNT. Two spectral components are observed: (i) a spectrum of 3 sharp lines of the spin label in aqueous solution, (ii) a spectrum of the spin label in the vesicle membrane (broader lineshape spectrum).

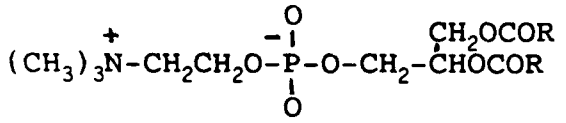
of the labels from the lipid vesicle into the surrounding aqueous solution. Knowing a compound's permeability helps greatly in evaluating it for pharmaceutical applications. This procedure gives one method of measuring the permeability.

By understanding the way in which a particular lipid interacts with a spin label, we can better understand the lipid's structure and its practical applications. The main purpose of this report is to demonstrate the state-of-the-art simulation techniques for analyzing and understanding ESR spin label spectra of lipid systems.

EXPERIMENTAL

Materials

Because the synthesis of GDNT or GDGT gives low yields, two more easily synthesized lipids were chosen to emulate the archaebacterial membranes. These are dimyristoyl phosphatidylcholine (DMPC) and diphytanoyl phosphatidylcholine (DPhPC). These lipids have molecular structures that are very similar to GDNT and GDGT, but possess only one phosphate head-group:



DMPC: R= $-(CH_2)_{12}CH_3$

DPhPC: R= $-[CH_2CH(CH_3)CH_2CH_2]_3CH(CH_3)CH_3$

Spin labels were incorporated into these systems by first mixing the labels with DMPC or DPhPC in chloroform, sonicating, and evaporating the chloroform by flowing argon through solution. The resulting powder was then dried under vacuum to remove all traces of chloroform. This produced the dry phase powder samples. The dry phase was hydrated using Hepes buffer solution to produce the aqueous phase samples.

PROCEDURE

We simulated ESR spectra that matched the experimental spectra of the compounds DMPC and DPhPC containing stearic acid spin labels I, II, and III in both their dry phase and aqueous phase at room temperature (See Appendix A and Appendix B). To calculate the data points of the simulated spectra, we used a

set of programs written in by J. Schneider and J. H. Freed of Cornell University. The following description indicates the input parameters of these programs. The first program is a FORTRAN program called LBLL that takes the data points entered by the user and places them in an input file for the ESR spectral calculation program. The needed data points are as follows:

- G-tensors (gx, gy, gz): These values are based on the spin label environment. For organic radicals such as these labels the value tends to be around g = 2.0023. The lower the value, the further the spectrum is shifted to the right on the magnetic field x axis. These numbers differed with each label and typical numbers are:

LABEL	G_x	G_y	G_z
I	2.0088	2.0062	2.0045
II	2.0087	2.0061	2.0046
III	2.0090	2.0073	2.0043

- Nuclear Spin: The programed value is twice the nuclear spin. For our purposes, we entered 2. (I = 1 for ^{14}N and 2 X 1 = 2).

- A-tensors (A_x, A_y, A_z): These values are entered in gauss and are used in the calculation of the hyperfine coupling interaction. Typical values are:

LABEL	A_x	A_y	A_z
I	4.7	4.7	38.1
II	4.8	4.8	35.7
III	4.4	4.4	36.4

- Static field: This is the value in gauss of the magnetic field.

- Diffusion tensors (D_{xy}, D_{zz}): These are the two values that are varied in order to produce the different spectra. The D_{xy} and D_{zz} tensors indicate the rate at which these chains move, the D_{xy} representing movement around the X-Y plane and the D_{zz} the movement around the Z-axis (Figure 1). In the spectrum, this generates linebroadening. As stearic hindrance becomes greater, a smaller diffusion coefficient is required for simulation. By experimenting with these values in the range of $1.0*10^8$ to $1*10^{10}$ for D_{xy} and $1*10^6$ to $10*10^8$ for D_{zz}, we can find a match for most of the compounds and spin labels we are using.

- Heisenberg Spin Exchange Frequency: This number denotes the spin exchange on neighboring sites due to random encounters.

- Number of terms in the potential: This number indicates a restoring potential which restricts the simulated label motion to a specified angle away from the starting symmetry axis.

- Angle Between Static Field & Local Director and the Diffusion Tilt Angle: Both are degree values and represent the angle of tilt between the label major axis and the applied magnetic field direction. Such a tilt may occur on the surface of thin films.

- Truncation Values & Lanczos/Conjugate Gradients steps: These values are assigned according to the degree of the diffusion tensors as follows:

10^7	6	3	2	2	2	steps=33
10^6	14	7	6	2	2	steps=100
10^5	30	13	10	2	2	steps=256
10^4	54	15	10	2	2	steps=447

- Calculation Type: We used the Lanczos method, which equals 0, but the Conjugate Gradients methods uses 1 and the FS is 2.

Once that data is entered, a file is created and coded under a two letter code entered by the user. Then the program EPRLL is called. This figures the EPR spectral calculations using the complex symmetric Lanczos algorithm.

After that, another file is created and called by the program TDLL, which processes the tridiagonal matrix generated by the Lanczos or Conjugate Gradients algorithms. This produces a file with the extension .FMT and contains three data values on a text line. The first number corresponds to the x value, an integer, and ranges from 1 to 1000. This scale will vary depending on the field sweep input parameter. The y value is the dispersion component of the spectrum and the z value is the absorption component of the spectrum.

Another FORTRAN program, FILTRAN transforms the XXXX.FMT file to another file under the name SYS.DAT that contains the data in a slightly different format (We transfer x values and z values to this file in order to produce an absorption spectrum). This can then be used by Hewlett-Packard's graphing and analysis program ASYST.

The final data file was then transferred over to the ASYST directory. Once in ASYST, it was necessary to write

another program under the name INPUT.COM to simply read the data from the SYS.DAT file and place the data into arrays. This program was written in ASYST's own programming language, which is similar to PASCAL stack procedures. The y-data, which represented the spectrum, was then pushed onto ASYST's stack and differentiated with the built-in function. By inspection we fitted the simulated version to the experimental one (See Appendix C). The diffusion tensors and tilt angles were calculated to be:

SPECTRUM	D_{XY}	D_{ZZ}	TILT
I-DPhPC-RT	$3.0*10^7$	$2.0*10^8$	40°
I-DMPC-RT	$3.6*10^6$	$2.7*10^8$	40°
II-DPhPC-RT	$5.0*10^7$	$2.0*10^9$	0°
II-DMPC-RT	$8.0*10^7$	$2.0*10^8$	0°
III-DPhPC-RT	$2.0*10^8$	$2.0*10^7$	0°
III-DPhPC-Dry	$8.0*10^7$	$2.0*10^8$	0°
III-DMPC-RT	$2.0*10^8$	$2.0*10^8$	0°
III-DMPC-Dry	$6.5*10^7$	$2.0*10^8$	0°

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note: In this table the spectrum notation refers to the spin label, the lipid system in which the label was placed, and the phase. For example, I-DPhPC-RT refers to spin label I (Fig. 1) in the lipid DPhPC in aqueous solution at room temperature. Dry refers to the dry powder phase. Tilt refers to the angle of tilt between the rotational diffusion z reference axis and the p-orbital direction of the ^{14}N of the spin label. These axes are often noncoincident in the region of the phosphate head-group.

These spectral values show that the labels in DMPC move more freely, especially since the linebroadening is less apparent than the labels in DPhPC.

DISCUSSION

Archaebacteria are a recently discovered family of micro-organisms consisting of methanogens or methane producers, halophiles which survive in high salt concentrations, and thermophiles which exist at high temperatures. The bipolar isopranyl ether lipids GDNT and GDGT are the predominant hydrolyzed lipids of the thermoacidophile Sulfolobus solfataricus. The natural environment under which these bacteria flourish is quite harsh. Their normal habitat is at 90°C and pH 2. The ability of these bacteria to withstand such highly acidic environments and such high temperatures makes them fascinating subjects for investigation.

The ESR studies which we have performed show that there is a significant difference in the fluidity of GDNT and GDGT in comparison to DMPC as measured by the ESR spectral lineshapes

shown in appendix A and appendix B. One comparison to support this is between spectrum II-DMPC-RT in appendix A and the spectrum marked as 0 minutes in Fig. 3. Although the purpose of Fig. 3 is to show the permeability changes of the DPhPC vesicle, this first spectrum at 0 minutes has features which indicate slower correlation times of label motion than in the II-DMPC-RT spectrum (Compare the lineshapes neglecting the 3 sharp peaks of Fig. 3). In other words, the broader lineshapes of the Figure 3 spectrum indicate a more restricted environment of label motion than in the II-DMPC-RT spectrum. This suggests that the GDNT membranes are more closely packed than in the DMPC membranes at similar concentrations and temperatures.

Further work on the GDNT and GDGT lipid systems will provide a complete data set using other spin labels. The purpose of this report is to demonstrate the simulation capability used for analyzing ESR spin label spectra in these systems.

CONCLUSION

We successfully fitted eight simulated spectra to the real spectra of stearic acid spin labels in dimyristoyl phosphatidylcholine and diphytanoyl phosphatidylcholine. These lipids emulate the GDNT and GDGT systems. Both aqueous and dry phases were studied at room temperature. By fitting the spectra and finding the rotation rates of the spin labels, we have found DPhPC to be more rigid than DMPC.

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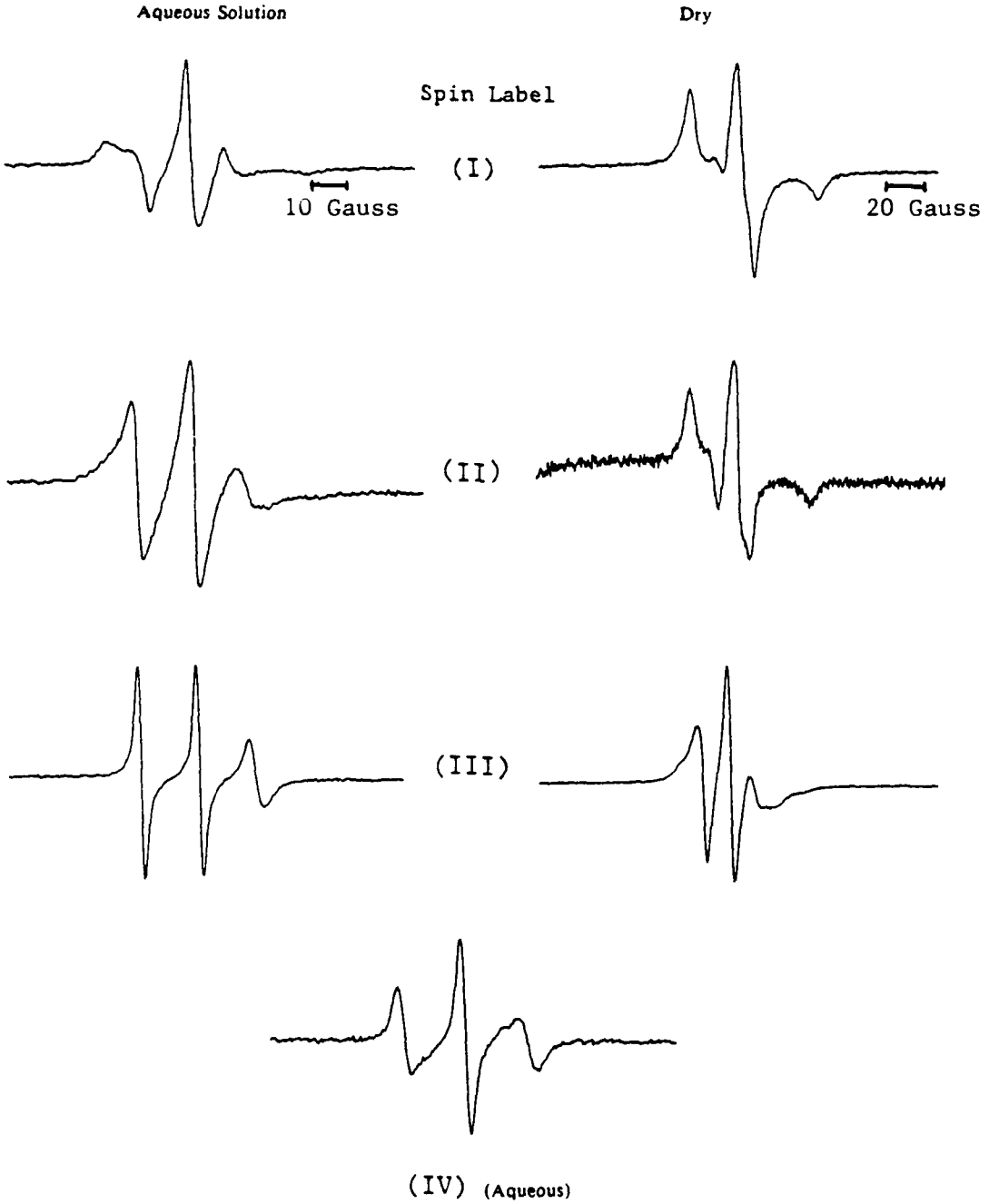
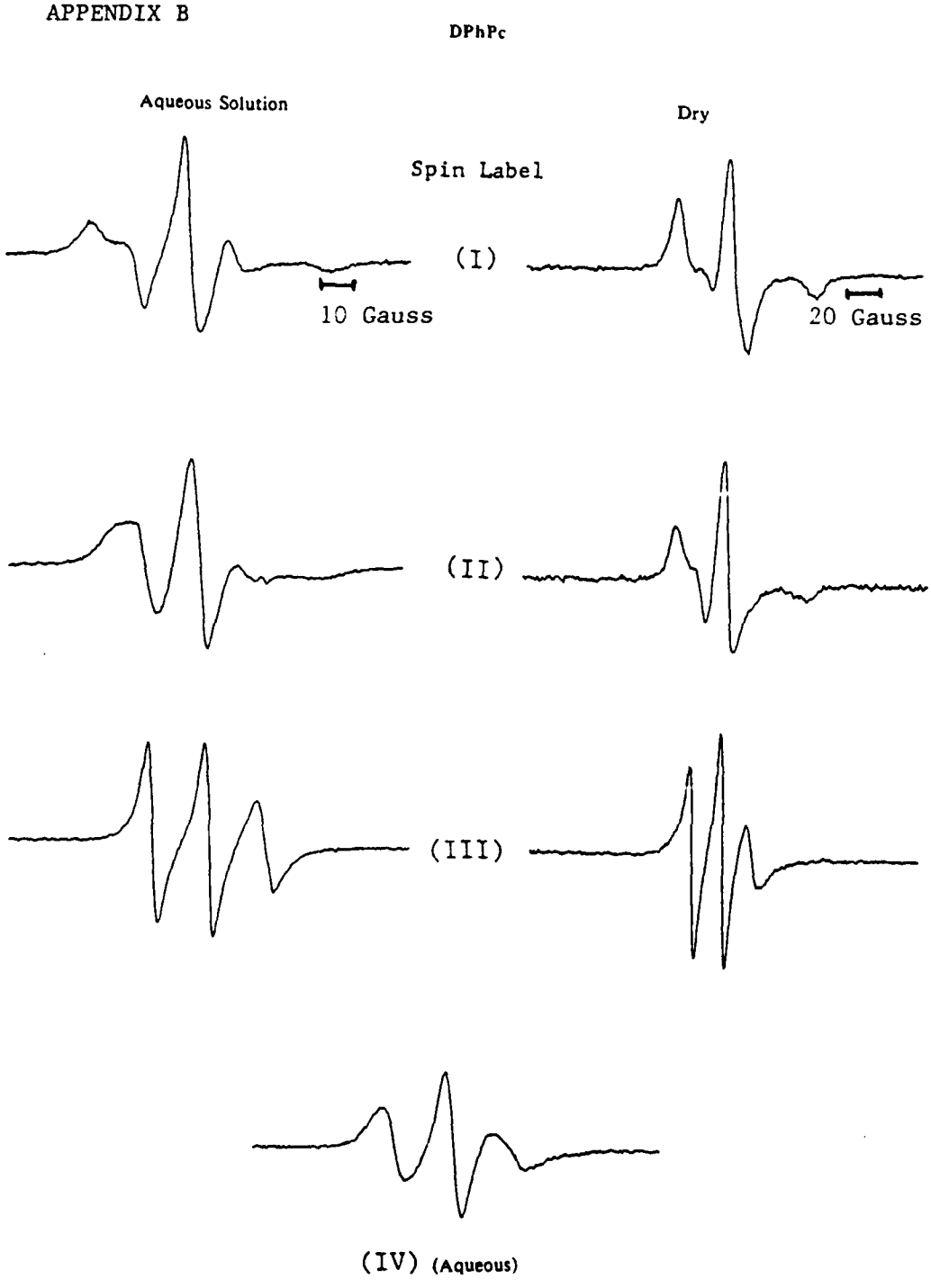
DMPC

APPENDIX A



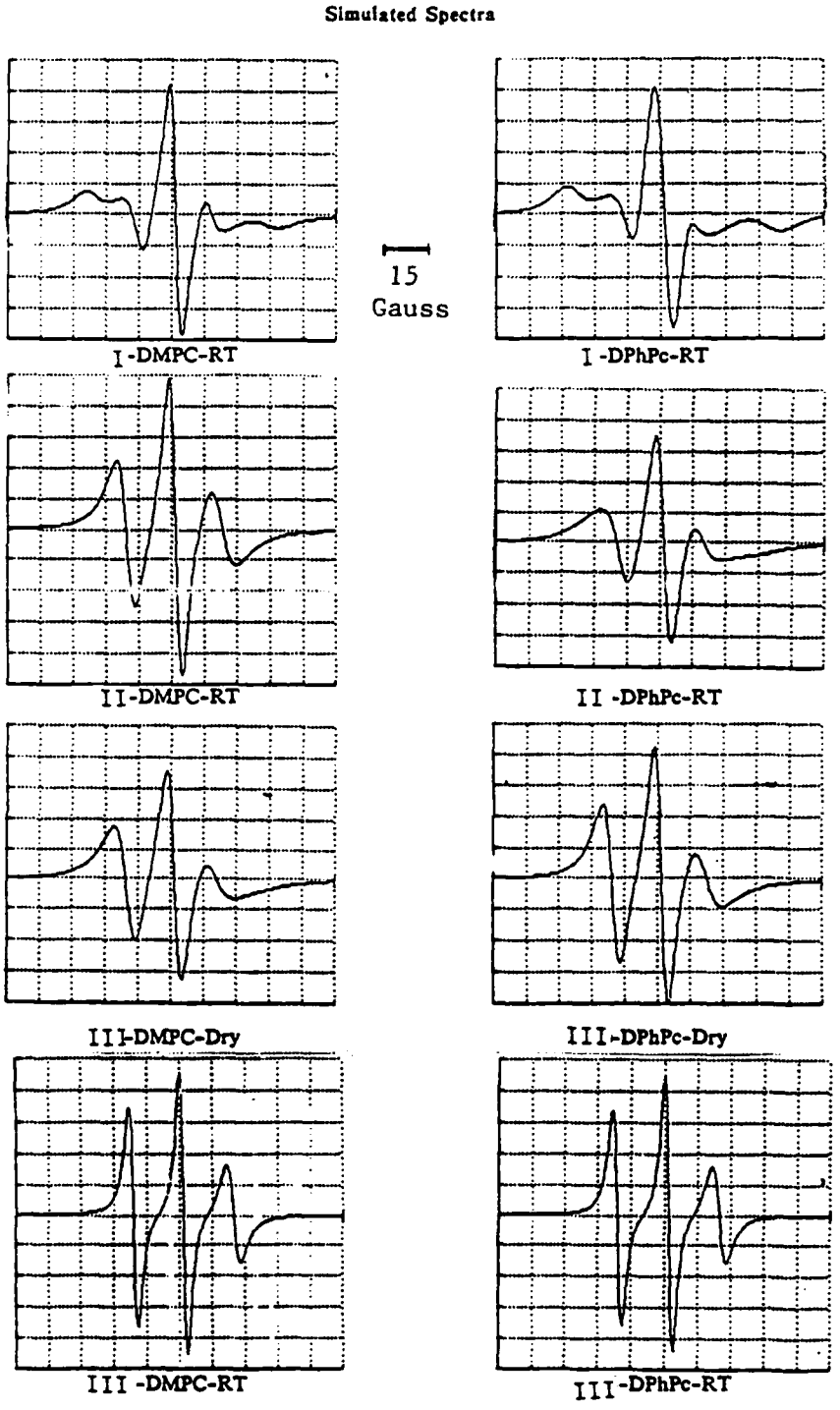
Experimental ESR Spin Label Spectra of Dry and Aqueous Phases of DMPC

APPENDIX B



Experimental ESR Spin Label Spectra of Dry and Aqueous Phases of DPhPc

APPENDIX C



Simulated ESR Spin Label Spectra of DMPC and DPhPc