Ressonância Paramagnética Eletrônica (RPE)

Introdução teórica e exemplos

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(Curso Kaline)

A Ressonância Magnética é uma espectroscopia de absorção

A amostra é colocada dentro de um campo magnético, e incide-se nela uma radiação:

RME ou RPE: microonda (~ GHz)

RMN: onda de rádio (~MHz)

A presença do campo magnético levanta a degenerescência dos níveis de spin eletrônico (RME) ou nuclear (RMN).

A "ressonância" acontece quando a energia da onda incidente é igual à separação dos níveis de energia:

radiação é absorvida

Ressonância Magnética

Ressonância Magnética Nuclear (RMN)

Ressonância Magnética Eletrônica (RME)

Ressonância Paramagnética Eletrônica (RPE)

Para núcleos, γ é muito bem definido:

^{1}H ($I = \frac{1}{2}$) $\gamma = 26753$ rad $G^{-1}s^{-1}$

^{2}H ($I = 1$) $\gamma = 4107$ rad $G^{-1}s^{-1}$

^{12}C ($I = 0$)

^{13}C ($I = \frac{1}{2}$) $\gamma = 6728$ rad $G^{-1}s^{-1}$

^{14}N ($I = 1$) $\gamma = 1934$ rad $G^{-1}s^{-1}$

Interação Zeeman

$$\mathcal{H}_{spin} = -\vec{\mu} \cdot \vec{H}$$

$$\vec{\mu} = \gamma \vec{J}$$

fator giromagnético

$$\mathcal{H}_{spin} = -\gamma \vec{J} \cdot \vec{H}$$

A RMN monitora todos os núcleos com spin $\neq 0$

Por ex., "enxerga" todos os prótons da molécula

A **RPE** é mais específica, pois "enxerga" somente **centros paramagnéticos**, que são poucos nas biomoléculas:

Metal centers

In proteins:

- Fe(II or III) in heme proteins

- Mn(II or III) in superoxide dismutase

- Mo(V or VI) in xanthine oxidase or sulphite oxidase

-etc.....

Free radicals

- Intermediates in biological redox reactions

- Radiation induced

- Stable radicals

(Melanin, cereals, etc...)

Spin labels

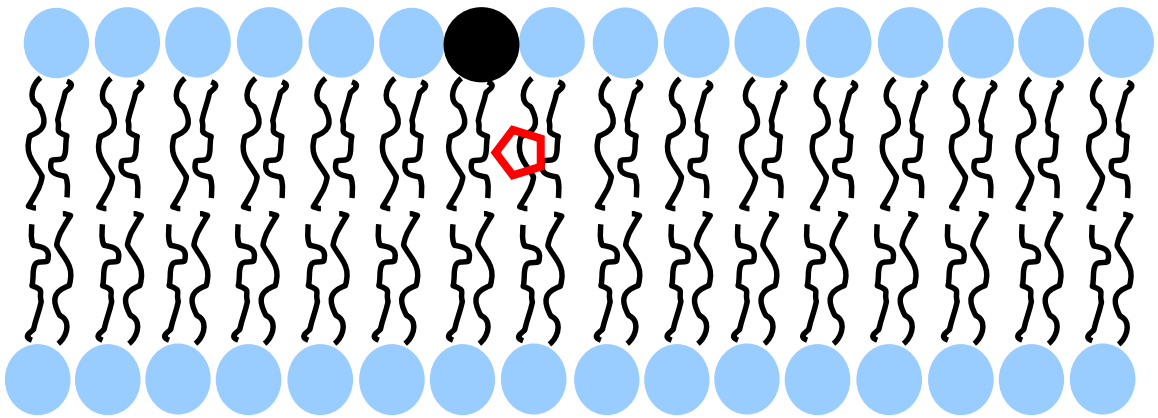
"Spin labels"

Marcadores de spin

Moléculas exógenas, paramagnéticas, incorporadas ao sítio a ser estudado

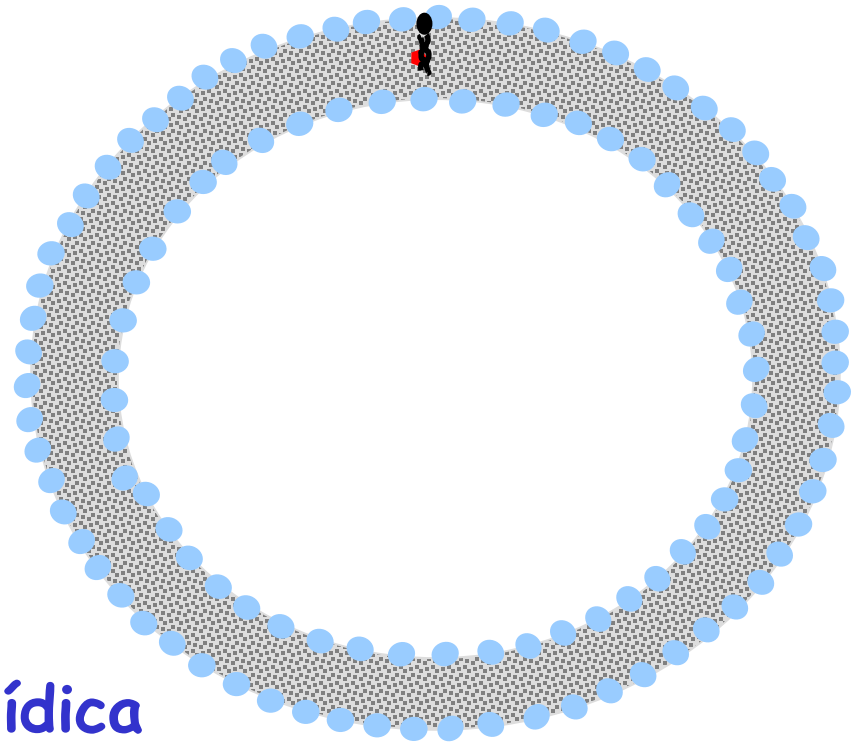
- ligadas covalentemente à molécula
- incorporadas a agregados de moléculas

Incorporadas a agregados anfifílicos em solução aquosa



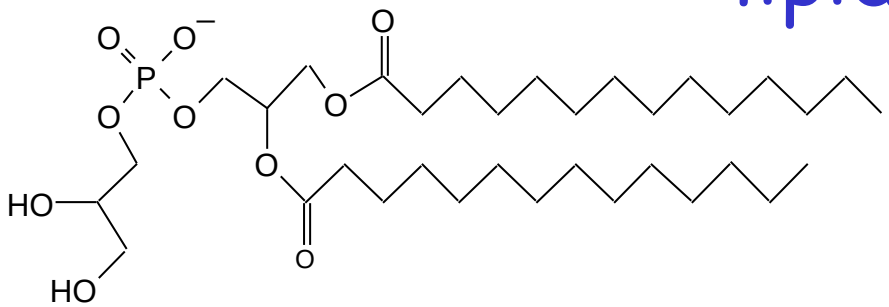
Marcadores de spin anfifílicos, derivados de:

- fosfolipídios
- ácidos esteáricos
- colesterol



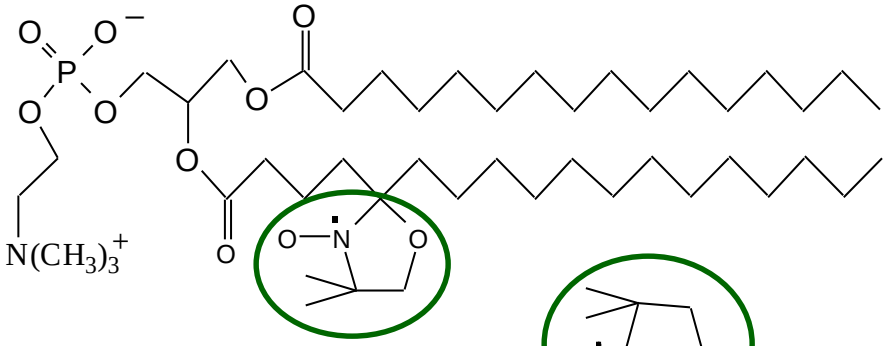
Incorporam-se à bicamada lipídica

lipídios e marcadores de spin

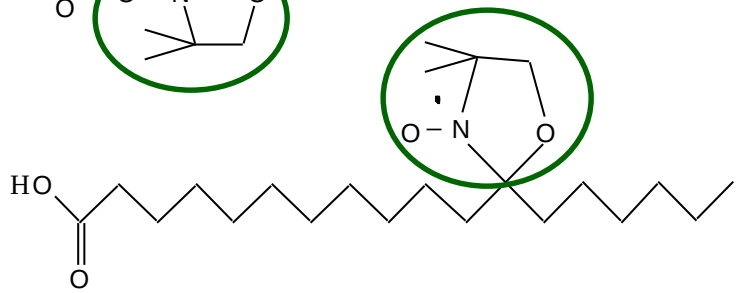


DMPG

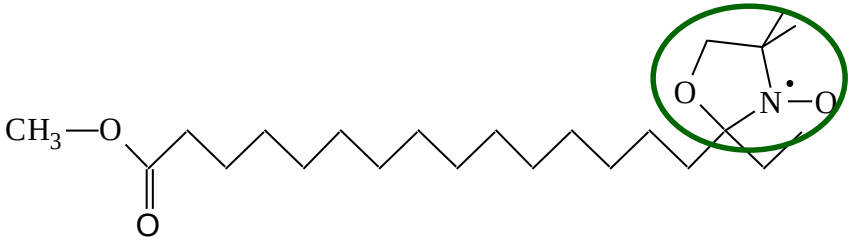
dimiristoil fosfatidil glicerol



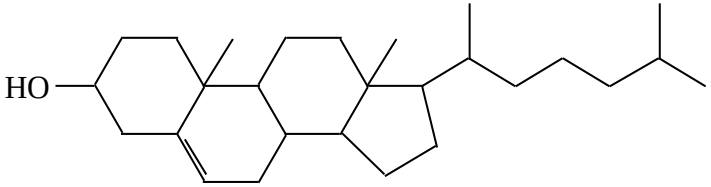
5-PCSL, fosfolipídio



12-SASL, ácido esteárico



16-MESL, metil éster do ácido esteárico



colesterol

O que podemos avaliar com medidas de RPE ?

- Estrutura do sítio onde se encontra a sonda paramagnética: ordem, fluidez e "polaridade" do sítio

Na **Ressonância Paramagnética Eletrônica** (RPE)

$$\vec{\mu} = \gamma_e \vec{J}$$

fator giromagnético para o elétron

$$|\gamma_e| \approx 10^3 \, |\gamma_p| \quad \begin{cases} w_e \approx GH_z \\ w_p \approx MH_z \end{cases}$$

Mas, em geral, usamos outra expressão:

$$\vec{\mu} = -g \frac{e\hbar}{2m} \frac{\vec{J}}{\hbar}$$

$\beta \equiv$ magneton de Bohr

$$\vec{\mu} = \gamma_e \vec{J} = -g\beta \frac{\vec{J}}{\hbar}$$

(para o "elétron livre" $g = 2{,}00232$)

$$\mathcal{H}_{spin} = -\vec{\mu} \cdot \vec{H}$$

sendo $\vec{\mu} = -g\beta \dfrac{\vec{J}}{\hbar}$ e $\vec{J} \equiv \vec{S} \Longrightarrow \mathcal{H}_{spin} = g\beta \dfrac{\vec{S}}{\hbar} \cdot \vec{H}$

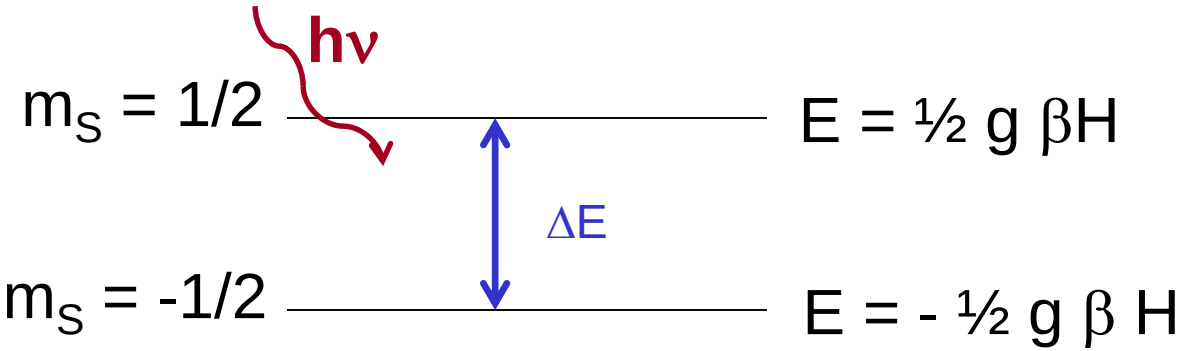
fazendo $\vec{H} \;//\; \hat{z}$ $\qquad \mathcal{H}_{spin} = g\beta H \dfrac{S_z}{\hbar}$

$S_z \rightarrow$ auto valores $\rightarrow m_s \hbar$

autoestados dessa Hamiltoniana:

$$E_{m_S} = g\beta H m_S$$

p. ex. $S = 1/2$, $m_S = +1/2$, $-1/2$



$$E_{m_S} = g\beta H m_S$$

$\Delta E = g\beta H$

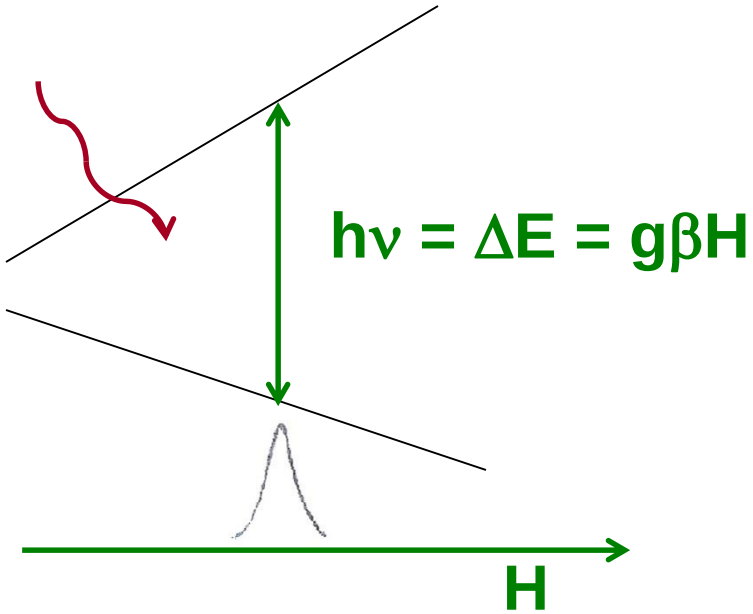
$\Delta m_S = \pm 1$

Na absorção de radiação:

$$h\nu = \Delta E = g\beta H$$

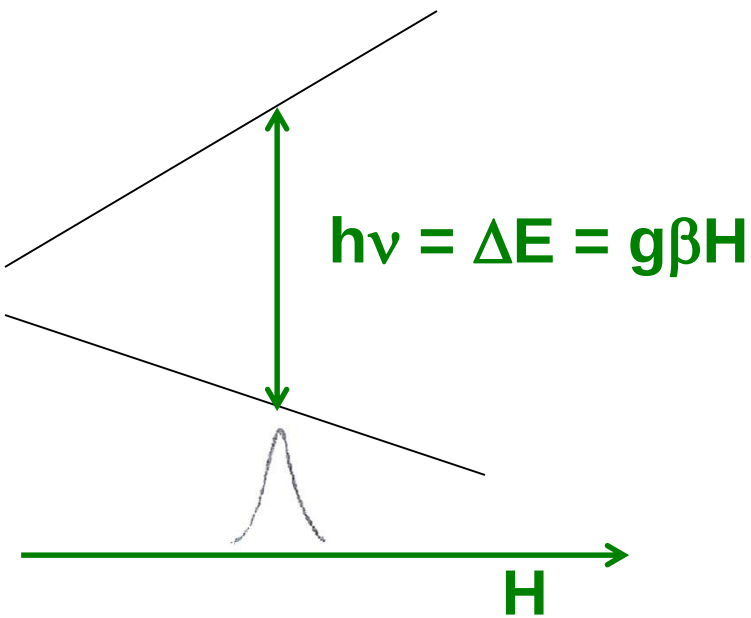
Como é feito o experimento de RPE:

ν fixa, varia-se H até se obter a ressonância



fator g

$$h\nu = g\beta H$$



Quando o experimento é feito, você **mede** o H onde acontece a absorção, portanto você **calcula** o fator g, dado que ν é conhecido.

para o "elétron livre" g = 2,00232

o H que ele "sente" é ~ H aplicado

Em geral, o elétron desemparelhado está em um orbital, e temos o acoplamento spin-órbita, anisotrópico. Portanto, para cada direção de aplicação de H, a interação Zeeman varia: interação de H com o momento angular orbital e momento angular de spin do sistema.

De uma maneira geral, escrevemos:

$$\mathcal{H}_{spin} = \frac{\beta}{\hbar}\vec{H}\cdot\overleftrightarrow{g}\cdot\vec{S}$$

no lugar de:

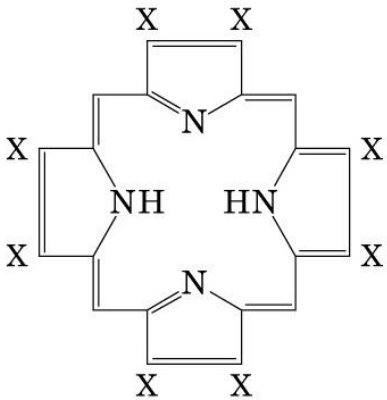
$$\mathcal{H}_{spin} = g\beta H\frac{S_z}{\hbar}$$

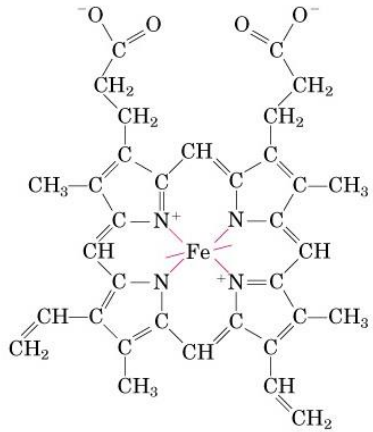
Sendo que é colocado no "tensor g", todo o desconhecimento que temos das interações do sistema, sendo H o campo externo, e este tensor g é medido experimentalmente, variando-se o ângulo de aplicação de H, com respeito à estrutura da molécula

$$h\nu = \beta\, g(\theta,\phi)H(\theta,\phi)$$

Para vários moléculas com centros paramagnéticos, principalmente metais, o trabalho do físico será o cálculo deste tensor g, dado que nele está embutido o acoplamento spin-órbita. Com ele, será, então, possível fazer estimativas dos orbitais do centro paramagnético, e comparar com parâmetros vindos, por ex., de modelagem.

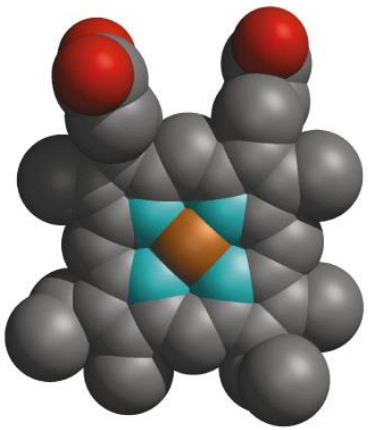
grupo heme

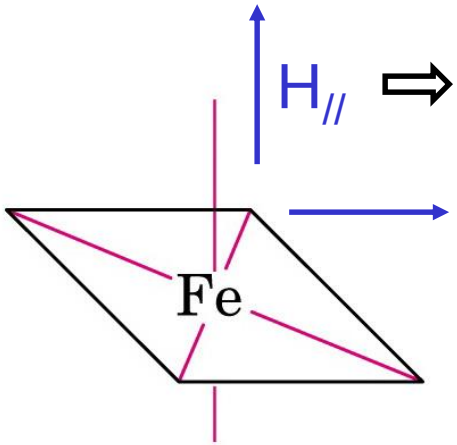




Fe^{3+} $(3d^5)$

spin alto ⇨ $S = 5/2$





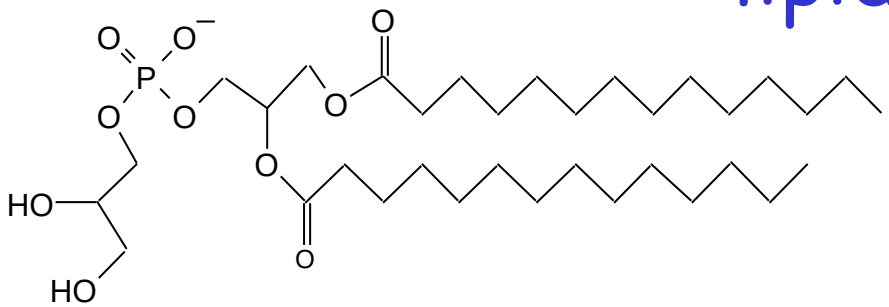
$H_{//}$ ⇨ $g_{//} = 2$

$H_{\perp}$ ⇨ $g_{\perp} = 6$

Ingram, 1957, estuda cristais de mioglobina, antes dos cálculos por raios-X: **duas moléculas por célula unitária e estrutura do centro ativo**

(http://courses.cm.utexas.edu/jrobertus/ch339k/overheads-1/ch7_heme.jpg)

lipídios e marcadores de spin



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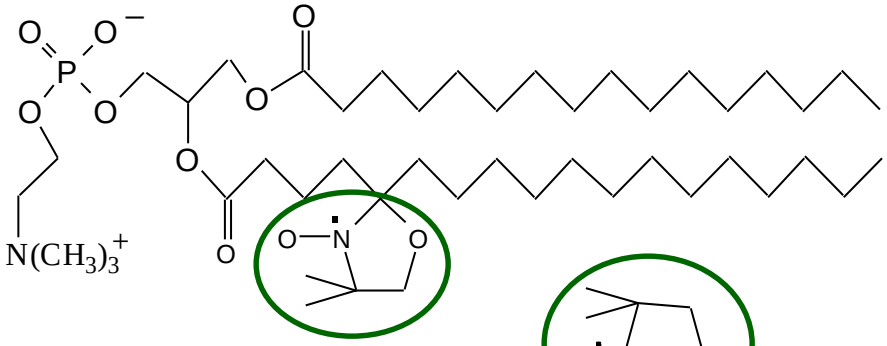

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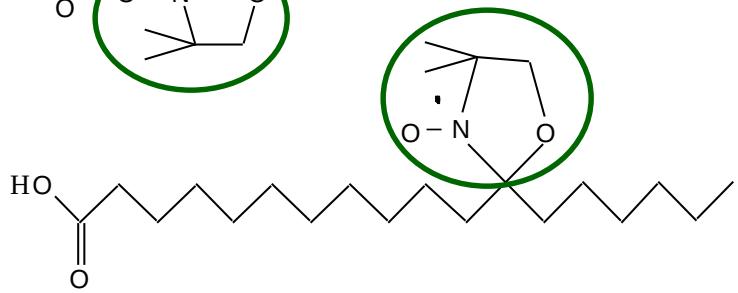

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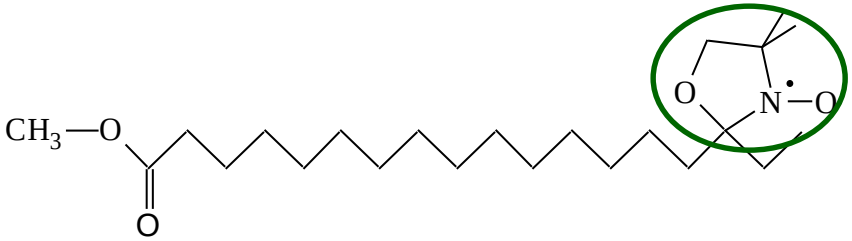

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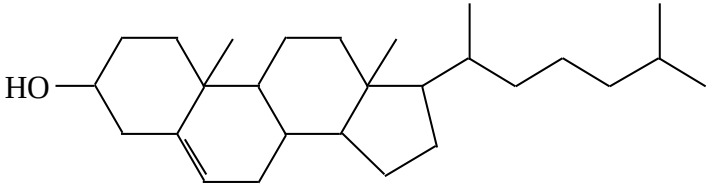
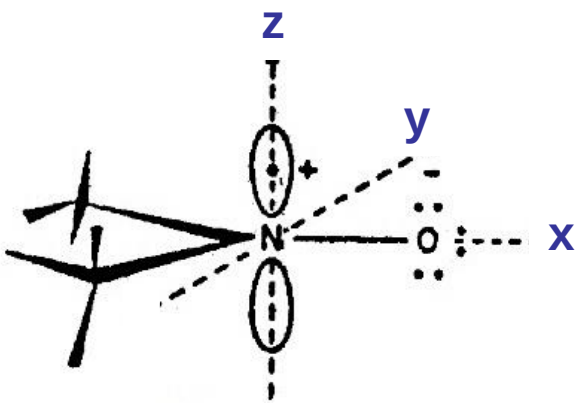


colesterol

nitróxido: usado como "marcador de spin"

radical livre, extremamente estável!

S = 1/2



$H_z \Rightarrow g_z = 2{,}0027$

$H_x \Rightarrow g_x = 2{,}0089$

$H_y \Rightarrow g_y = 2{,}0061$

(elétron livre $g = 2{,}0023$)

Fator g, nas 3 direções, não são muito distintos.

Elétron desemparelhado localizado principalmente no orbital p do nitrogênio.

Interação hiperfina

Interação do elétron desemparelhado com os núcleos vizinhos

Interação clássica entre dois dipolos magnéticos: do elétron e do núcleo

$$\vec{\mu}_e = -g\beta\frac{\vec{S}}{\hbar} \qquad \vec{\mu}_N = \gamma\vec{I}$$

$$\boxed{\mathcal{H}_{anisot} = \frac{\mu_o}{4\pi\hbar} g\beta\gamma\left\{\frac{3(\vec{S}\cdot\vec{r})(\vec{I}\cdot\vec{r})}{r^5} - \frac{\vec{S}\cdot\vec{I}}{r^3}\right\}}$$

Interação de contato (de Fermi)

$$\boxed{\mathcal{H}_{isot} = \frac{8\pi}{3\hbar} g\beta\gamma\left|\Psi(0)\right|^2 \vec{S}\cdot\vec{I}}$$

$$\boxed{\mathcal{H}^{hiperfina} = \mathcal{H}_{anisot} + \mathcal{H}_{isot}}$$

$$\mathcal{H}^{\text{hiperfina}} = \vec{S} \cdot \overleftrightarrow{A} \cdot \vec{I}$$

Sendo que no tensor A, que pode ser medido, é colocada toda a interação hiperfina desconhecida,

que será, portanto, medida experimentalmente.

$$\mathcal{H}_{\text{spin}} = \frac{\beta}{\hbar} \vec{H} \cdot \overleftrightarrow{g} \cdot \vec{S} + \frac{1}{\hbar^2} \vec{S} \cdot \overleftrightarrow{A} \cdot \vec{I}$$

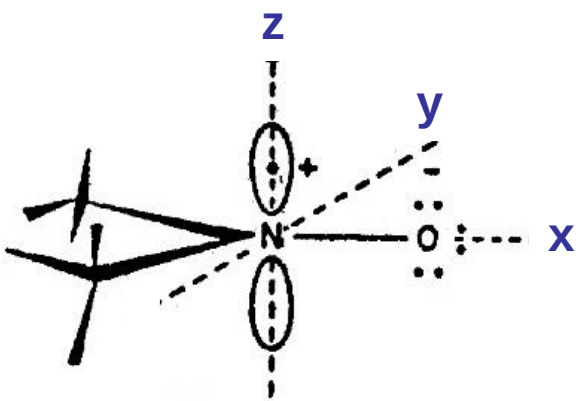
$\leftrightarrow$

Interação Zeeman

Interação hiperfina

Para o nitróxido:

$S = \frac{1}{2}$ e $I = 1$ (interação com o spin nuclear do ^{14}N)



$H_z \Rightarrow g_z = 2{,}0027$, $\mathbf{A_z = 32\ G}$

$H_x \Rightarrow g_x = 2{,}0089$, $\mathbf{A_x = 6\ G}$

$H_y \Rightarrow g_y = 2{,}0061$, $\mathbf{A_x = 6\ G}$

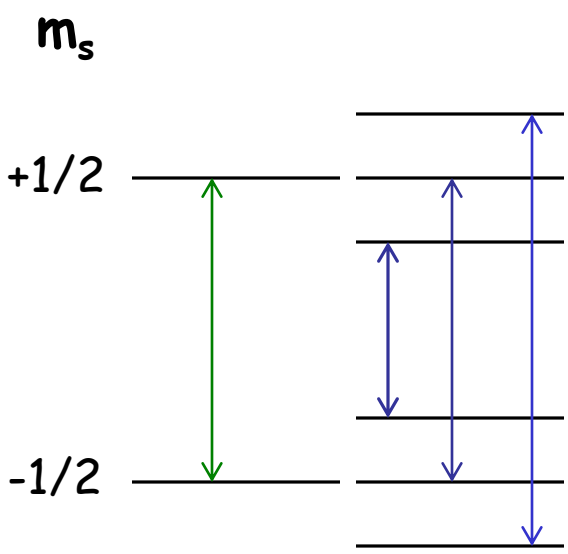
Como estes valores são medidos?

Por ex., $\vec{H} // \hat{z}$ e **todas as moléculas ordenadas**

$$\mathcal{H}_{spin} = \frac{\beta}{\hbar}\vec{H}\cdot\overleftrightarrow{g}\cdot\vec{S} + \frac{1}{\hbar^2}\vec{S}\cdot\overleftrightarrow{A}\cdot\vec{I} \qquad \mathcal{H}_{spin} = g_z\frac{\beta}{\hbar}HS_z + \frac{A_z}{\hbar^2}I_zS_z$$

S = ½ e I = 1

$$E = g_z\beta H m_S + A_z m_S m_I$$



m_I	
+1	$E = ½\, g_z\beta H + ½\, A_z$
0	$E = ½\, g_z\beta H$
-1	$E = ½\, g_z\beta H - ½\, A_z$
-1	$E = -½\, g_z\beta H + ½\, A_z$
0	$E = -½\, g_z\beta H$
+1	$E = -½\, g_z\beta H - ½\, A_z$

$$\left(\begin{array}{c}\Delta m_S = \pm 1 \\ \Delta m_I = 0\end{array}\right)$$

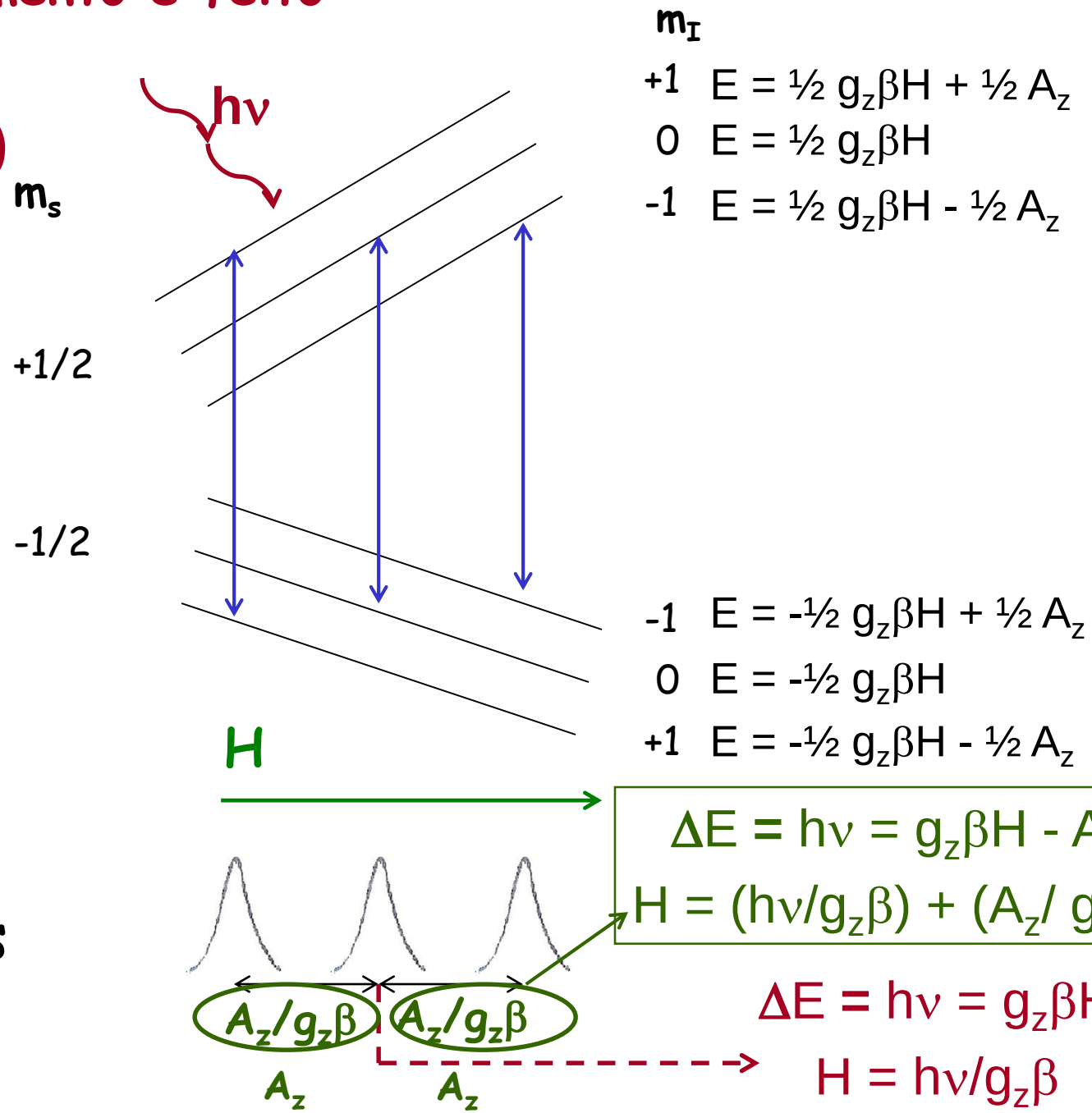
Como o experimento é feito

hν fixa (micro-onda)

Varia-se o campo magnético aplicado

$$\left(\begin{array}{l}\Delta m_S = \pm 1 \\ \Delta m_I = 0\end{array}\right)$$

Mede-se os valores de g e A

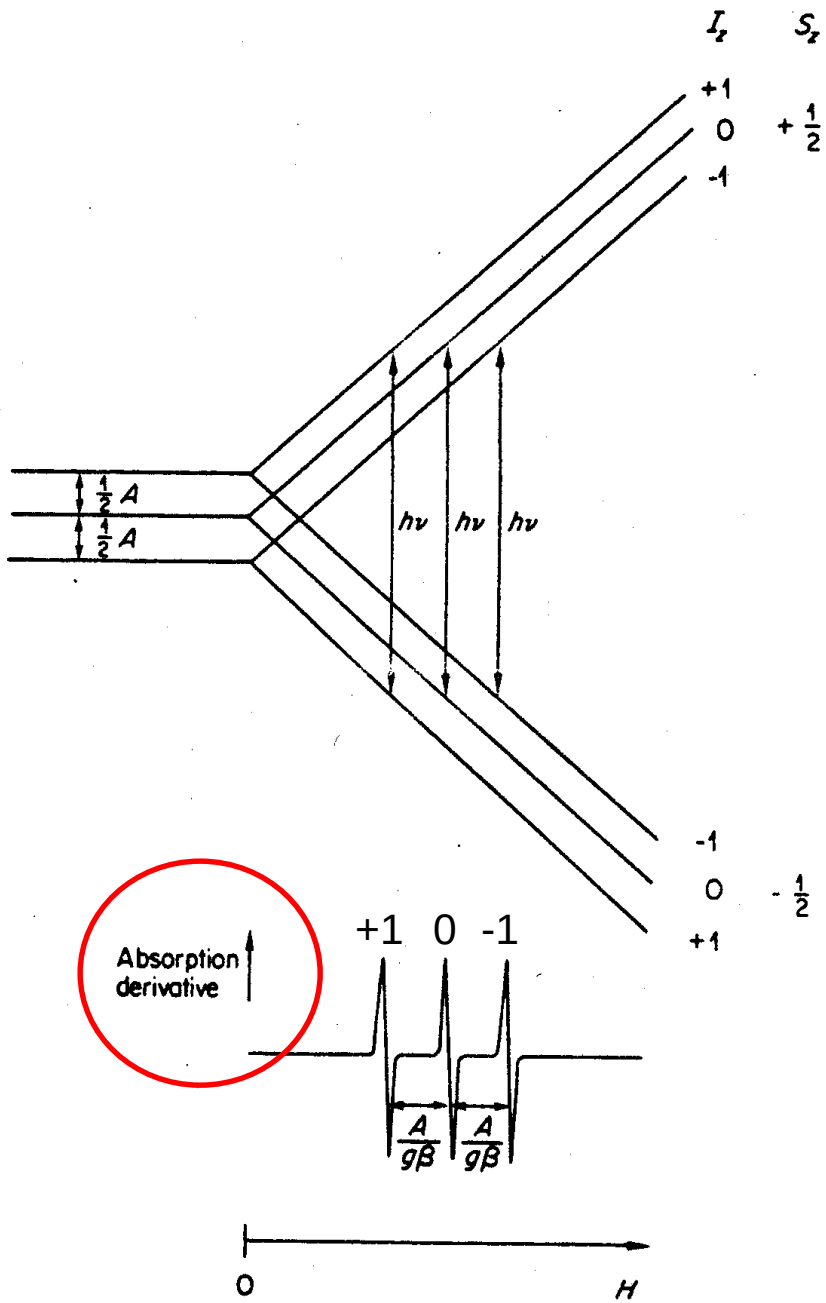


$$\Delta E = h\nu = g_z\beta H - A_z$$

$$H = (h\nu/g_z\beta) + (A_z/ g_z\beta)$$

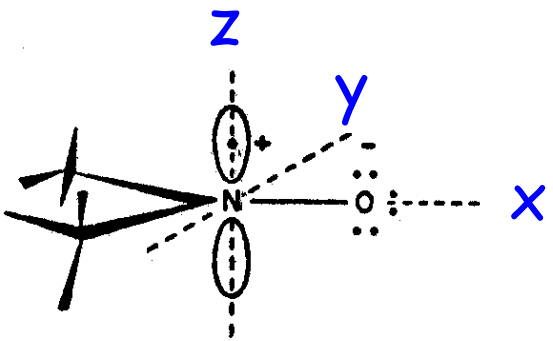
$$\Delta E = h\nu = g_z\beta H$$

$$H = h\nu/g_z\beta$$


I_z
S_z
+1
0
-1
$+\frac{1}{2}$
$\frac{1}{2}A$
$\frac{1}{2}A$
$h\nu$
$h\nu$
$h\nu$
-1
0
+1
$-\frac{1}{2}$
Absorption derivative
+1 0 -1
$\frac{A}{g\beta}$
$\frac{A}{g\beta}$
0
H

(Marsh, 1981)

Lembrando a simetria do grupo nitróxido

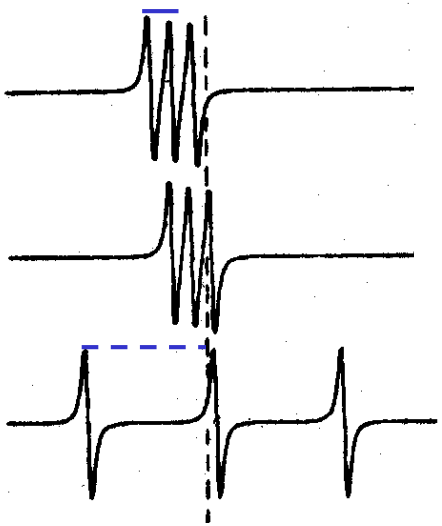


Em caso de um **cristal**, onde o nitróxido foi incorporado como impureza, mas está ordenado

A_{xx} = 6 G

A_{yy} = 6 G

A_{zz} = 32 G

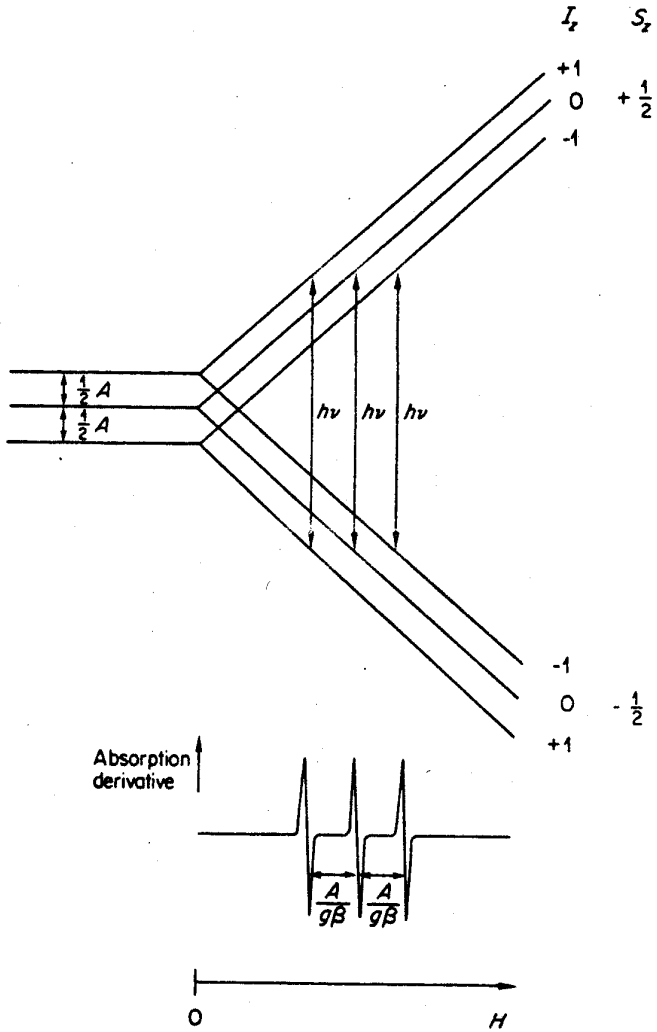


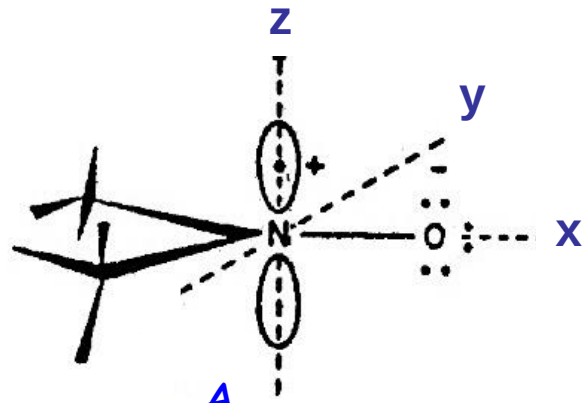
H//x

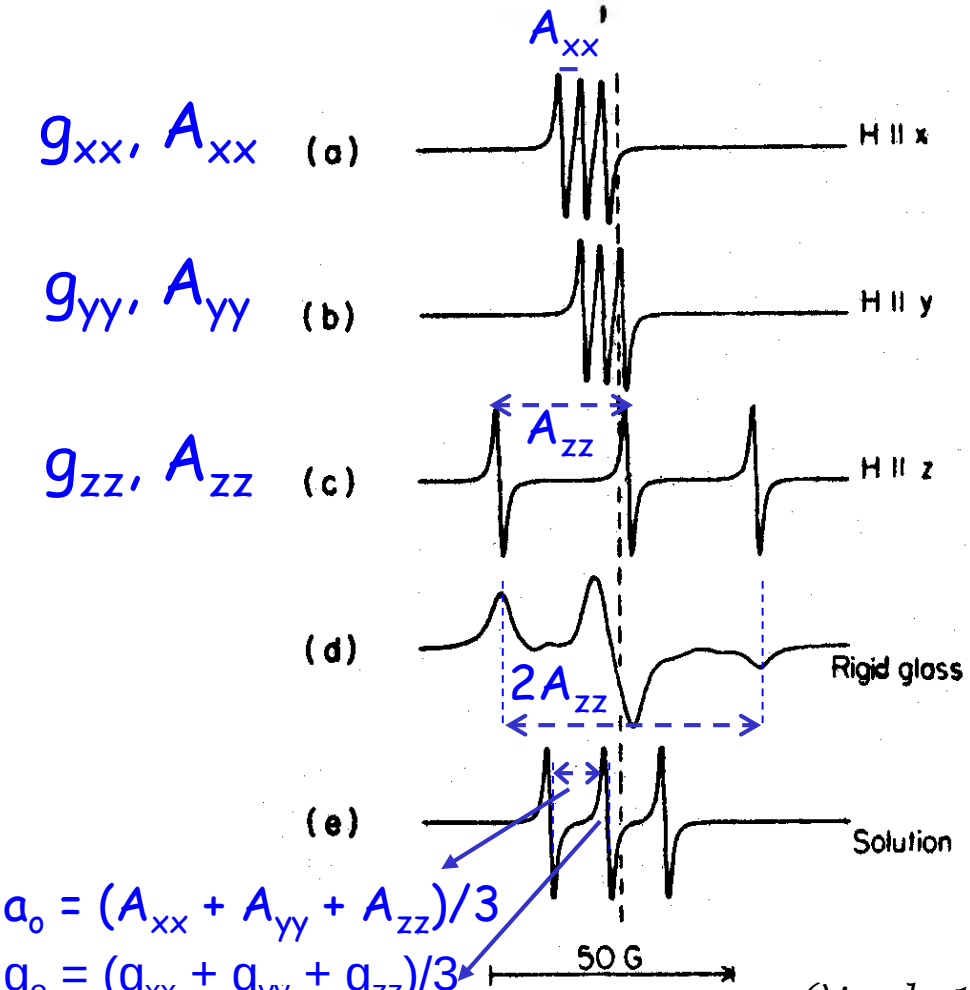
H//y

H//z

Portanto, os valores do desdobramento hiperfino, $A(\theta,\varphi)$, ou $A(\theta)$, dado que existe uma simetria axial, variam entre 6 e 32 G



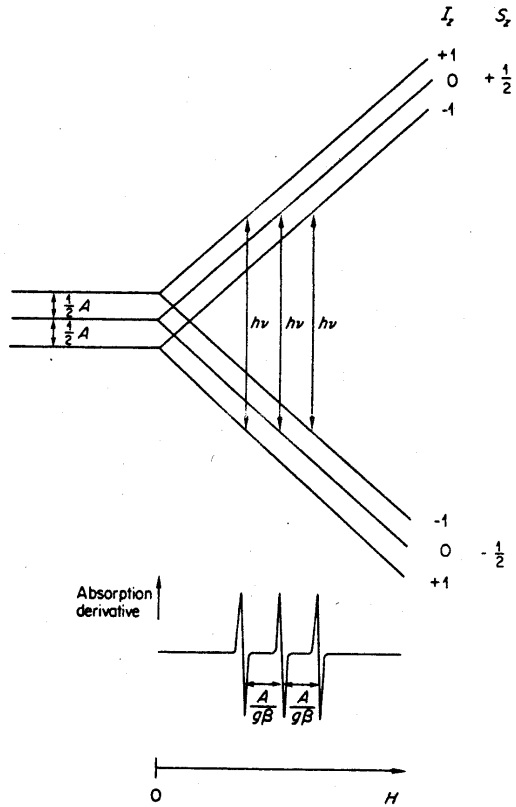


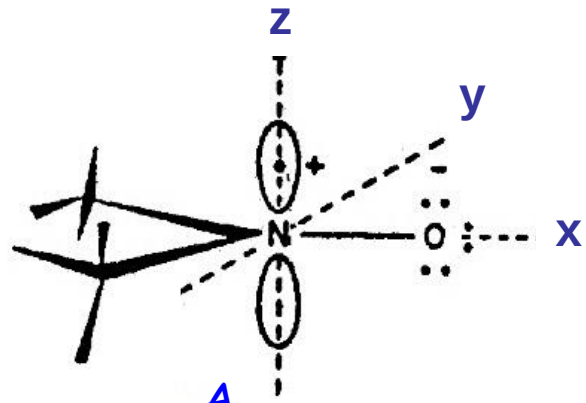


$a_o = (A_{xx} + A_{yy} + A_{zz})/3$

$g_o = (g_{xx} + g_{yy} + g_{zz})/3$

(Marsh, 1981)





crystal

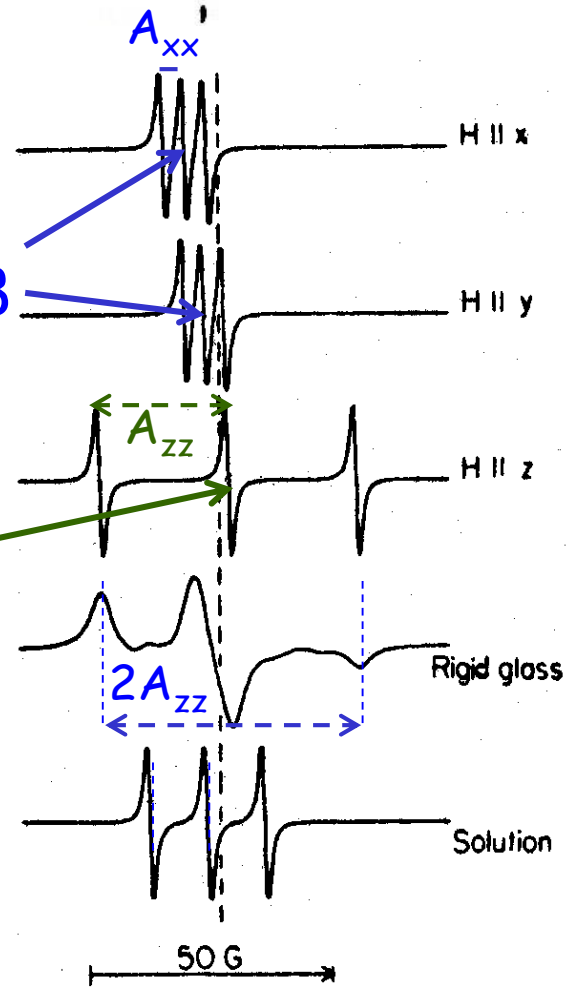
$A_{xx} \sim A_{yy} \sim 6$ G

$g_{xx} \sim g_{yy} \sim 2.008$

$A_{zz} \sim 32$ G

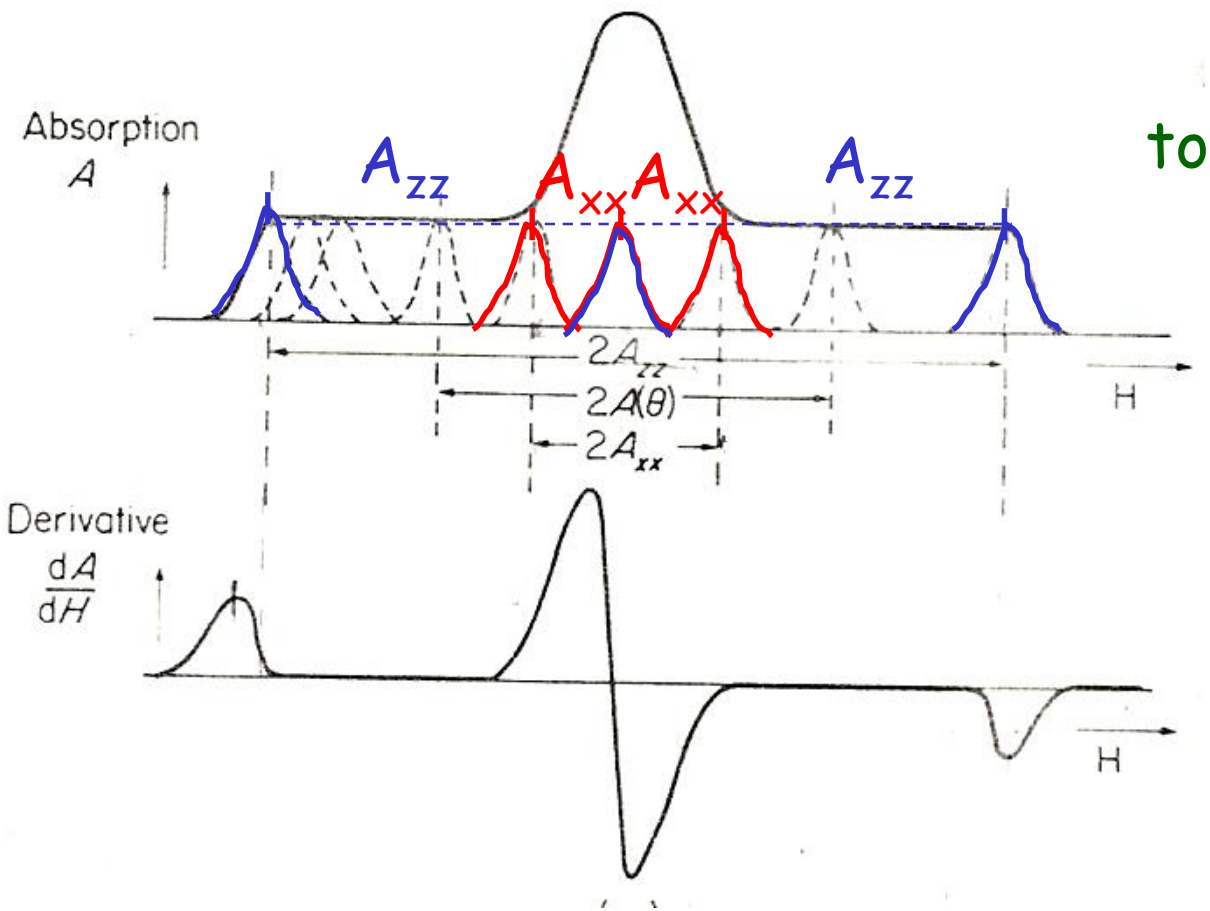
$g_{zz} \sim 2.003$

movimento rápido



"espectro de pó"

Soma dos espectros obtidos com o marcador de spin em todas as direções



Marcador de spin totalmente imobilizado

Deste tipo de espectro, mede-se somente o $2A_{zz}$

(Knowles, Marsh, Rattle, 1976)

Forma e posição das linhas do sinal de RPE informam sobre o microambiente onde se encontra o nitróxido:

- fluidez

-polaridade

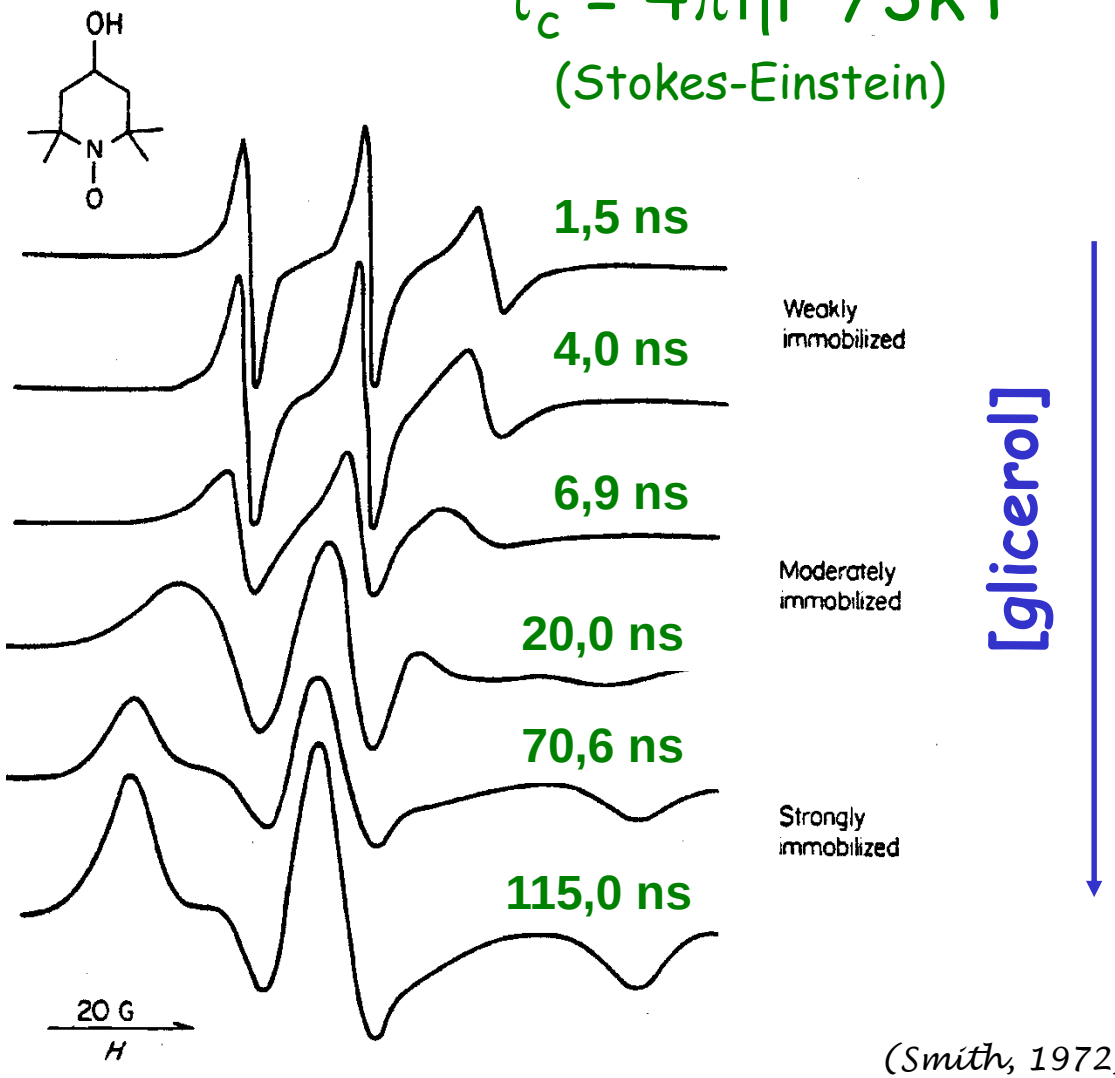
Processos de relaxação do sistema estão relacionados ao movimento dos centros paramagnéticos

Tempol em meios de diferentes viscosidades

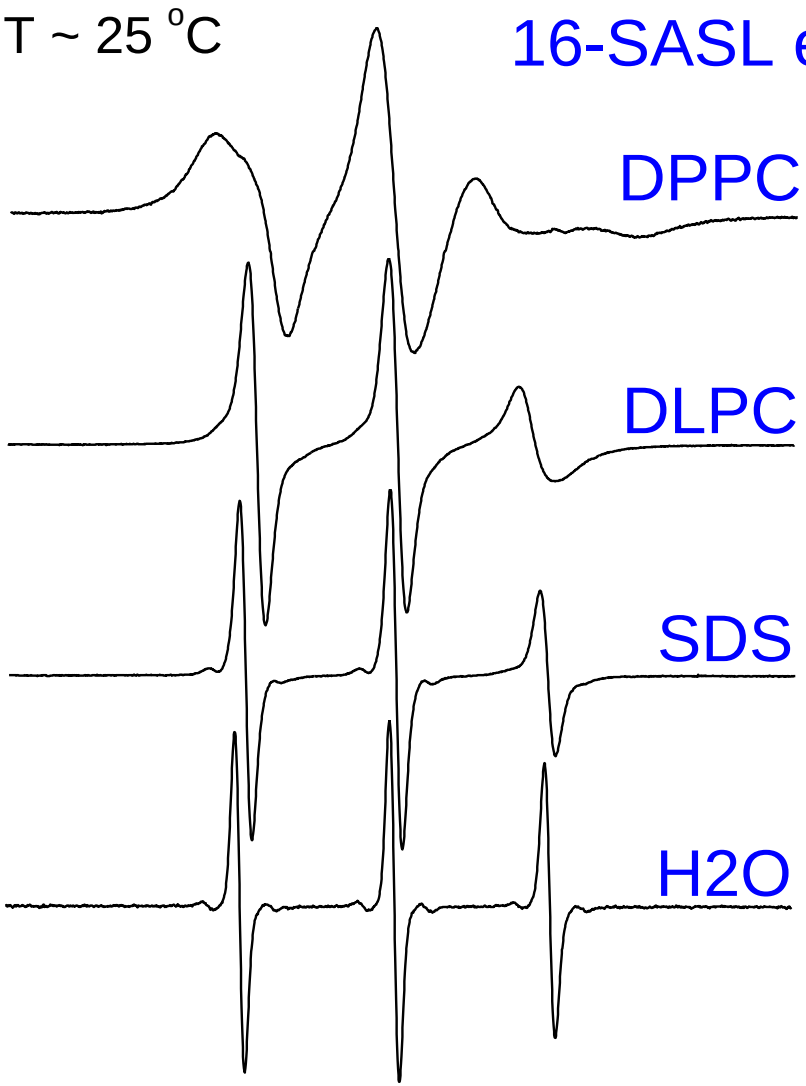
tempo de correlação rotacional

$$\tau_c = 4\pi\eta r^3/3kT$$

(Stokes-Einstein)



(Smith, 1972)



Em bicamada, abaixo da T_m do fosfolipídio

Em bicamada, acima da T_m do fosfolipídio

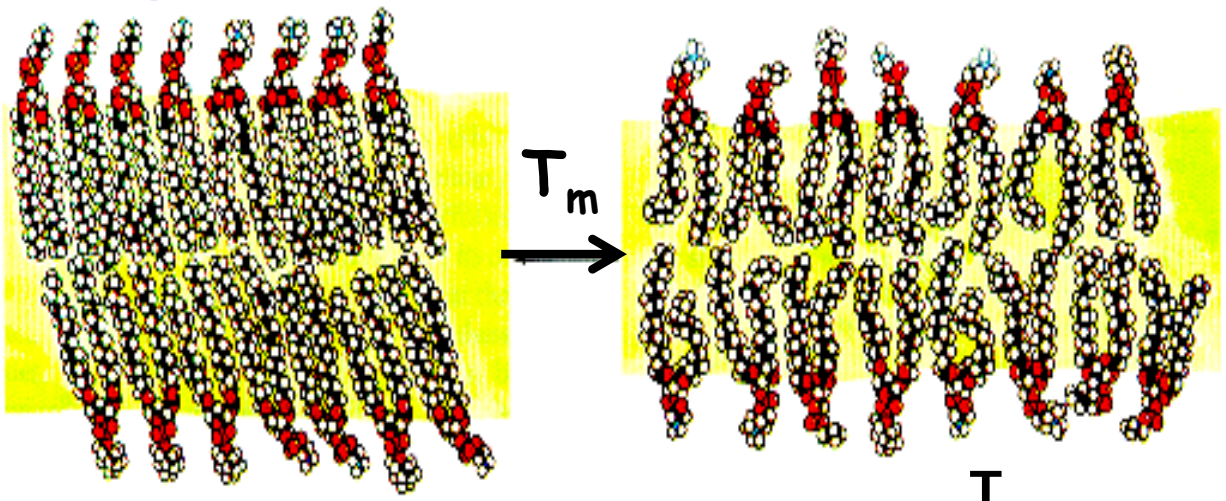
Em micelas

Livre, em solução

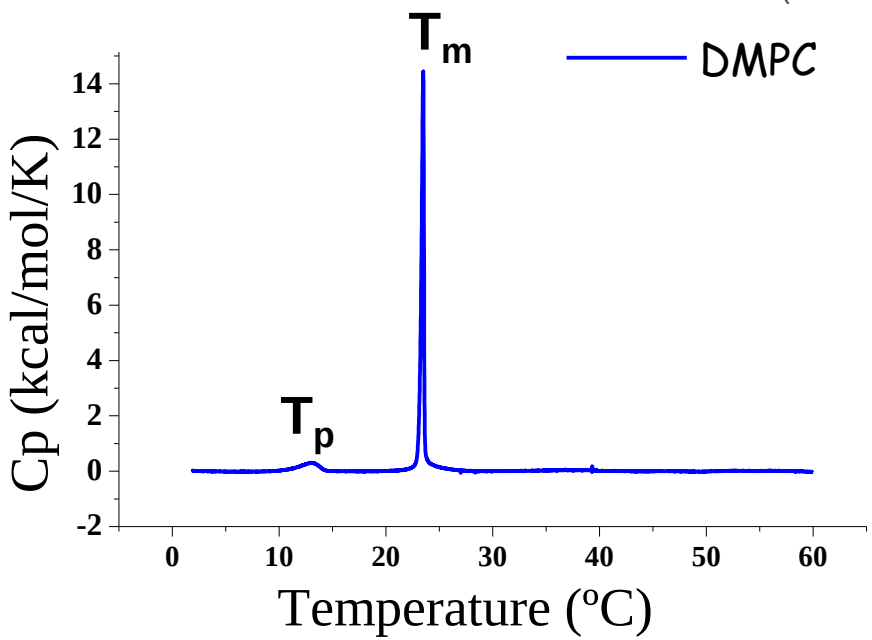
BRUKER

Muitas membranas lipídicas apresentam uma transição de fase gel/fluido

gel **fluido**

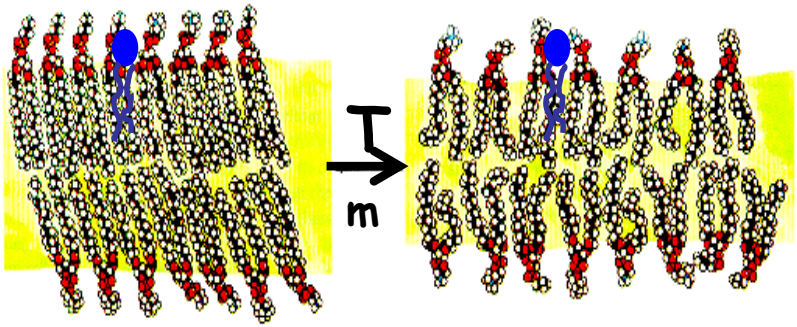


(Voet, Voet, 1990)



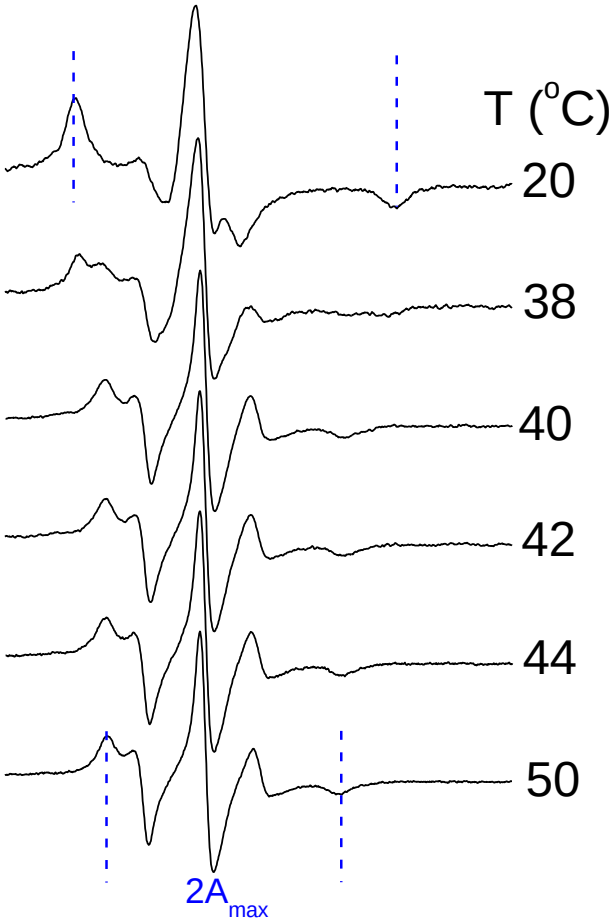
Podendo ser detectada por medidas de absorção de calor:

calorimetria



Caso um marcador de spin esteja incorporado à bicamada lipídica, seu espectro de RPE é sensível à transição gel-fluido do lipídio

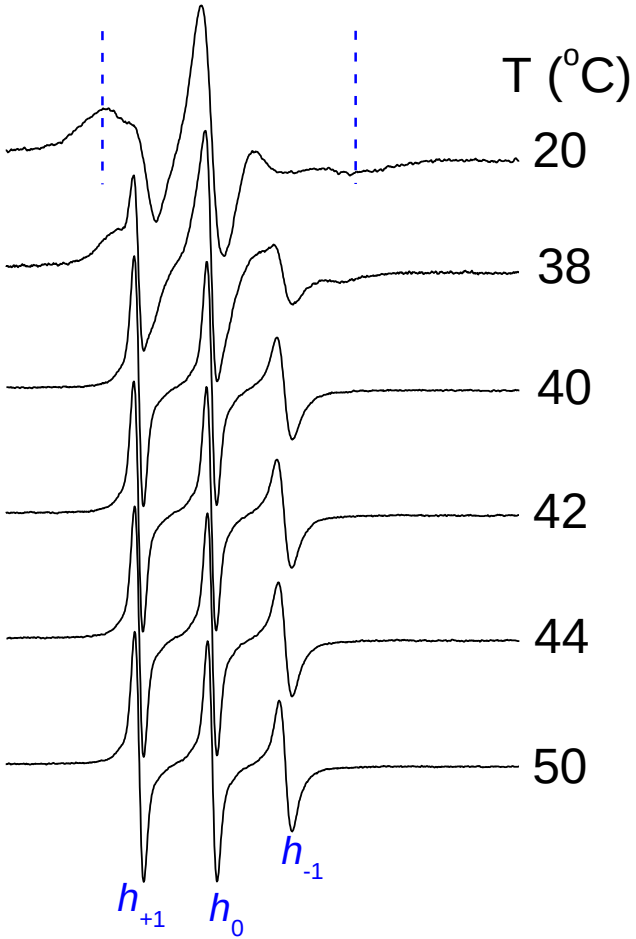
5-PCSL in DODAB



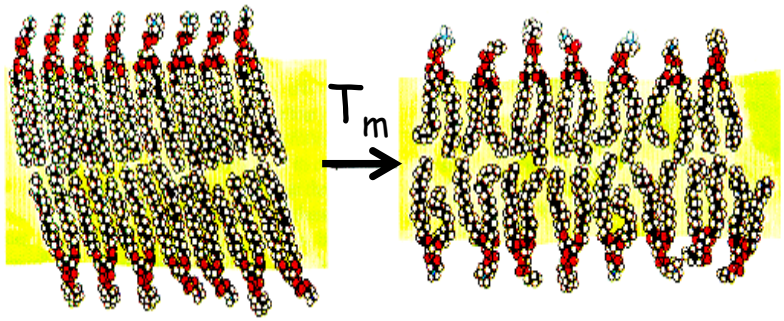
gel

fluida

16-PCSL in DODAB



Water penetration into lipid bilayers



(Voet and Voet, 1990)

H_2O crosses lipid bilayers

$[H_2O]$ inside the bilayer?

- Neutron diffraction
- X-ray diffraction
- NMR

→ "no substantial water beneath the C=O groups"

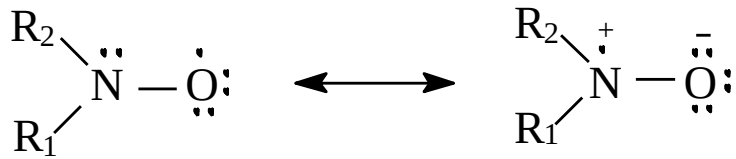
- Fluorescence probes
- ESR probes

→ "water penetration into the bilayer core"

Nitroxide spin labels are sensitive to environment polarity

(Griffith, Dehlinger, Van, 1974)

non polar medium



polar medium

unpaired electron density mostly localized on the O-atom

⬇

low a_o

unpaired electron density mostly localized on the N-atom

⬇

high a_o

- polar solvents
- electron transfer complex
- electric fields
- **H_2O hydrogen bonding**

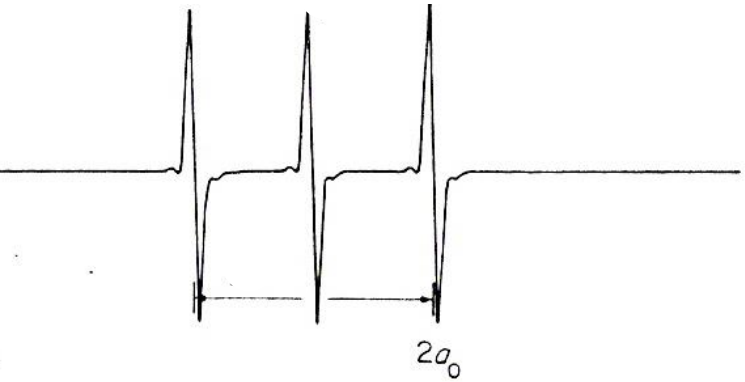
$[H_2O]$ increases $\Rightarrow$ higher a_o

Podemos, então, medir

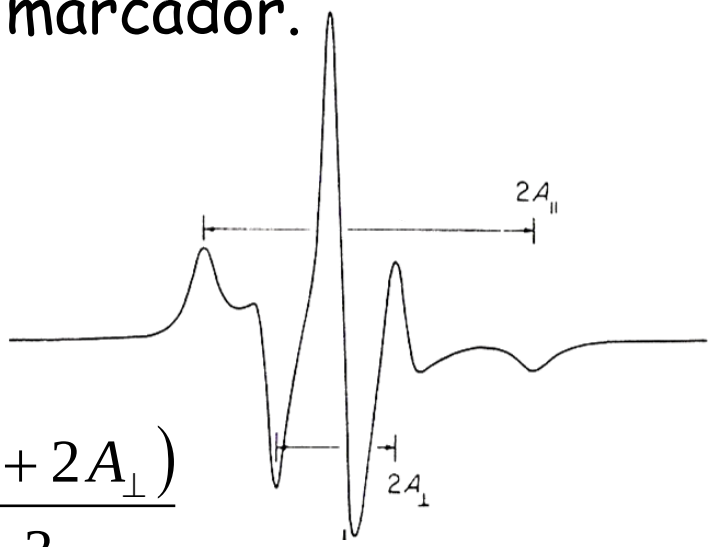
"polaridade" do meio ou "hidratação" da sonda:

probabilidade de formação de ligações de H com o grupo –NO do marcador de spin

Quanto maior o desdobramento hiperfino isotrópico (a_0), maior é a hidratação do micro-ambiente onde se encontra o grupo –NO do marcador.



$$a_0 = \frac{\left(A_{//} + 2A_{\perp}\right)}{3}$$

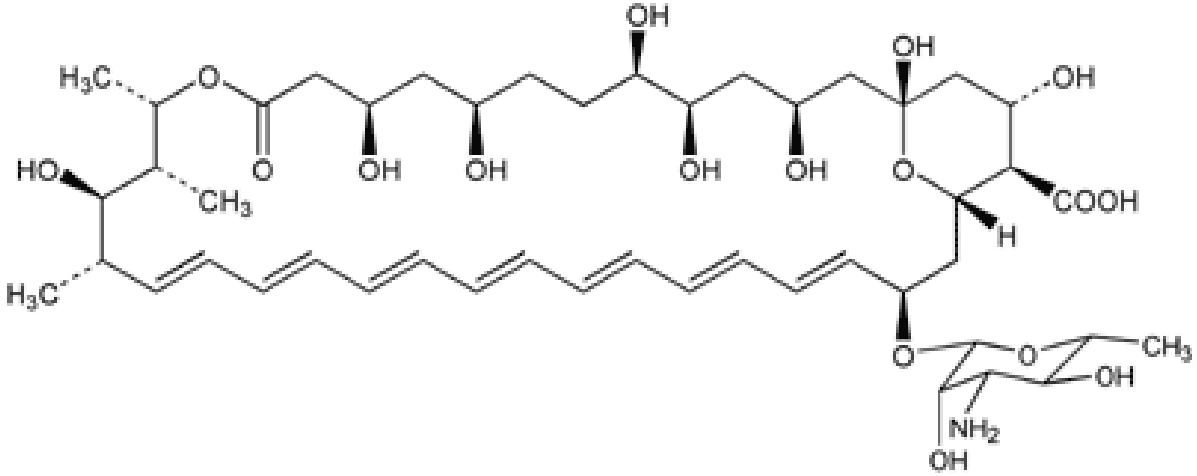


Tiago R. Oliveira

The interaction of the polyene antibiotic **amphotericin B** with **DODAB** aggregates: a structural characterization

(Oliveira, Benatti, Lamy, BBA 1808, 2629-2637, 2011)

Amphotericin B



- One of the oldest and most widely used drugs for the treatment of systemic fungal infections.

- Very low solubility in aqueous medium: forms large aggregates.

- Highly toxic: meanly hemolysis and nephrotoxicity, the latter possibly related to large AmB aggregates.

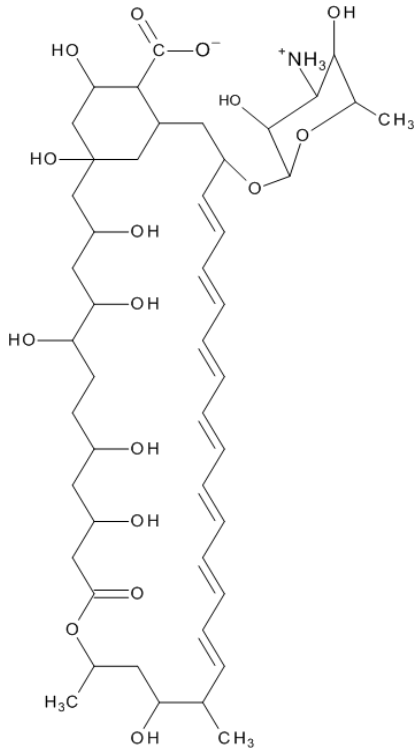
- Fungizone, a commercial formulation: AmB/deoxycholate mixed micelle.

Fungizone, AmB/deoxycholate mixed micelle: formation of large AmB aggregates upon dilution.

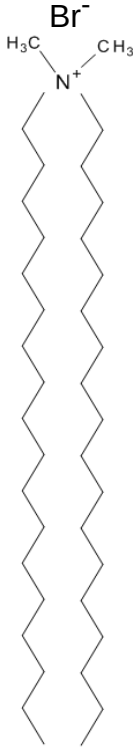
(Lamy-Freund, Ferreira, Schreier, BBA 1989)

Possibly lower toxicity with **AmB monomers**: several formulations have been tried.

AmB



DODAB



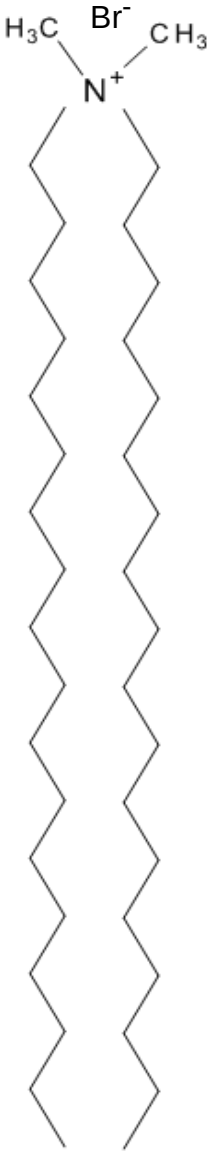
In sonicated **DODAB** dispersion, **AmB** was said to be **monomeric** and **less nephrotoxic.**

(Vieira, Carmona-Ribeiro, JCI, 2001;
Lincopan, Mamizuka, Carmona-Ribeiro, JAC, 2005)

DODAB is a synthetic cationic amphiphile

AmB-DODAB: a possible pharmaceutical formulation

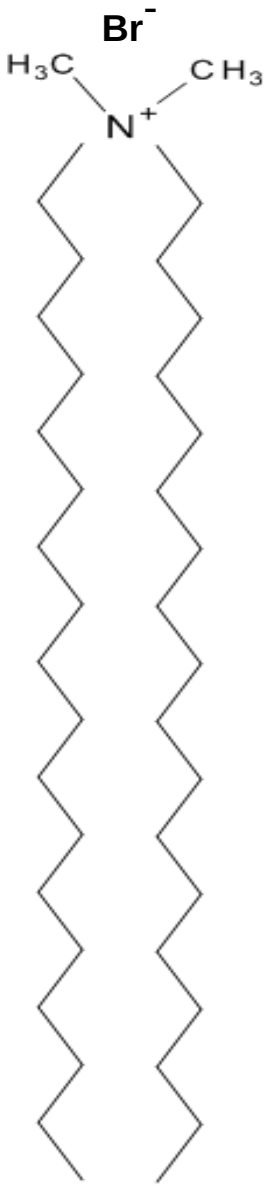
DODAB



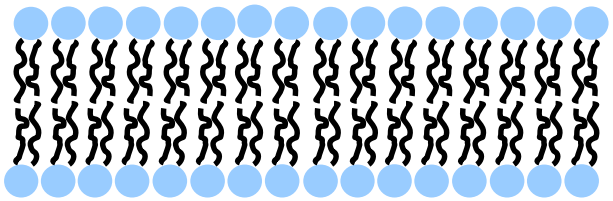
DODAB, a cationic amphiphile, has been found to be toxic, but has also been found to be an effective adjuvant, enhancing the level of immune response.*

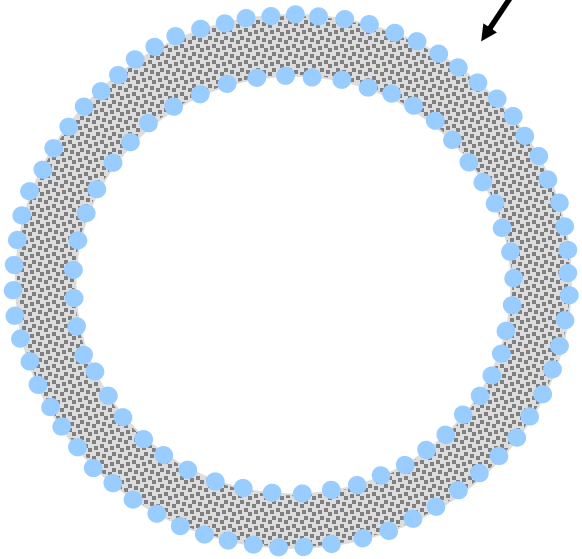
**Gall, Immunology 11, 369, 1966*
Holten-Andersen et al. , Infect. Immun. 72, 1608, 2004
…

DODAB

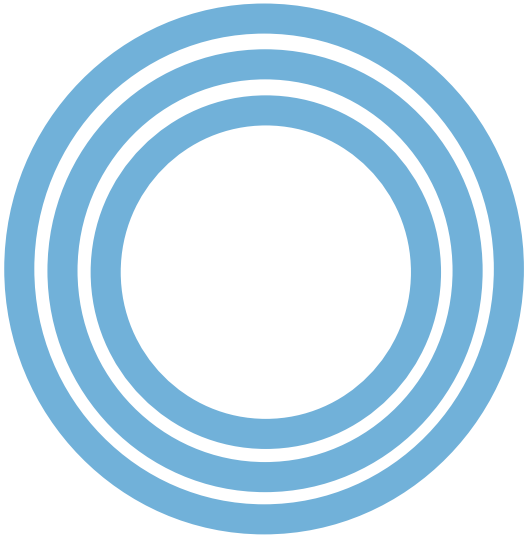


DODAB aqueous dispersion: highly polydisperse, with very large unilamellar vesicles



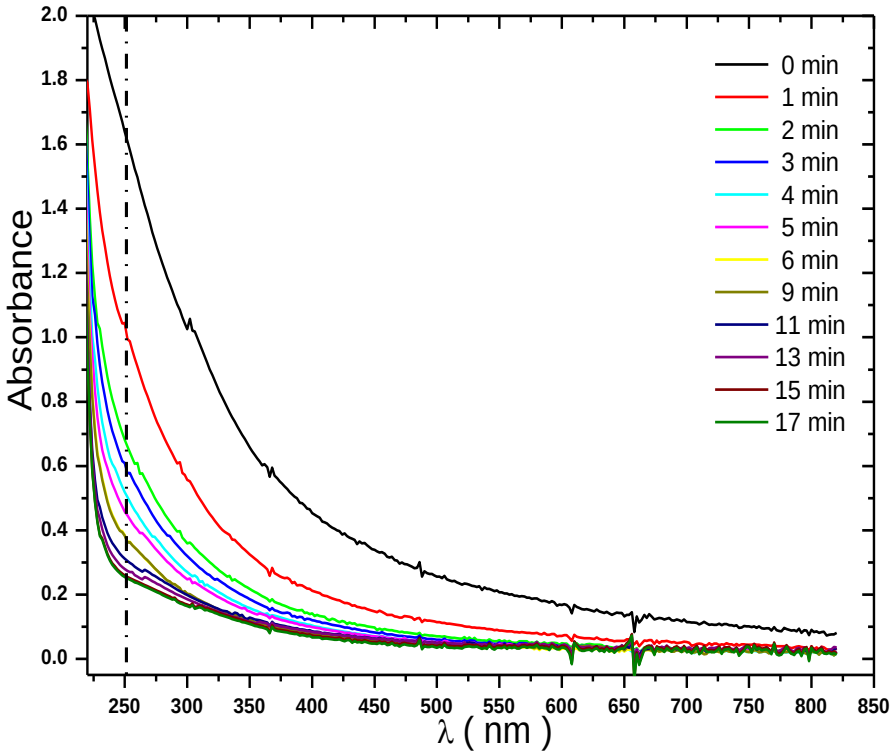


unilamellar vesicles

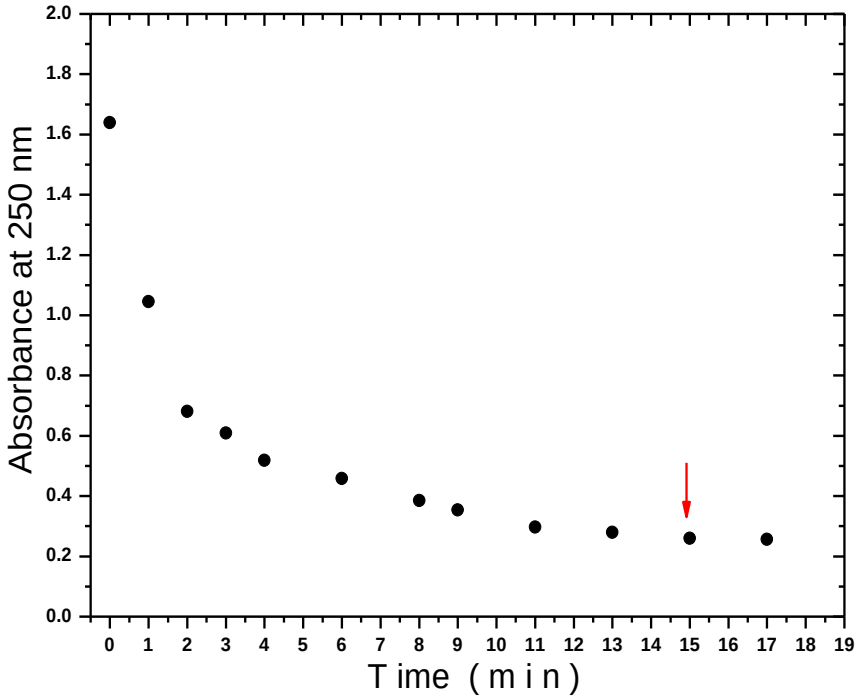


multilamellar vesicles

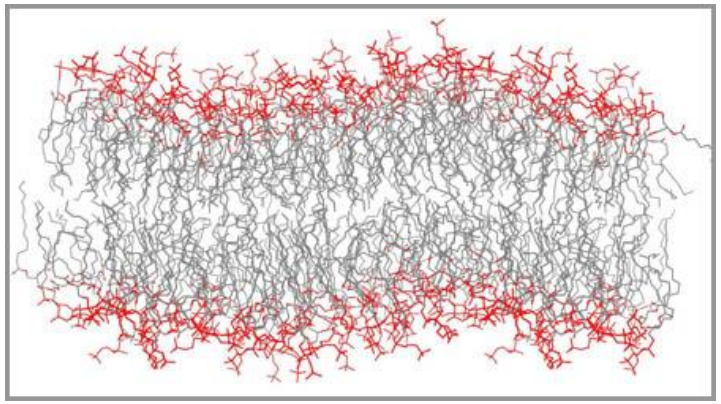
turbidity



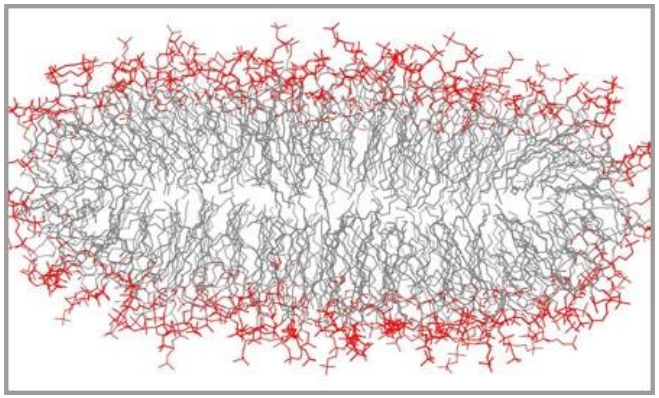
Sonicated DODAB^S



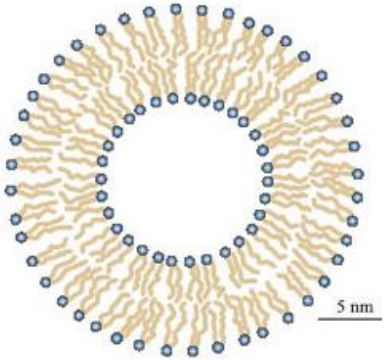
Sonication leads to bilayer fragments (bicelles) and/or very small vesicles



bicelles



very small vesicles



(http://www.chemistry.emory.edu/faculty/kindt/molecular.htm)

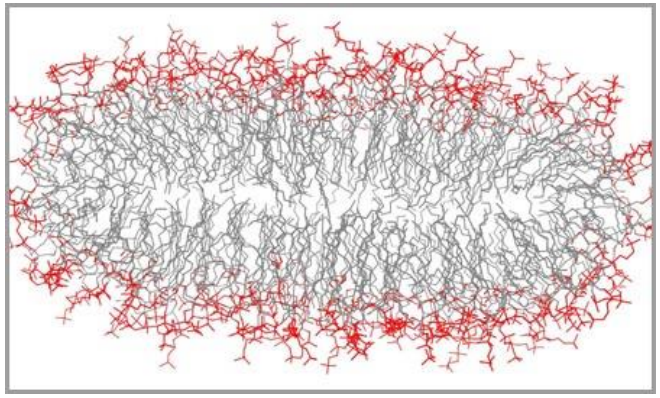
Bilayer fragments (bicelles) in sonicated DODAB dispersions ($DODAB^S$)

Pansu, Arrio, Roncin, Faure, J. Phys. Chem., 1990 (DODAC)

Andersson, Hammarstrom, Edwards, J. Phys. Chem., 1995

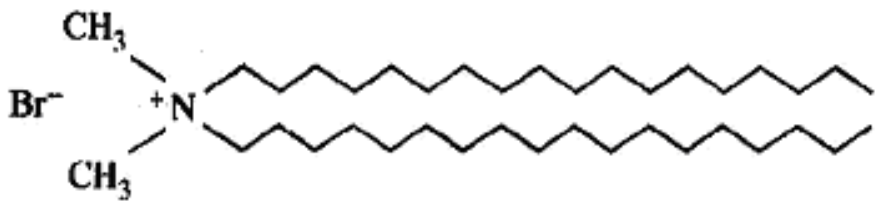
→ Benatti, Feitosa, Fernandez, Lamy-Freund, Chem. Phys. Lipids, 2001

Cocquyt, Olsson, Olofsson, Van der Meeren, Langmuir, 2004

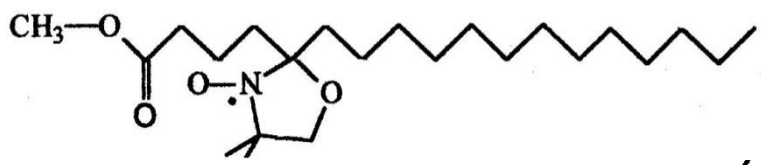


Spin labels incorporated into **DODAB** aggregates to investigate their structure

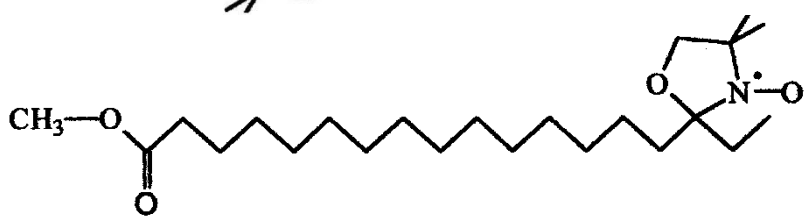
DODAB

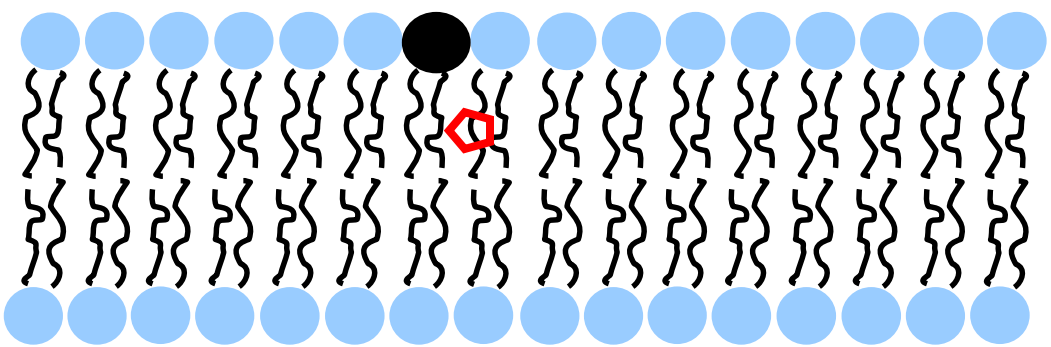


5-MESL

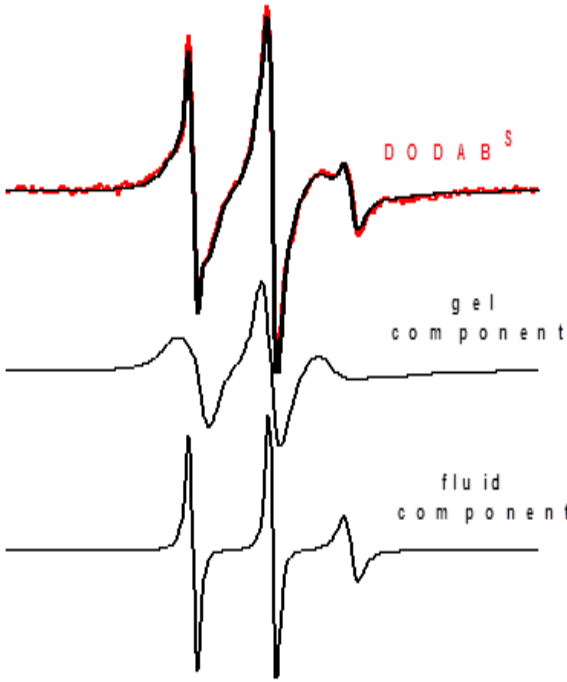


16-MESL





16-MESL in DODAB^S



Computer simulations of the ESR spectra

(Freed, 1976; Schneider and Freed, 1989; Budil et al., 1996)

Density Matrix formalism

Quantum mechanical Liouville equation of motion

$$\frac{\delta\rho}{\delta t} = -i\,[H(t), \rho]$$

$H(\Omega) = H_o + H_1(\Omega)$

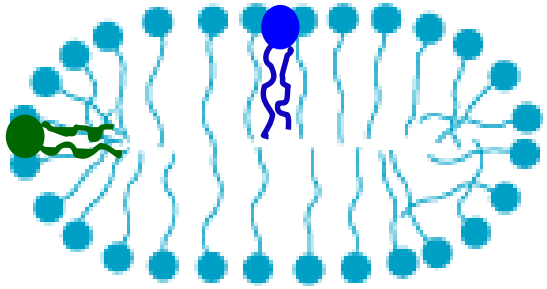
H_o = Zeeman interaction + Hyperfine interaction

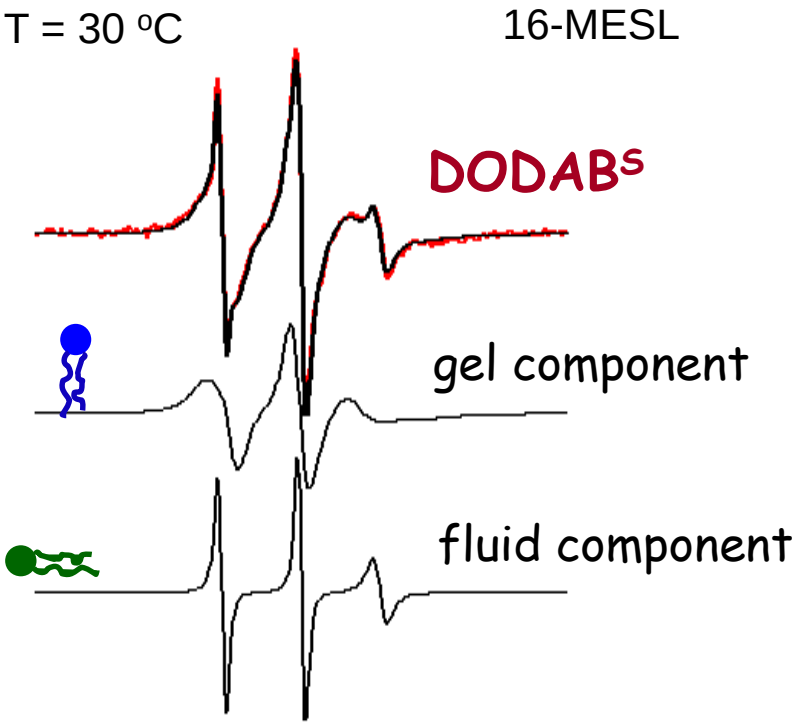
$H_1(\Omega)$ = perturbation depending on the random variable Ω

Stochastic Liouville equation of motion

16-MESL in sonicated DODAB, below DODAB T_m

It is possible to analyze the two components of the ESR signal separately





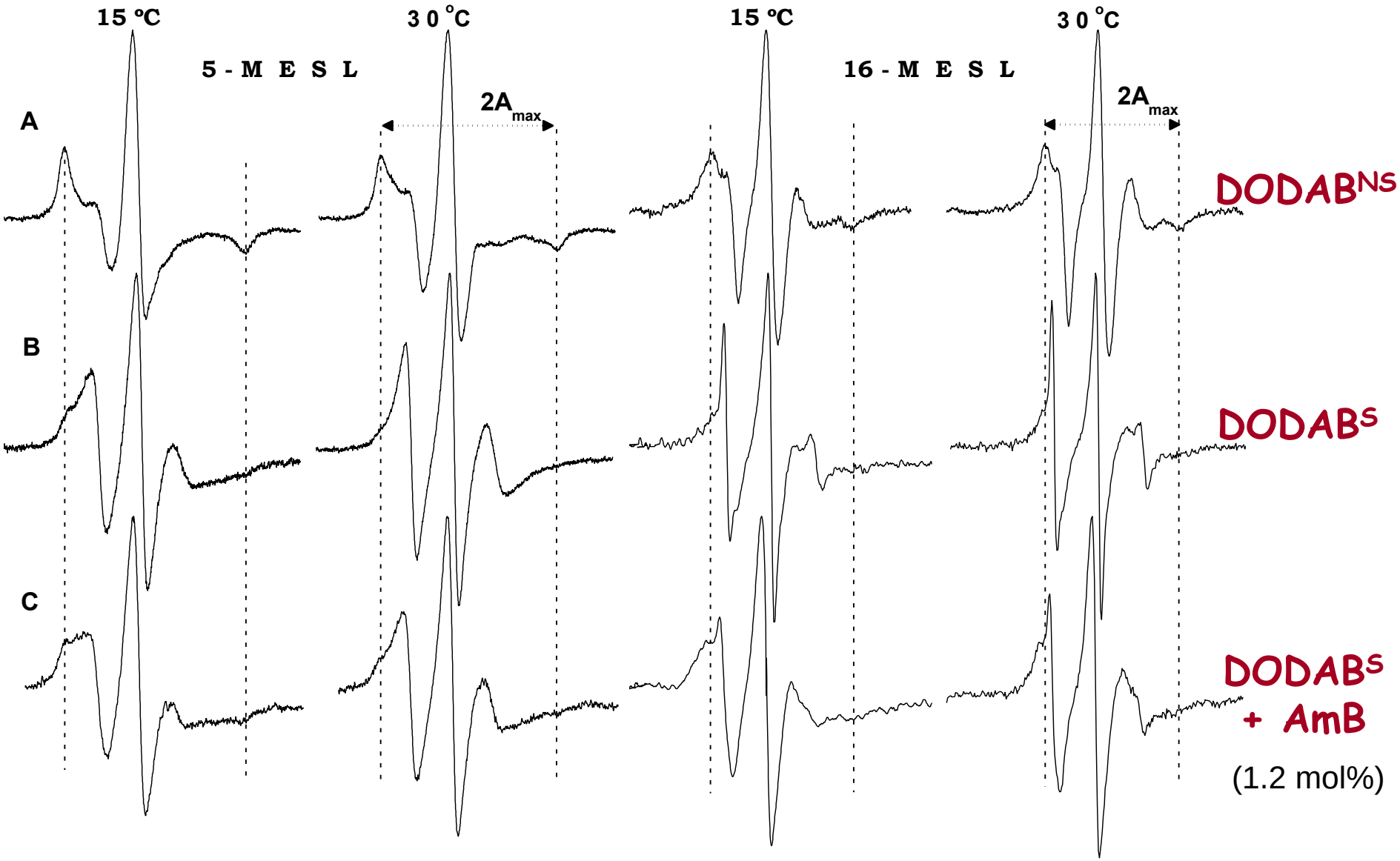
So, with ESR, we can separately monitor the two environments in a bicelle: the bilayer and the edges

What happens when we add AmB to DODAB?

What can spin labels tell us?

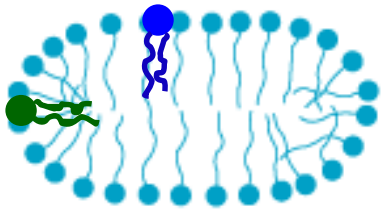
(Ribeiro, Benatti, Lamy, BBA, 2011)

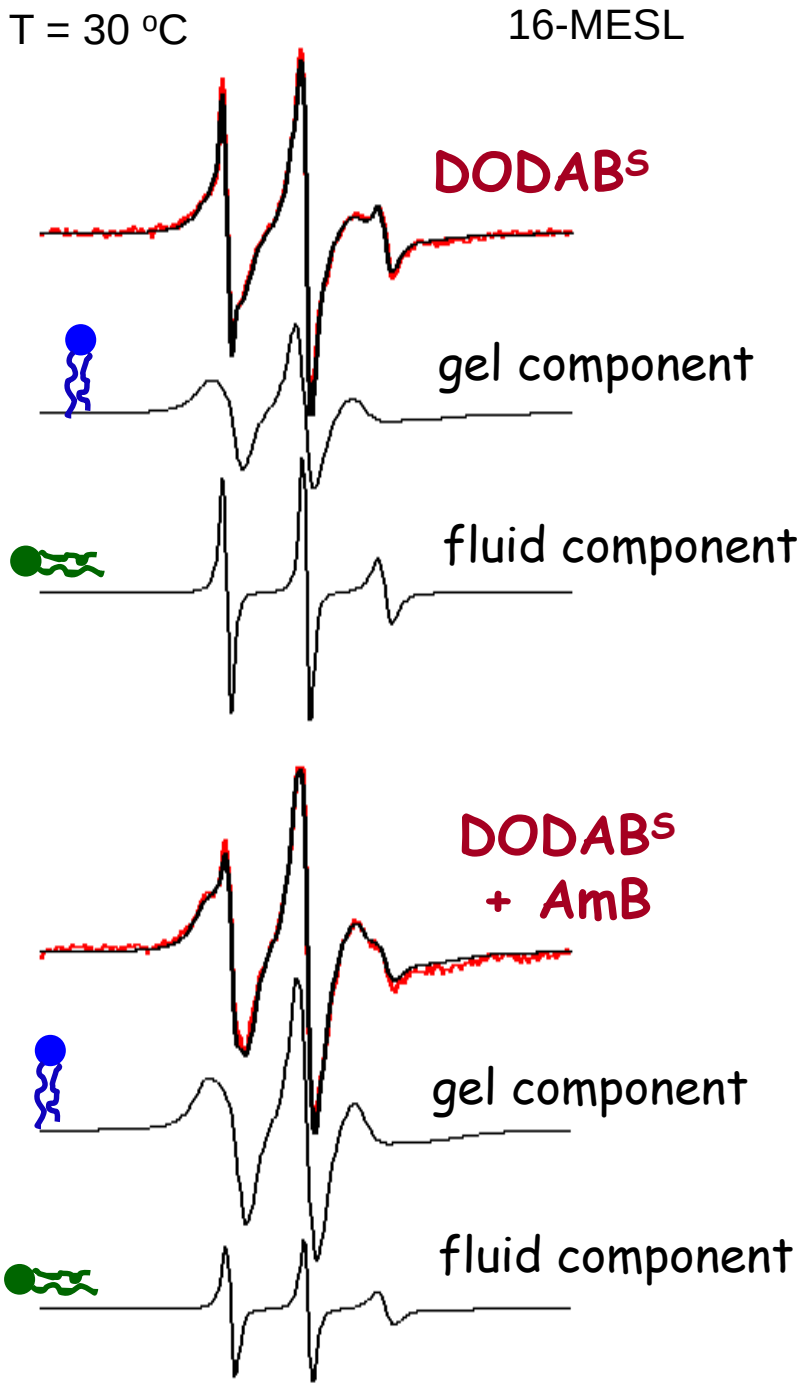
Below DODAB T_m



It is possible to analyze the two components of the ESR signal separately

It is possible to know the effect of AmB in each component, hence at the two different bicelle domains

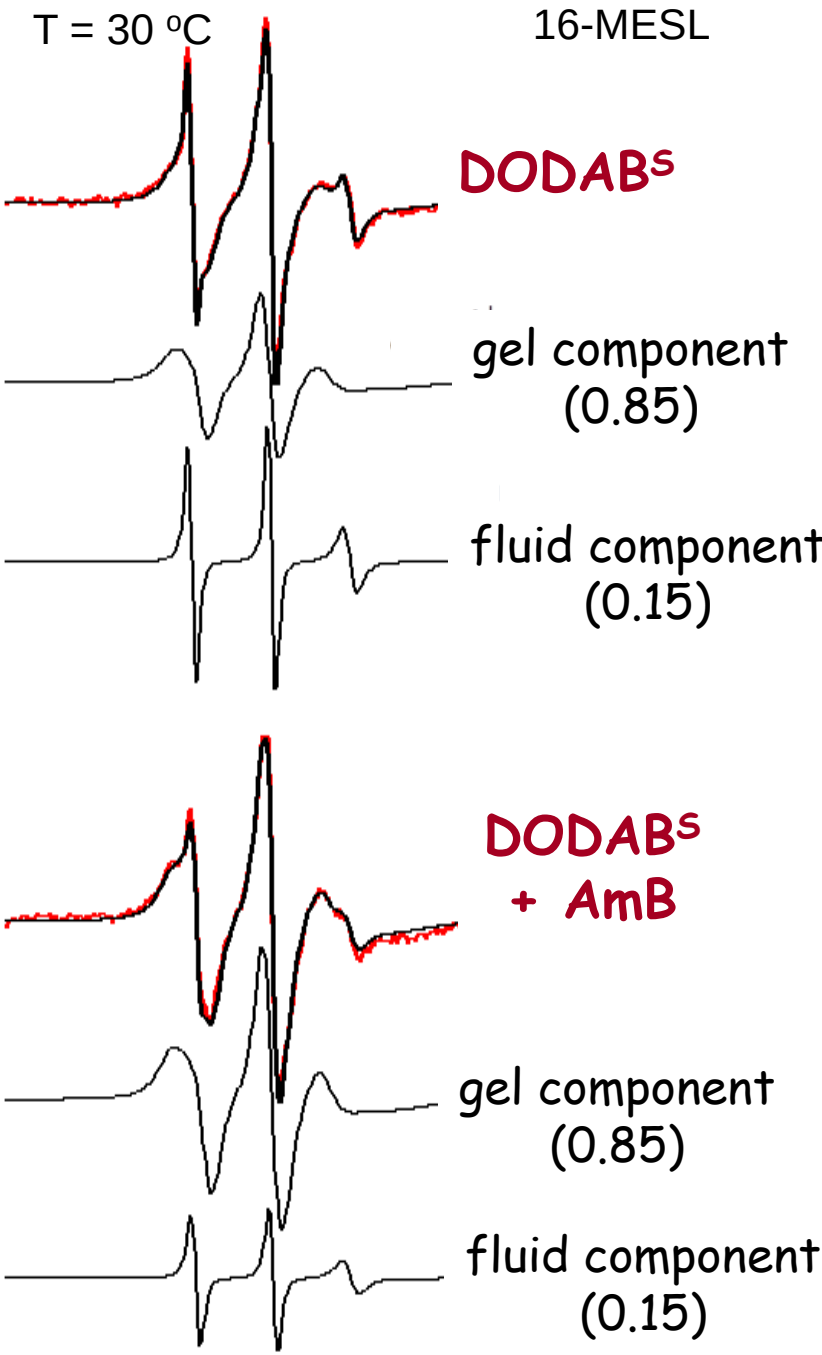




Binding of AmB does not alter relative fractions of gel and fluid domains in DODAB aggregates:

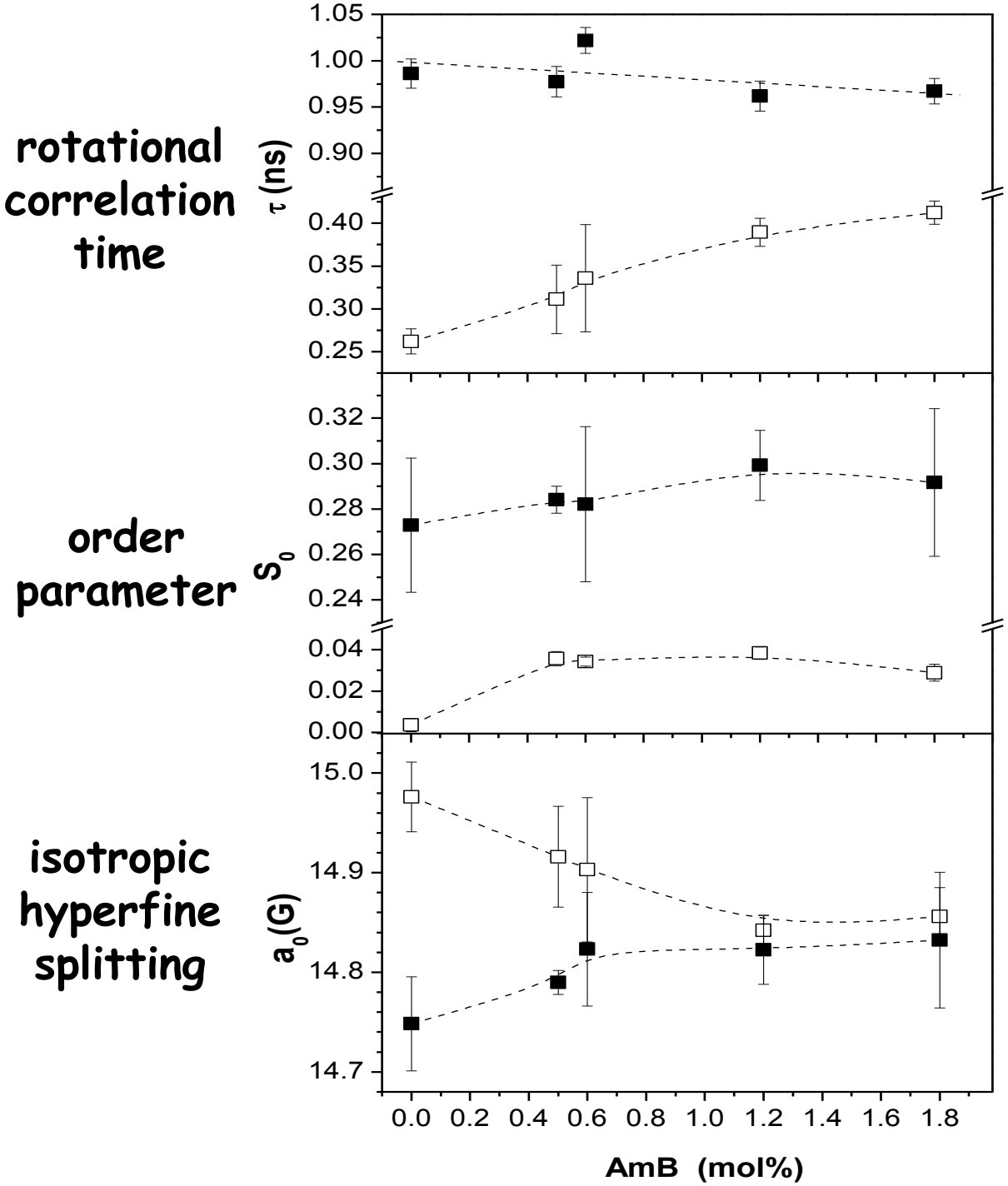
AmB does not rearrange the aggregates

The effect of AmB on the gel and fluid domains can be analyzed separately



■ gel-component
□ fluid-component

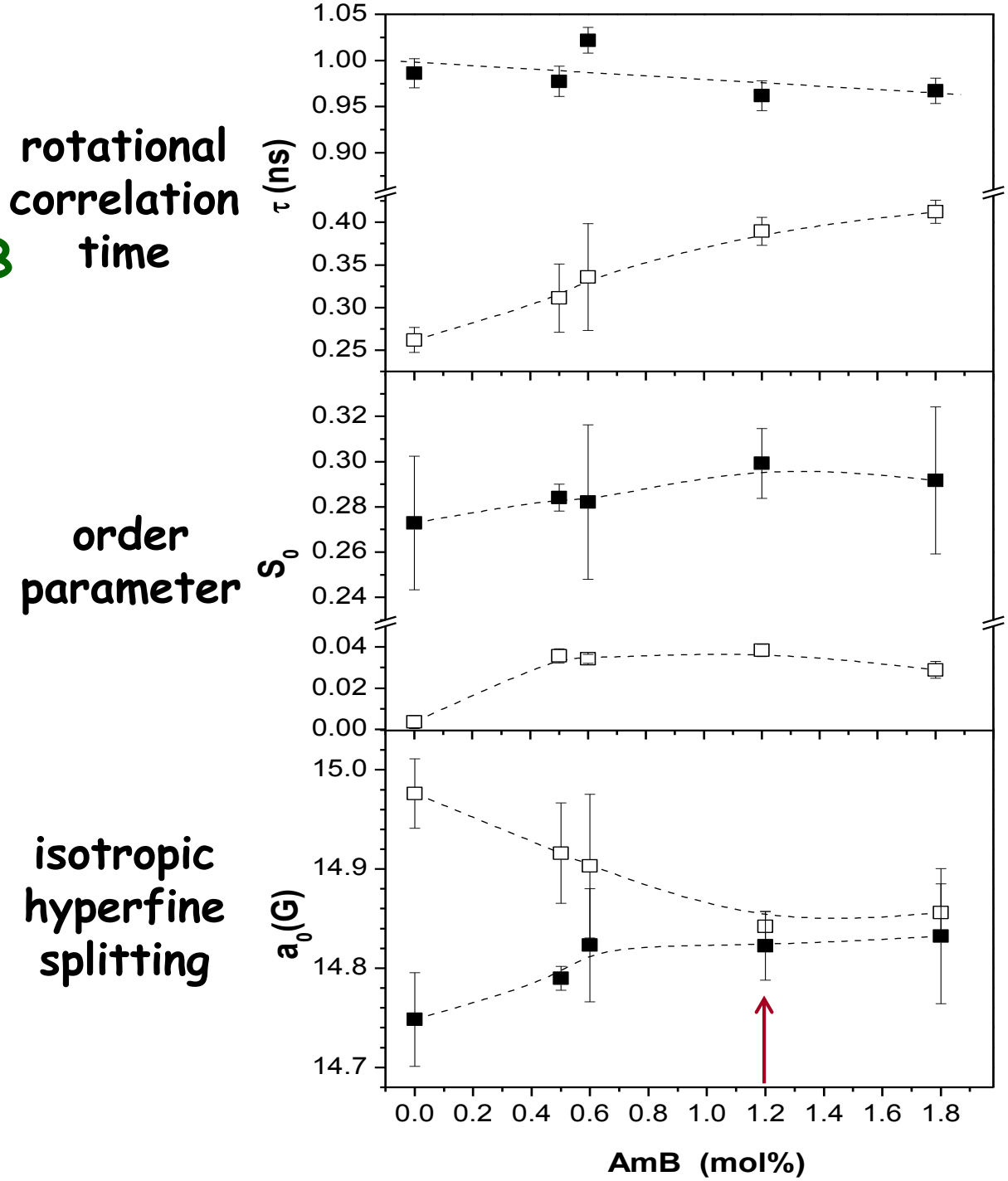
Spectral changes were found to be mostly due to alterations on the fluid domain



■ gel-component
□ fluid-component

AmB rigidifies DODAB bicelle fluid domains: increases τ and S_0. And decreases the domain polarity.

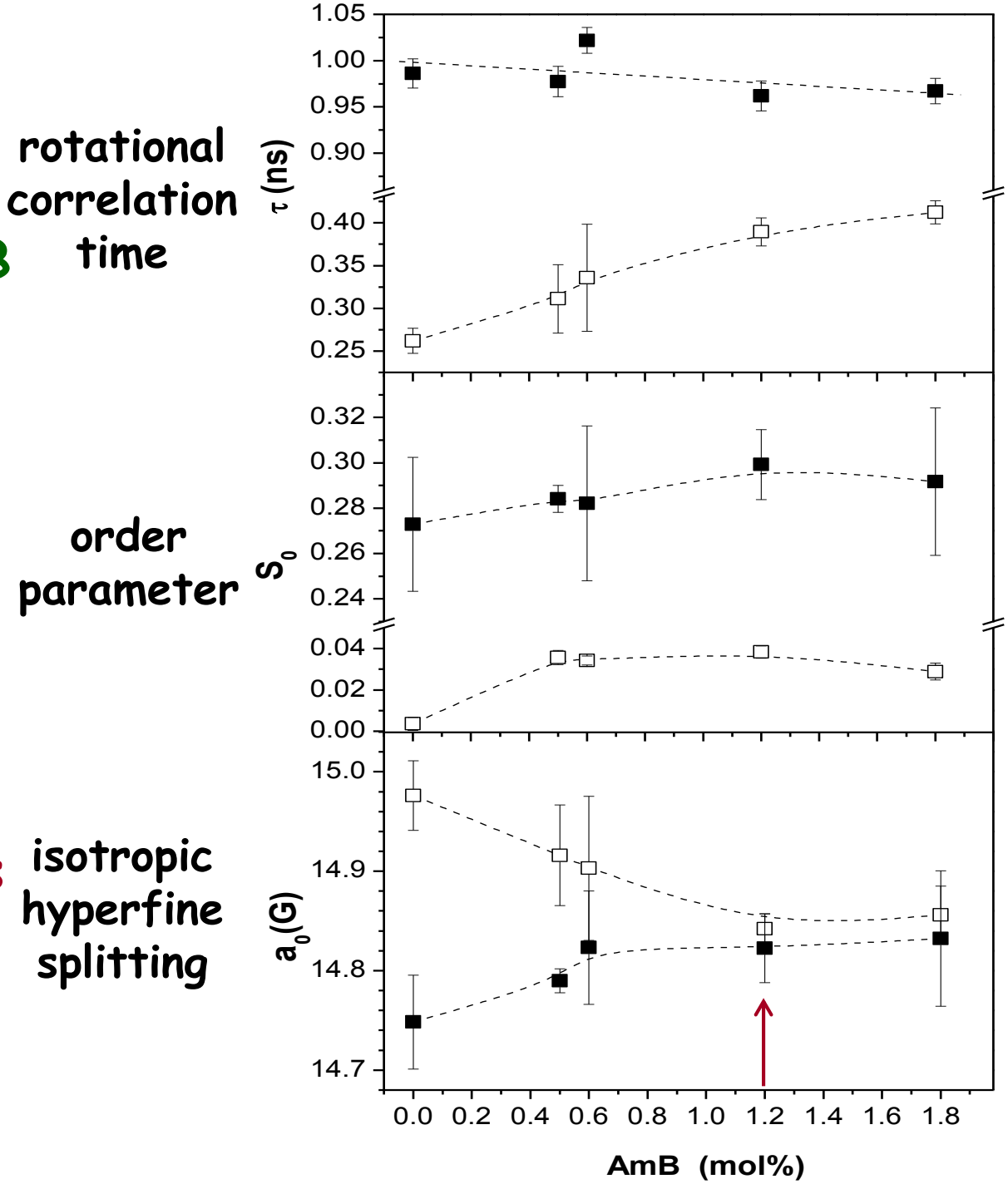
1.2 mol% is a limit AmB concentration: also found limit for bound **monomeric** AmB (optical parameter)



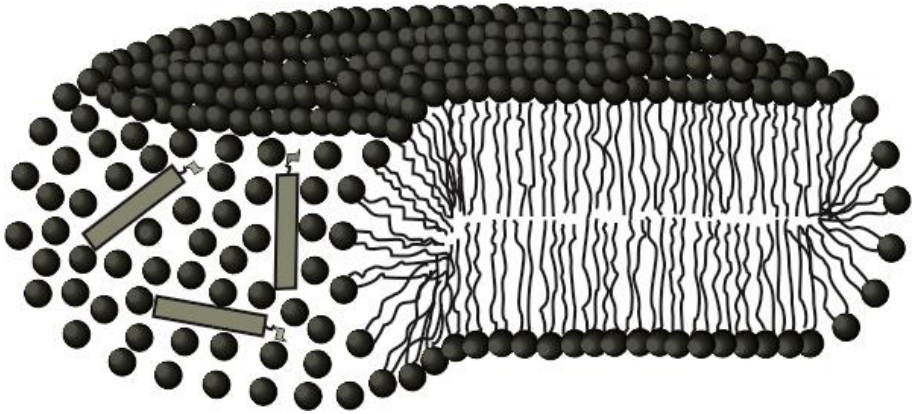
■ gel-component
□ fluid-component

AmB rigidifies DODAB bicelle fluid domains: increases τ and S_0. And decreases the domain polarity.

Aggregated AmB does not seem to be bound to DODAB

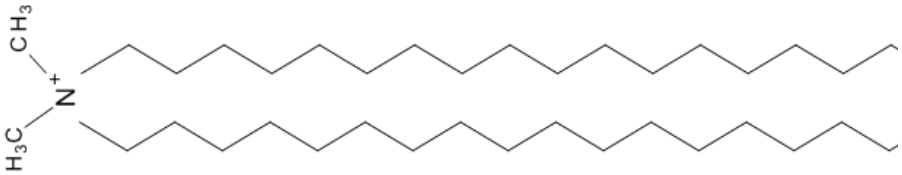


Schematic representation of AmB bound to a DODAB bicelle

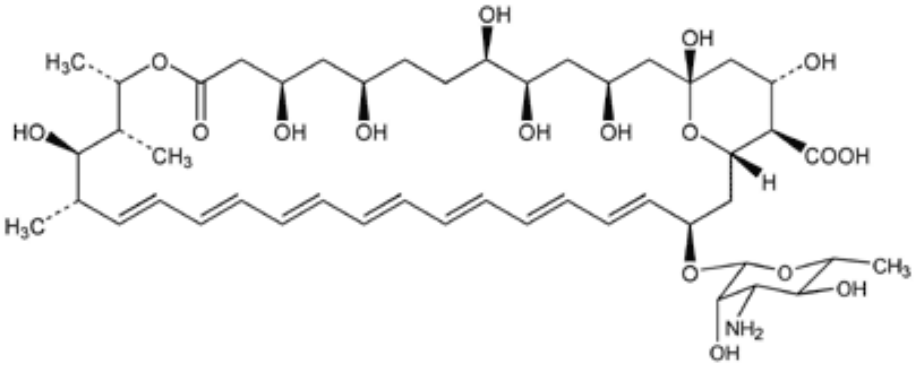


1.2 mol% bound AmB per DODAB

DODAB



AmB



Main conclusions

- A new optical parameter for the relative quantitation of amphiphile-bound **monomeric** AmB

- Calculation of maximum monomeric AmB bound to bicelles and vesicles

- Optical absorption, DSC and ESR show AmB aggregates are in water in DODAB dispersions

- **ESR spectra analysis clearly shows that AmB binds at DODAB bicelle edges**

- **ESR analysis discussed here could be used with other drugs or other AmB carriers**

Principles of Magnetic Resonance – Slichter

Electron Paramagnetic Resonance – Weil, Bolton, Wertz

Principles of Electron Spin Resonance – Atherton

Magnetic Resonance of Biomolecules – Knowles, Marsh, Rattle

Spin Labeling, Theory and Applications – Berliner (Ed.)

Biological Magnetic Resonance, Vol8 – Spin Labeling, Theory and Applications – Berliner, Reuben (Eds.)