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SPIN-PROBE SPIN-LABEL STUDIES OF SODIUM DODECYL SULFATE MICELLES

by

Barney L. Bales[2,5], Marilia P. Linhares[1,3] and

Maria Conceição Barbosa Lima[1,4]

[1]Centro Brasileiro de Pesquisas Físicas - CBPF/CNPq
Rua Dr. Xavier Sigaud, 150
22290 - Rio de Janeiro, RJ - Brasil

[2]Departamento de Física - PUC-RJ

[3]Instituto de Física - UFRJ

[4]Instituto de Física- UERJ - RJ

[5]On leave from the Department of Physics and Astronomy,
California State University at Northbridge, Ca. USA.

ABSTRACT

The magnetic interactions of paramagnetic ions with nitroxide labels in micelles was determined by using spin-label, spin-probe technique. The results from EPR spectrometer shows that the line broadening is dominated by exchange interactions. This results points to a rapid averaging of dieletric environments.

Key-words: Magnetic interactions-Micelles-Spin-Label, Spin-Probe

INTRODUCTION

There has been a tremendous resurgence of interest in organized chemical systems such as micelles in recent years. Of particular interest is the ability of these systems to catalyze a wide spectrum of chemical reactions, therefore, information on location and accessibility of a substrate solubilized in a micelle is important.

A modest amount of work employing solubilized paramagnetic molecules studied by electron spin resonance spectroscopy (ESR) has appeared over the past several years. [2-11] Most of this work has employed nitroxide spin labels, taking advantage of their stability and simple spectra, to investigate rotational and translational motion, polarity and partioning between the micellular and aqueous environments. A potentially powerful method to study the accessibility of paramagnetic ions to a solubilized nitroxide radical has been mentioned [5] but not developed.

The magnetic interactions of paramagnetic ions with nitroxide labels, under suitable conditions, can lead to useful information and forms part of a general experimental strategy that has come to be called the spin-label spin-probe technique.

Spin-label Spin-probe Technique

In principle, any two paramagnetic particles may be used but, in practice, the "label" is almost always a nitroxide free radical and the probe is a paramagnetic ion. A full discussion of the technique at its present level of development is given by Hyde [12], to which the reader is directed for details. Briefly, the probe interacts with the label via magnetic dipolar and spin exchange interactions [12,13] these interactions being manifested in a broadening of the ESR lines of the label in the motional narrowing region. Both interactions lead to a broadening that is proportional to the probe concentration in homogeneous systems but the physical interpretation depends critically on an understanding of the relative importance of the two interactions. This is so because relative translational motion between the label and the probe affects the two interactions in the opposite way: increased relative motion tends to lead to an increase in collision frequency, which increases exchange broadening while

this same motion tends to average the dipolar interaction and therefore decrease the line broadening. For example, in a model of Brownian diffusion in a homogeneous liquid involking the Stokes-Einstein model, $\Delta Hpp - \Delta H^{o}pp = (A_1 T/\eta + A_2 \eta/T)C$ where ΔHpp is the peak-to-peak linewidth, in derivative form, in the presence, and $\Delta H^{o}pp$ in the absence, of probes of molar concentration C. The absolute temperature is T, the viscosity is η and the constants A_1 and A_2 contain magnetic and geometric parameters as well as other constants

The first term is due to exchange and the second to dipolar interactions. In the study of homogeneous liquids, in which the simple diffusion model seems to work pretty well[13], experiments at variable temperature or viscosty or both serve to separate the two interactions.

This experimental strategy of variable temperature studies can not be relied upon to effect the separation in inhomogeneous systems such as micelles, membranes, and biological macromolecules because the assumptions in the simple model are suspect. Therefore, separation of exchange and dipolar interactions, always a problem, is more severe in the study of micelles and we suspect that it is for this very reason that the method has not yet been extensively used in these systems.

The purpose of the present paper is to describe experiments to separate the exchange and dipolar interactions at constant temperature. Much to our surprise, these measurements show that, in the case of fatty acid spin labelled sodium dodecyl sulfate (SDS) micelles at room temperature, the concentration-dependent linebroadening due to paramagnetic cations is dominated by the exchange interaction. This is surprising because in the case of other nitroxide labels, the opposite has been reported to be true[5,11].

THEORY

The theory of exchange and dipolar contribution to concentration broadening of ESR lines in the motional narrowing region has been worked out by several authors[13-17]. We draw heavily on the review by Molin et al[13].

Electron spin exchange in liquids between a label and a probe broadens the lines of the label spectrum due, essentially, to a lifetime limiting process brought about by collisions between the two paramagnetic particles. In the so-called strong collision limit, invariably observed in low viscosity liquids, the exchange frequency, W_{ex}, is equal to the collision frequency, T_2^{-1}, which in the brownian diffusion limit is

$$T_2^{-1} = 4\Pi d\ DN \qquad (1)$$

Where T_2 is the mean time between collisions, d is the effective distance for spin exchange, D is the relative translation diffusion coefficient between the label and the probe and N is the probe density. The line broadening, is given by

$$\Delta Hpp - \Delta H^{0}pp = 2W_{EX} \,/\, \gamma\sqrt{3} = 2\ T_2^{-1} \,/\, \gamma\sqrt{3} \qquad (2)$$

Note that Eq. (2) does not have a statistical factor found in similar expressions for spin exchange between like particles[13]. Thus, the line broadening,

$$\Delta Hpp - \Delta H^{0}pp = [8\Pi dD/\gamma\sqrt{3}\,]\,N \qquad (3)$$

Where the quantity in brackets is not a function of the specifc magnetic properties of the probe.

In liquids, concentration broadening due to the dipolar interaction is due to the not-quite complete averaging of this interaction by either translational motion or the spin lattice relaxation of the probe. Which of these two time dependent phenomena is effective in a particular experiment depends on the relation ship between the relaxation time and the characteristic time of translational diffusion. If diffusion dominates,

$$\Delta Hpp - \Delta H^{0}pp = [(^{32\Pi}/_{81\sqrt{3}\gamma})\gamma^{2}\ \gamma_{\rho}^{2}\ h^{2}\ Sp(Sp+1)(dD)^{-1}]N \qquad (4)$$

where the subscript p refers to the probe. If, on the other hand, spin relaxation of the probe dominates,

$$\Delta Hpp - \Delta H^{0}pp = [(^{32\Pi}/_{9\sqrt{3}\gamma})\ \gamma^{2}\ \gamma_{\rho}^{2}\ h^{2}\ Sp(Sp+1)t_{1p}]\ N \qquad (5)$$

where T_{1p} is the spin lattice relaxation of the probe.

EXPERIMENTAL STRATEGY

According to Eqs. (3), (4), and (5) the label line broadening depends on the probe density in the same way, thus to distinguish mechanisms we are forced to examine the parameters inside the square brackets in the three equations, at constant temperature. Our strategy is to use a series of paramagnetic probes in which the parameters $\gamma_p^2\ S_p\ (Sp + 1)$ and T_{1p} cover a wide range. See Table 1 which actually gives the experimental value of the magnetic moment squared, μ^2_{eff}, instead of $\hbar^2\ \gamma_p^2\ S_p\ (S_p + 1)$. The basic assumption is that all of these cations bind to the micelles with resident times large compared with any of the ESR characteristic times. This is a good assumption as shown by NMR studies[18]. The square bracket in Eq. (3) is expected to be very nearly constant as a function of type of cation while eqs (4) and (5) would vary as μ^2_{eff}. Since translational diffusion times to average dipolar coupling are of the order 10^{-10}S, one would expect Eq. (4) to apply to ions like Mn^{2+}, Cu^{2+}, and Gd^{3+}, while Eq. (5) would apply for Ni^{2+} and Co^{2+}, ions like Fe^{2+} and Fe^{3+} would be intermediate.

In addition to paramagnetic cations, we also bind diamagnetic ions to the micelle surface is a control to show line broadening is produced only by magnetic interaction. In this work, we maintain ion concentrations at an average of less than one ion per micelle in order to minimize the salt effect on structure. This is orders of magnetude less than salt concentrations found to alter the structure[1].

We use 0.1M SDS solutions which, with an average of 62 molecules per micelle, produces 1.6mM concentration of micelles. The label concentration is limited to 0.2mM, labelling, on average, one-eight of the micelles and leading, according to the Poisson distribution, to less than 1% of the micelles labelled with two or more labels.

Clearly theories developed for homogeneous systems must be applied with care to micellar systems. For example, one does not expect the density N in Eqs. (3), (4), and (5) to be the average density in the sample, rather an effective density - much higher

than the average-since both labels and probes congregate in and around the micelle volume. Too, one does not expect to observe a linear behavior of linewidth with N over a wide range as predicted by theory because the experimental spectrum would be a superposition of spectra due to labelled micelles that have zero, one, two, etc... probes associated with that micelle. Nevertheless we may proceed with confidence by fixing N at a constant, small value for all ions reasoning that whatever the effective density is, and whatever the superposition might be, these well be similar from ion to ion especially of we fix N to be such that less than one ion per micelle is utilized. Thus use a 1.0mM probe concentration.

Experimental

SDS micelles were prepared to 0.2M distilled water. The spin labels used were doxyl labeled stearic acids labeled at the 5, 10, 12, and 16 position which we call 5FASL, 10FASL, etc. also the ester of 5FASL was used in one series of experiments. These labels were purchased from molecular probes and were used without further purification. The labels were dissolved in chloroform and devided into a series of vials such that each vial had approximately 1mg of label and the chloroform was evaporated. These vials were stored in the refrigerator until used.

Labeled micelles were prepared by adding 0.2M SDS to a label-containing vial to produce 0.4mM label. The solution was stired for about one-half hour. The final samples were prepared by adding equal volumes of water or paramagnetic ion solutions to the labeled micelles producing the final concentrations of 0.2 mM label in 0.1M SDS. These samples were not degassed because the results one practically independent of the small oxygen induced broadening because this effect contributes equally to ΔH_{pp} and ΔH^{o}_{pp} and because the effect is small in aqueous solutions anyway. The samples were sealed in 75μℓ disposable pipets which were then housed in a quartz tube to make the ERS measurements.

The ESR spectra were measured on a Varian E÷ Line spectrometer using 100 kHz modulation. Power saturation and modulation broadening curves were run in order to select parameters that negligibly affected the spectra. All spectra were run at room temperature T = 296$\pm$2k,

RESULTS

All spectra were the familiar three-line, symetric nitroxide spectra. The line separation and the line width alternations were measured to yield hyperfine coupling constants and rotational correlation times respectively. The usual "B" and "C" coefficients[19] were measured and the rotational correlation times calculated from[19].

$$T_b = 6{,}1 \times 10^{-10} |B| \Delta H_{pp}^2 \qquad (6)$$
$$T_c = 6{,}0 \times 10^{-10} C \Delta H_{pp}^2$$

For isotropic rotation $T_b \approx T_c$, we have not corrected our results for unresolved hyperfine structure, so Eq. (6) will only provide an estimate of the relative rotational correlation times, Table 2 show the results. These results show the trend often observed[20] in which the mobility of the doxyl group increases as a function of the distance from the head group. We do not believe that any conclusion can be drawn concerning the spatial profile of the viscosity of the micelle: These results could very well represent the intrinsic flexibility of these labels thenselves, it is interesting that near the head group (5FASL) the mobility of the acid and ester is the same.

The values of the ^{14}N, hyperfine coupling constants are given in Table 3. These values, which are a measure of the dielectric constant of the medium in which the doxyl group resides, are remarkably similar to one another and reflect an environment similar to ethanol.

At this point, we should mention that there are a variety of time-dependent and pH dependent effects on the measured parameters and, for this reason, all of the work reported here was carried out in micelles "aged" for several hours, after being prepared and measured shortly after addition of the spin labels. It will be of interest to investigate "aging" effects that occur over 5-7 days using the present methods in the future.

The results of line broadening produced by 1mM concentrations of the various ions in this study are given in Table 4. Non paramagnetic ions such as Mg^{2+} and Zn^{2+} produce no effect, within experimental error and the outstanding feature of the rest of the data is that the

broadening is very similar. Since Eqs. (4) and (5) predict a factor of 18 variations due to the factor μ_{eff}^2 and a one or two orders of magnitude difference due to the factor T_{1p}, we conclude that line broadening is dominated by exchange interactions.

DISCUSSION

These results demonstrate the very fluid nature of solubilized fatty acid spin labels in SDS micelles because the dipolar interaction is effectively averaged even for ions such as Cu^{2+}, Mn^{2+}, and Gd^{3+}. Such a fluidity is also reflected in the fast rotational correlation times in Table 2. The fluid nature of the micelle may also explain the puzzling similarity of the hyperfine coupling constants in Table 3: in a less fuid model one would expect a trend from 5 FASL to 16 FASL reflecting a trend toward a hypperfine coupling constants near 14G, similar to hydrocarbon environments [2]. The present results point to a rapid averaging of dielectric environments.

Since exchange interactions dominate in this system, the broadenings in Table 4 are proportional to the collision frequency between the doxyl group and the surface-bound probe. This one sees that 16 FASL collides with the surface ion about half as often as the others which are remarkably similar to one another. It will be interesting to construct models to interpret the variation in line broadening with doxyl position but this must wait the development if a more adequate theory and measurements over a wider probe concentration range.

TABLE 1-PARAMETERS OF THE PARAMAGNETIC IONS USED AS SPIN PROBES

Ion	T_{1p}	M_{eff}^2
Cu^{++}	$\sim 3 \times 10^{-9}$	3.6
Mn^{++}	$\sim 2 \times 10^{-9}$	24
Gd^{+++}	$10^{-9} - 10^{-10}$	64
Ni^{++}	$\sim 4 \times 10^{-12}$	10
Co^{++}	7×10^{-13}	23
Fe^{++}	$< 10^{-11}$	29
Fe^{+++}	$< 10^{-11}$	35

TABLE 2 - ROTATIONAL CORRELATION TIMES (SECOND/RADIAN) FOR FATTY ACID SPIN LABELS IN FRESH MICELLES OF SODIUM DODECYL SULFATE (0.1M) T= 296K

	T_b[a]	T_c[a]
5FASL	$9,1 \times 10^{-10}$	$10,8 \times 10^{-10}$
10FASL	7,3	7,9
12FASL	7,2	8,3
16FASL	2,8	3,3
5FASL-ESTER	9,5	11,5

[a] Calculated from eq.(6) Units.

TABLE 3 - HYPERFINE COUPLING CONSTANTS (GAUSS) FOR FATTY ACID SPIN LABELS IN SODIUM DODECYL SULFATE MICELLES (0.1M) T = 296K

5 FASL	15.16 ± 0.02
10 FASL	15.39 ± 0.00
12 FASL	15.35 ± 0.01
16 FASL	15.32 ± 0.00

TABLE 4 - LINE BROADENING (GAUSS) INDUCED BY 1mM ADDED IONS IN FATTY ACID SPIN LABELS IN SODIUM DODECYL SULFATE MICELLES (0.1M) T= 296K

	Mg^{++}	Cu^{++}	Ni^{++}	Co^{++}	Mn^{++}	Fe^{++}	Fe^{+++}	Gd^{+++}
5 FASL[a]	0.00	0.13	0.19	0.22	0.12	-	-	0,29
10 FASL[b]	0.05	0.15	0.25	0.25	-	-	-	-
12 FASL[b]	0.05	0.24	0.17	0.21	-	-	-	-
16 FASL[c]	0.00	0.08	0.08	0.11	-	0.10	-	-
5 FASL-ESTER[a]	0.02	0.17	0.18	0.25	0.10	0.41	0.50	0.36

[a]Error ± 0.05G., [b]Error ± 0.08G, [c]Error ± 0.036

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