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**Spin-Label-Spin-Probe Studies of
Bimolecular Encounters in Micelles**

Final Report

Barney L. Bales

February 26, 1991

U. S. Army Research Office

Contract Number DAAL03-88-K-0006

California State University
at Northridge

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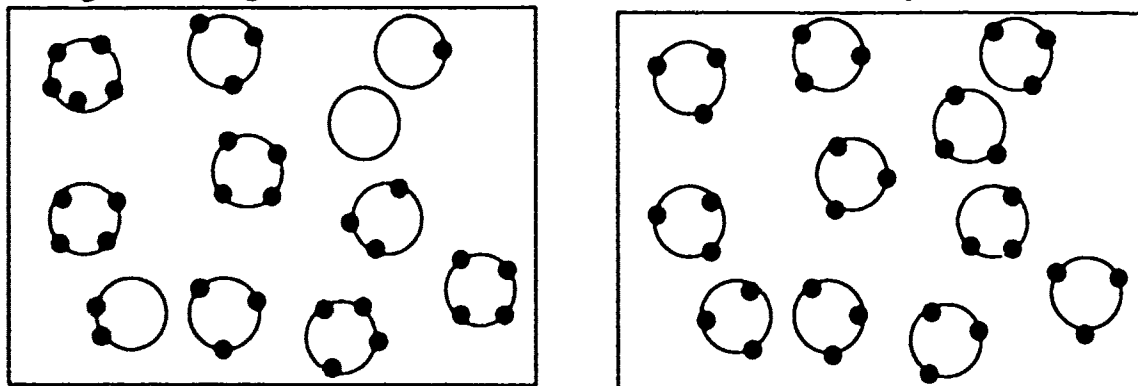
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A. STATEMENT OF THE PROBLEM STUDIED

A new method, based on EPR, to study the bimolecular collision rate between additives at the surface of micelles was developed. In order to study the collision rate in these *compartmentalized* systems, a detailed understanding of the number distribution function of the additives is crucial. To fix the ideas, we show below, schematically, two systems each having an average number of additives $\langle N \rangle = 3.0$. The system on the left



has one micelle unoccupied, one singly occupied, etc. What we mean by the *number distribution* of additives is the list of probabilities P_j of finding a micelle having j additives. A completely random distribution of the additives is described by the Poisson distribution. The system on the right has $P_3 = 1$ and all other $P_j = 0$ and is highly improbable. Implicit in the above schematic representation are the following: (1) the residence time of an additive on a given micelle is long compared with the measurement time and (2) all of the additives are associated with some micelle. In our new method, stable nitroxide spin labels are used as the additives and thus may be measured with EPR. One of the advantages of EPR is that the first assumption is true. The second is true to a high degree of approximation or not depending on the nature of the additive spin label. In all of the work carried out during this contract period, the additives were very sparingly soluble in water and in every case except one, showed no appreciable presence in water. The EPR spectra of the spin label additives are different in water and in micelles, so an internal check is available. In future work, it will be straightforward to extend our experimental and theoretical methods to cases in which additives are partitioned between the micellar region and the aqueous region.

A primary goal of this project was to develop a method to measure the collision rate between additives on the same micelle. This is accomplished by deducing the collision-induced linewidth broadening of the ESR spectrum and is illustrated schematically in Figs. 1 and 2.

SILLI

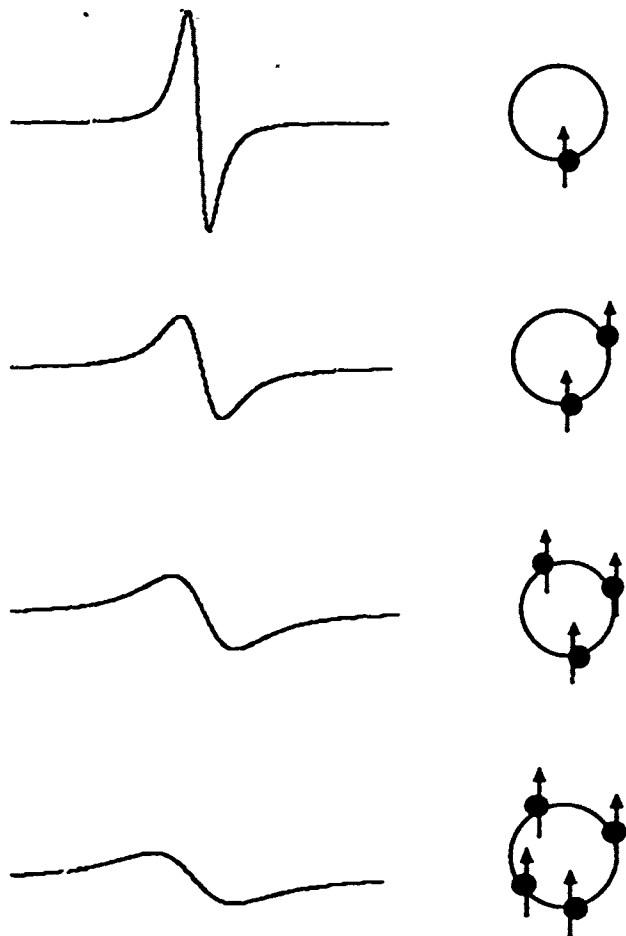


Fig. 1. Schematic representation of the SILLI experiment. Labels of the same kind are added to a micellar solution to a known average number per micelle $\langle N \rangle$. There are ensembles of micelles that have $N = 0$ labels (not shown because no EPR signal is observed), and ensembles having $N = 1, 2, 3, 4$, etc. labels. Each ensemble produces a distinct EPR signal, shown schematically to the left, which is broadened due to spin-spin interactions. The doubly-integrated intensity of the EPR is proportional to N , thus the top spectrum is one-fourth as intense as the bottom spectrum even though the latter is shorter since it is broader. The observed EPR signal is the sum of the above signals each weighted by the probability that a micelle contains N labels. Proper deconvolution yields the individual spectra as well as the probabilities rather than a global average. The line broadening yields the bimolecular collision rate between the probe and the label which is assumed to be of the form $\delta_A \cdot (N-1)$.

SLASP

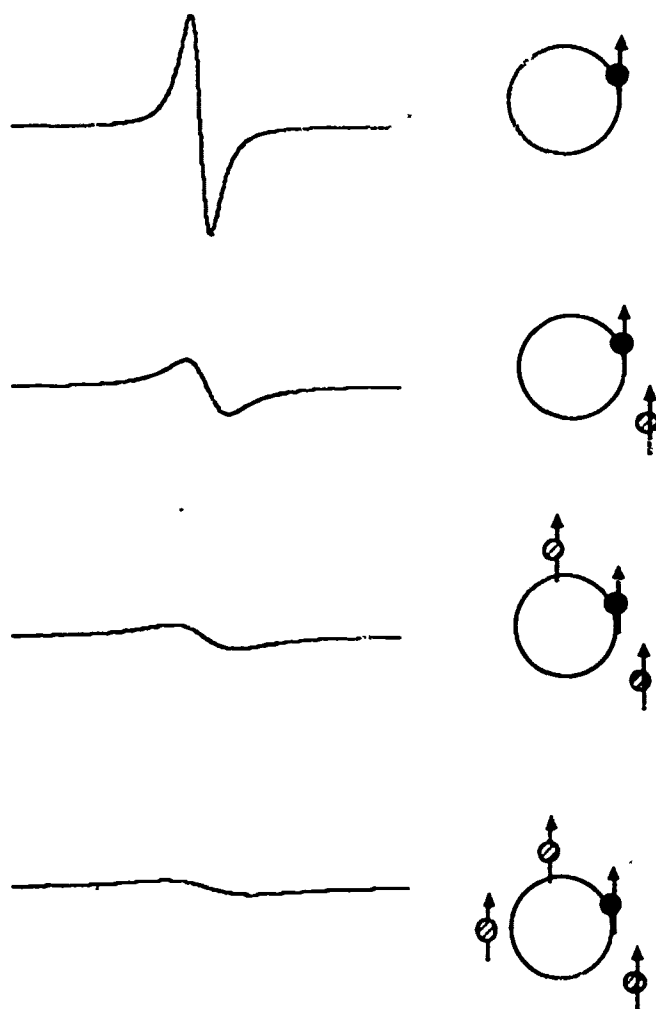


Fig. 2. Schematic representation of the SLASP experiment. Labels are added to a micellar solution in small concentration such that most micelles contain none a few contain one, and very few contain two. Only those micelles that contain one label are shown, since they provide practically all of the EPR signal. Probes (paramagnetic ions, complexes or other labels) are added to a known average number per micelle $\langle N \rangle$. There are ensembles of micelles that have $N = 0, 1, 2, 3$, etc. probes. Each ensemble produces a distinct EPR signal, shown schematically to the left, which is broadened due to spin-spin interactions. The doubly-integrated intensity of the EPR is constant vs. N , so the signal heights decrease drastically because of the increased linewidth. The observed EPR signal is the sum of the above signals each weighted by the probability that a micelle contains N labels. Proper deconvolution yields the individual spectra as well as the probabilities rather than a global average. The line broadening yields the bimolecular collision rate between the probe and the label assumed to be of the form $\delta_A \cdot N$.

B. SUMMARY OF THE MOST IMPORTANT RESULTS

Development of a new method. The method of SLASP has proved to be a more effective method to study bimolecular collision rates and number distributions that we had hoped at the time we wrote the proposal. In every micellar system studied, using every spin label additive attempted, we have been able to get very good data. This means that the collision rates in all of the systems we have studied have been just about right to get good measurements. This is an accident of nature. If the rates had been too fast, the EPR line would have been broadened too much to be easily measured and vice versa. In addition, we have shown that the SILLI experiment is equally useful.

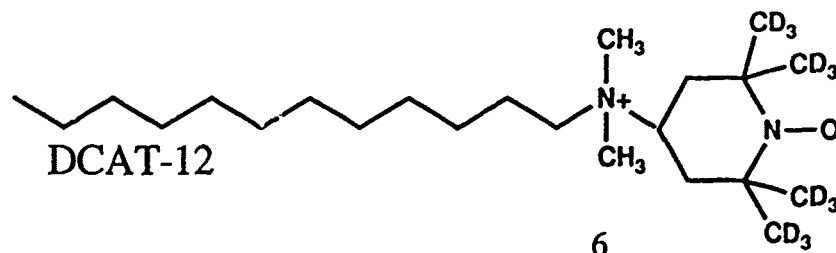
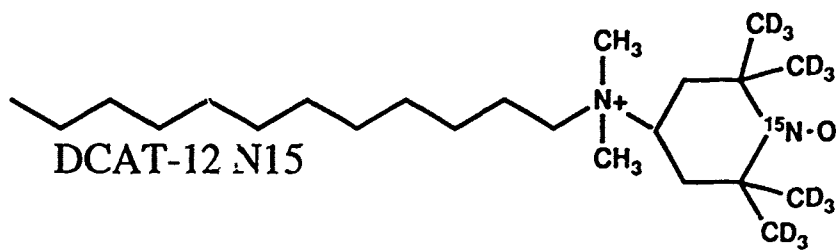
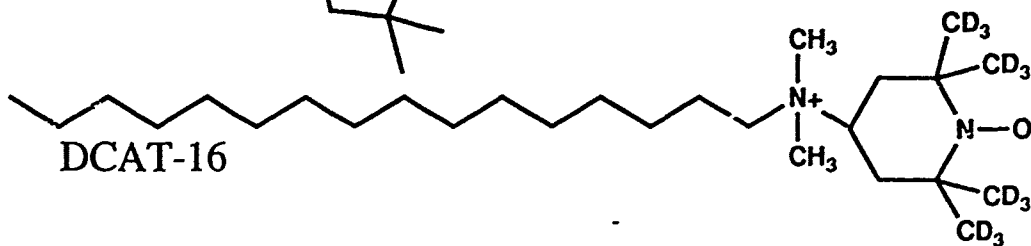
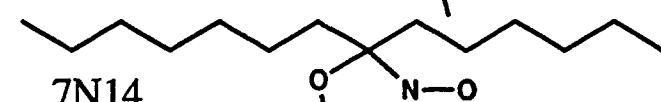
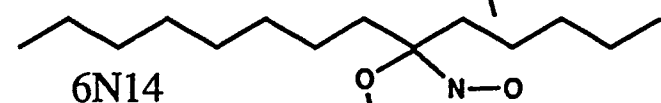
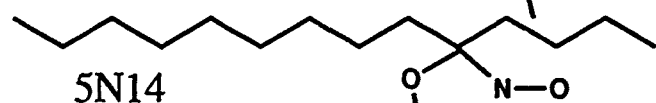
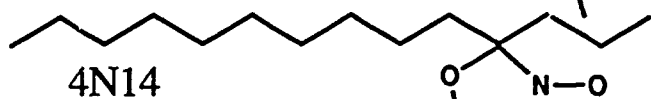
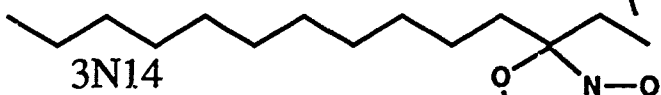
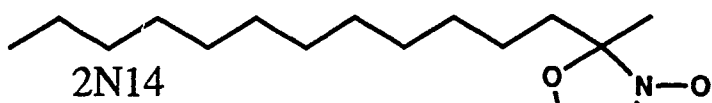
Synthesis of spin-label additives. Dr. Francis Harris has synthesized the spin labels shown on page 6. The acronyms used in this report are indicated next to each label.

Characterization of spin label additives. The hyperfine structures of labels 2N14, 3N14, 3N14, 4N14, 5N14, 6N14, and 7N14 have been completely characterized[6]. This characterization is necessary in the SLASP and SILLI methods because the unresolved hyperfine structure of the spin label must be taken into account[2].

SLASP in anionic micelles. DCAT-16 has been used in extensive SLASP measurements using Cu^{++} , Co^{++} , Ni^{++} , Gd^{+++} , Fe^{+++} , and Mn^{++} in sodium dodecylsulfate[1]. These measurements show that the broadening mechanism is spin exchange with collision rates on the order of 10 MHz. Only small departures from Poisson number distributions are found in this system.

A series of fatty acid spin labels in sodium dodecylsulfate using the same divalent cations as above led to essentially the same conclusions[8]. The fatty acid spin labels were labeled at various positions and a dependence of the collision rate on the *position* was found.

The collision rate was shown to be a very sensitive function of added salts under the experimental conditions of the last paragraph[7].



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SILLI experiments. Departures from Poisson distributions SILLI experiments were performed using 5N10 and fatty acid spin labels in cationic, anionic, and neutral micellar solutions[4]. Large departures from the Poisson distribution were postulated in order to satisfactorily interpret the results[5].

Theory of number distributions in micellar systems. A theory, based on the grand canonical ensemble, was developed to allow the incorporation of additive-additive interactions in the calculation of number distribution functions.[5]

A, rather unique, SILLI experiment was performed in CTAB micelles using the divalent anionic nitroxide free radical Fremy's salt[3]. These additives reside in the aqueous fraction near the micelle surface. Unexpectedly, in view of Deaton's findings[4, 5], the Poisson distribution worked quite well. The collision frequencies were an order of magnitude smaller in these experiments than in all of the others, presumably due to coulomb repulsion.

Considerable progress has been made on the deconvolution of EPR spectra in micellar solutions in both the SLASP and the SILLI experiments. These are nonlinear least-squares fits of the composite spectra using the Levenburg-Marquardt algorithm.

Software development. EPR data were collected by an IBM 9000 work station interfaced with the spectrometer. Computer programs have been developed to handle and analyze the data as outlined in Table 1.

Table 1. Computer Programs Written to Handle and Analyze EPR Data in the SLASP and SILLI Experiments.

Name	Language	Purpose	Comments
Six programs	BASIC	Find V_{pp} and ψ versus $\langle N \rangle$ for given additive-additive interactions and δ_A . [5]	Implemented on a Macintosh SE
LYB2TXPC	BASIC	Translate binary files in the IBM 9000 to ASCII files and transfer them to a 386 clone	
LOWFIT	C	Least-squares fit of spectra at $\langle N \rangle = 0$ to a Voigt shape	Implemented on a 386 clone.
SLASP	C	Least-squares fit of spectra at low $\langle N \rangle$ to two terms (zero and singly-occupied). Variable P_0 , P_1 , and δ_A .	
SILLI	C	Least-squares fit of spectra at low $\langle N \rangle$ to two terms (Singly and doubly-occupied). Variable P_1 , P_2 , and δ_A .	
POISSLSP	C	Least-squares fit of spectra at intermediate $\langle N \rangle$ to five terms Fixed P_j given by Poisson. Variable δ_A .	
POISSILI	C	Least-squares fit of spectra at intermediate $\langle N \rangle$ to five terms Fixed P_j given by Poisson. Variable δ_A .	
PROBSLSP	C	Least-squares fit of spectra at intermediate $\langle N \rangle$ to five terms. Fixed P_j given by grand canonical ensemble Variable δ_A .	
PROBSILI	C	Least-squares fit of spectra at intermediate $\langle N \rangle$ to five terms. Fixed P_j given by grand canonical ensemble Variable δ_A .	
HIFIT	C	Least-squares fit of spectra at intermediate $\langle N \rangle$ to a specified number of terms. Fixed δ_A variable P_j .	Linear problem.

Training of students. A large number of students received first-hand research experience in the EPR lab during this contract. Five students wrote master's theses based upon their original work. Two students wrote the bulk of the computer programs and many other participated in the data processing phase of the work.

C. LIST OF ALL PUBLICATIONS AND TECHNICAL REPORTS

"Inhomogeneously Broadened Spin-Label Spectra", Barney L. Bales, *Biological Magnetic Resonance*, **8**, 77-130 (1989).

"EPR Studies of Micelles as Compartments", Barney L. Bales and Jack Deaton, Proc. CRDEC Conference on Chemical Defense Research, 31-37 (1989).

"Micelles as Compartmentalized Systems", Jack Deaton , Master's Thesis in Physics and Astronomy, California State University at Northridge, December 1989.

"EPR Studies of Fremy's Salt in Micelles", Ronald Blum , Master's Thesis in Physics and Astronomy, California State University at Northridge, May 1990.

"Cationic Spin-Label-Spin-Probe Studies of Micelles", Sabah Alnaemi, Master's Thesis in Physics and Astronomy, California State University at Northridge, May 1990.

"Effect of Impurities on the Bimolecular Encounters on the Surface of Micelles" Helen Solomon, Master's Thesis in Chemistry, California State University at Northridge, May 1990.

"Hyperfine Structures of Doxyl Labeled N-alkyl Chains by NMR and ESR", Donna Mareno, Master's Thesis in Chemistry, California State University at Northridge, May 1991.

D. LIST OF ALL PARTICIPATING SCIENTIFIC PERSONNEL

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Scott Kelly

David Rapkin

Daniel Kinzek

Donna Mareno

Sabah Alnaemi

Garen Moradkhanian

Yakem Habtemarian

Felipe Hervias

Jennifer Hunyhn

Helen Solomon

Jack Deaton

E. REPORT OF INVENTIONS: NONE

E. REFERENCES

1. Alnaemi, S. Cationic Spin-Label-Spin-Probe Studies of Micelles. Master's Thesis, California State University at Northridge, 1990.
2. Bales, B. L. Inhomogeneously Broadened Spin-Label Spectra. *Biological Magnetic Resonance*. 8: 77-130, 1989.
3. Blum, R. EPR Studies of Fremy's Salt in Micelles. Master's Thesis, California State University at Northridge, 1990.
4. Deaton, J. Micelles as Compartmentalized Systems. Master's Thesis, California State University at Northridge, 1989.
5. Deaton, J. and B. L. Bales. Micelles as Compartmentalized Systems. 12th International EPR Symposium. 1989.
6. Mareno, D. Hyperfine Structures of Doxyl-Labeled N-alkyl Chains by NMR and EPR. Master's Thesis, California State University at Northridge, 1991.
7. Solomon, H. Effect of Impurities on the Bimolecular Encounters at the Surface of Micelles. Master's Thesis, California State University at Northridge, 1990.
8. Stenland, C. and B. L. Bales. Spin-Label-Spin-Probe Studies of Sodium Dodecylsulfate Micelles. 11th International EPR Symposium. 1988.