The infrared chemiluminescence spectrometer used has been described previously.[1] It used a liquid-nitrogen-cooled germanium detector similar to that used by Khan. The reaction temperature was measured with a glass thermmistor probe. Catalase (11 800 Sigma units/mg) was obtained from Sigma Chemical Co., St. Louis, MO. All experiments were done at an ambient temperature of 24 °C.

Figure 1 shows the time course of the infrared emission through a 1670-nm interference filter. It is similar in half-life and intensity to that resulting from the injection of 1.5 mL of H_2O heated to 51 °C into an equal volume of H_2O at 24 °C. Figure 2 shows the time course of the temperature is similar in both systems. The half-life of the temperature decay is of the same order of magnitude as the half-life of the infrared emission. Table I presents the spectral distribution of the observed radiation in both systems, corrected for the transmission curves of each interference filter. The emission spectrum is the same for each system and consistent with thermal radiation. The absence of detectable emission through the 1880- and 1968-nm filters is due to steep decline in detector sensitivity for wavelengths greater than 1600 nm. Consistent with past studies excluding singlet oxygen as a product of the catalase–H_2O_2 system, no emission near 1268 nm was detected.[7] Using the H_2O_2 + HOCl reaction as a calibrating source of singlet oxygen emission, an upper limit of 0.02% can be placed on any singlet oxygen produced.[3]

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Real Two-Dimensional NMR Solvent Suppression Technique

E. Guittet, M. A. Delsuc, and J. Y. Lallemand*

Laboratoire de R.M.N.
Institut De Chimie Des Substances Naturelles
91190 GIF sur YVETTE, France

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Suppression of the solvent signal in ^{1}H NMR is of utmost importance, particularly in the studies of biological molecules and for in vivo applications. Although many different techniques have been recently reported, their applicability in two-dimensional (2-D) NMR has not been investigated. We present here a COSY-type experiment wherein two-dimensional solvent peak elimination permits us to observe signals obscured by the solvent or protons in slow exchange with the solvent. This technique is extendable to most 2-D experiments.

In order to run NMR experiments in the presence of strong solvent signals two different problems have to be faced. The undesired signal has to be attenuated at an early stage of the acquision, in order to enhance the acquisition dynamics, limited mainly by the analog-to-digital converter. This is generally achieved by the nonexcitation of the solvent signal (Redfield "2-1-4",[1,2] soft pulses,[2] composite pulses[3]) or alternatively by the attenuation of its resonance (saturation, WEFT[4]). On the other hand the residual acquired signal can be canceled by use of further suppression techniques such as DSA, ADA,[5] or filtering.

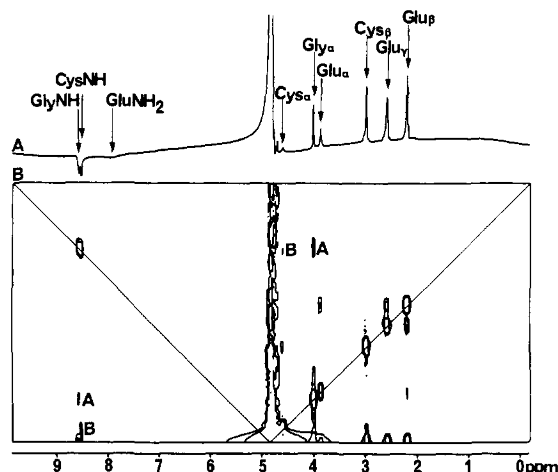

Figure 1. Spectra of glutathione 65 mM in H_2O (10% D_2O, pH 3.00, 19 °C), acquired on a BRUKER WM 400 spectrometer. (A) JR spectrum showing the characteristic phase inversion of this technique. (B) The modified JR sequence COSY spectrum shown in magnitude. A 64*1024 data matrix was acquired, 128 scans per FID for a total time of 8 h. A saturation pulse of 1 s was applied after each scan followed by a relaxation time of 2 s.

The need in 2-D experiments of hard pulses with definite flip angles leads to the preferential use of attenuation techniques such as WEFT or saturation.[6,7] However, their use is restricted to nonexchangeable protons or long T_1 molecules. The use of semiselective pulses in 2-D has been restricted for the moment to the read pulse in the NOESY experiment.[8,9] However, in this very special experiment, the mixing pulse and the read pulse are distinct and significant decay of the transverse H_2O magnetization takes place during the mixing period.[8]

In order to develop one-scan solvent suppression techniques that would be operative for 2-D applications, we therefore turned to those that use hard pulses and whose structure itself could be included as such in the pulse sequence. The jump and return (JR[10]) and the COSY pulse sequences present the required correspondence. The JR experiment consists in applying two $\pi/2$ pulses and making the phase of the second pulse such as to bring back the solvent magnetization to its original equilibrium state. The 2-D extension presented here also uses only two $\pi/2$ pulses and is based on increasing the time between these two pulses by the usual incremented time of the COSY experiment. This leads to the nonexcitation of the solvent diagonal line of the 2-D spectrum. It is worth noting that since all resonances experience the preparation pulse and the mixing pulse, a true two-dimensional solvent suppression is achieved. In fact the zero signal area is a hole centered at the point on the spectrum at $w_1 = w_2 = w_{\text{solvent}}$ coordinates and not a furrow lying along $w_2 = w_{\text{solvent}}$ as in the other solvent suppression techniques. As a consequence a proton under the solvent line will show its correlation peaks with other protons at their full intensity. It should be noted that the phase of the second pulse is fixed by the phase of the first, which hampers any quadrature detection in the w_1 dimension and leads to folding in this dimension. The only disadvantage of this method would be for a situation in which two coupled signals are symetrically placed on each side of the solvent. In this case correlation peaks would be lost.

Figure 1B shows the COSY spectrum of glutathione (Glu-Cys-Gly) 65 mM in H_2O. A saturation pulse has been introduced that randomizes the spin distribution and thus allows shortening of the necessary relaxation time. Although this ensures a better

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reproductibility of the experiment, it is in no way an absolute necessity and can be omitted. It is interesting to note the off-diagonal correlations of the amide resonances with the protons (A, Gly; B, Cys). The Glu-NH resonance is severely exchange broadened and hence is unobservable for all practical purpose. The JR spectrum of glutathione in the same conditions is shown (Figure 1A), presenting the characteristic phase inversion of this technique.

Applications of this general method can be developed for most 2-D experiments. As an example, this "phase" sequence can replace the preparation and evolution periods[11] of the 2-D NOE (followed by a selective pulse[8,9]) or the evolution and mixing periods of the multiple-quantum experiments.

We think that this new methodology should not be restricted to the specific cases discussed in this paper (non-deuterated solvents, under-solvent correlations) but should prove useful as an alternative approach for the residual protonated solvent suppression in most experiments.

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Spontaneous Vesicles

James E. Brady,[†] D. Fennell Evans,*[†] B. Kachar,[‡] and B. W. Ninham[§]

Department of Chemical Engineering and Materials Science, University of Minnesota Minneapolis, Minnesota 55455
Laboratory for Neurobiology, NINCDS National Institutes of Health Bethesda, Maryland 20205
Department of Applied Mathematics Research School of Physical Sciences Australian National University Canberra ACT 2600, Australia

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Understanding the structure, stability, and dynamics of vesicles is important in elucidating biological self-assembly and in devising strategies for practical use of stable vesicles. The most widely studied vesicle systems are prepared using lecithin or double-chained surfactants.[1] Such systems require sonication to facilitate vesicle formation, are unstable, and invariably revert to the bilayers from which they emerged. Didodecyldimethylammonium hydroxide (DDAOH) is an exception to this rule. In previous reports[2–4] we have shown DDAOH vesicles form spontaneously, survive freeze–thaw cycles, and appear stable. Vesicles prepared from lecithin and DDAOH conceivably represent extremes in a spectrum of behavior regarding ease of vesicle formation and stability.

We have now found a wide range of didodecyldimethylammonium (DDA) salts form vesicles with surprising ease. In Figure 1 we show pictures of vesicles prepared from DDA carboxylates and polymerized acrylate obtained using video-enhanced differential interference contrast microscopy (VEDICM).[5–7] VEDICM, which employs a light microscope equipped with Nomarski optics,[8] a video camera, and frame processor, permits real time imaging of very fine structural details at the resolution limit of the light microscope (1500–2000 Å) and the detection of (diffraction enlarged) structures as small as 300 Å. The dynamics, growth, fusion, and flocculation of surfactant microstructures and other colloidal suspensions are readily visualized on a television screen. The frame processor permits real time image analysis and enhancement, freeze-frame, slow-motion, or time-lapse capability. Quantitative evaluation of data may be carried out with available image analysis technology (e.g., developed by NASA for satellite photograph analysis). The method is direct, rapid, independent of physical models (cf. light scattering etc.), and relatively free of artifacts, involves no sample alteration, and immediately reveals the presence of polydispersity.

As a guide to interpreting VEDICM images, we mention the following points. Images of particles smaller than the resolution limit of the microscope ($\lambda/2$, λ = wavelength of illuminating light) are diffraction enlarged relative to the true particle size. The highlighting of images produced by Nomarski optics may be altered by adjustment of movable beam combiner such that the *edges* of a *uniform* specimen can appear as dark on a light background or light on a dark background or so the entire image appears shadow cast.[8] Since the optical system is sensitive to differences in refractive index at edges, species such as vesicles will yield donut-like images.

Didodecyldimethylammonium salts were prepared by combining DDAOH (ion exchanged, REXYN 201 Fisher, from the bromide salt) with a stoichiometric amount of the appropriate acid.[2] Previously encountered ambiguities in the determination of DDAOH were avoided by employing a water–methanol mixture (10:90 v/v) as the titration medium. In this solvent system DDAOH behaves as a simple strong base, the titration products are soluble, pH electrode response is rapid and reproducible, and fouling of the reference electrode (saturated calomel) is absent. Hydrochloric acid served as titrant and was standardized with sodium borate decahydrate.

Two polymerized acrylate samples, one sonicated the other not sonicated prior to polymerization, were prepared with hydrogen peroxide as the initiator (100 μL of 30% H_2O_2 per 10 μL of sample; surfactant concentrations of 5.5, 6.4, and 55.0 mM).

The didodecyldimethylammonium salt samples were prepared for VEDICM analysis by depositing a small drop of solution on a microscope slide and placing a cover slip on top. This method of preparation subjects the surfactant solution to a small uncontrolled amount of shear as the fluid flows between the two glass surfaces. In a second series of experiments shear was minimized by permitting water to diffuse into a concentrated surfactant solution contained between the slide and coverslip, and the emergence of vesicles was followed as the solution was diluted. The VEDICM equipment was described in detail previously.[4]

Didodecyldimethylammonium fluoride, formate, acetate, propionate, butyrate, glycinate, tartarate (as dianion), and oxalate (as $C_2O_4^{2-}$) give clear isotropic solutions up to at least 0.1 M which have low to moderate viscosities (<500 cp at 0.13 M).

In concentrated solutions ($\sim$0.13 M) no evidence of vesicle formation is detected by VEDICM. Dilution of these solutions by a factor of 100–10 000 yields vesicles, see Figure 1. This behavior parallels that observed for DDAOH.[2,3] In concentrated DDAOH solutions, small micelles with aggregation numbers of $\sim$40 are obtained by the double-exponential fluorescence decay method;[9] dilution yields vesicles. The existence of DDAOH vesicles has been established by VEDICM, electron microscopy, and light-scattering measurements.[2–4] The transformation of DDAOH and DDA carboxylate micelles to vesicles appears surprisingly rapid ($\sim$60 s). Samples of DDA formate subjected to freeze–thaw cycles yield similar to those shown in Figure 1c. This provides a crude indication the DDA carboxylate vesicles

[†] University of Minnesota.
[‡] National Institutes of Health.
[§] Australian National University.

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