Resonance Raman Analysis of Fluorescent Compounds Using Micellar Solutions and Ultraviolet Laser Excitation

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Micelles composed of specifically functionalized surfactants can be used to great advantage in resonance Raman spectroscopy. In addition to circumventing the problem of luminescence background, micelles allow the use of aqueous solvents which stabilize labile species and improve the signal-to-noise ratio. The first examples of micelle-mediated resonance Raman analysis of fluorescent compounds using ultraviolet excitation are given.

Index Headings: **Micelles; Resonance Raman spectroscopy of fluorescent compounds; UV lasers.**

INTRODUCTION

The use of ultraviolet lasers is becoming increasingly popular in resonance Raman spectroscopy (RRS). One reason is because of the greater number and types of compounds amenable to RRS analysis in this spectral region.[1] It is well known that interfering fluorescence limits the practicality and widespread use of RRS. Unfortunately, the background luminescence also becomes increasingly critical at shorter wavelengths. Exciting in the ultraviolet region may also increase impurity and/or solvent interferences, photochemical reactions, and photodecompositions.

There have been several attempts to alleviate or circumvent the luminescence problem with the use of instrumental approaches such as coherent anti-Stokes Raman spectroscopy (CARS),[2,3] inverse Raman spectroscopy,[4,5] Raman-induced Kerr-effect spectroscopy,[6] time-resolved Raman spectroscopy,[7] modulation techniques,[8–10] and Fourier transform methods.[11] All these techniques require additional and, in many instances, complex hardware. These approaches must be considered not a complete solution to the luminescence problem, but rather an improvement in certain circumstances. Previously reported methods of quenching[12] and drenching[13] can be used in some cases in which a minimum amount of luminescence can be tolerated.

Specially functionalized micelles often have the ability to negate the interfering analyte and/or impurity luminescence, so that resonance Raman spectra can be obtained for a variety of compounds, including those that absorb and luminesce in the ultraviolet region. Micelle-mediated resonance Raman spectroscopy (MMRRS) has been shown to be an effective technique for analyzing low concentrations of highly luminescent molecules.[14] This was done with visible lasers and without the complex instrumentation necessary for most of the previously mentioned techniques. In this work, MMRRS is shown to be even more effective and more widely applicable when used in conjunction with ultraviolet laser excitation.

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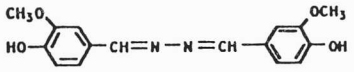
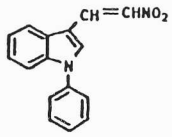
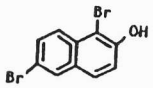
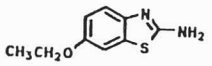
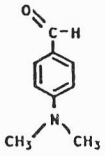
TABLE I. Compounds analyzed by micelle-mediated resonance Raman spectroscopy using UV-laser excitation (325 nm).

Compound	Structure
1. Vanillin azine	
2. 3-(2-Nitrovinyl)-1-phenyl indole	
3. 1,6-Dibromo-2-naphthol	
4. 2-Amino-6-ethoxythiazole	
5. 4-(Dimethylamino)benzaldehyde	

EXPERIMENTAL

Materials. Concentrated solutions (2000 ppm) of 4-(dimethyl amino) benzaldehyde, 2-amino-6-ethoxythiozole, vanillin azine, 3-(2-nitrovinyl)-1-phenyl indole, and 1,6-dibromo-2-naphthol (from Aldrich Chemical Co., Milwaukee, WI, and used as received) were made with HPLC grade methanol (Fisher Scientific, Plano, TX). These solutions were diluted to 5×10^{-3} **M** (unless otherwise stated) with 0.11 **M** brominated Brij 96. The solutions were transferred into quartz capillaries (2-mm i.d. by 5 cm) for measurement. The derivitization of the nonionic Brij surfactant was described previously.[15]

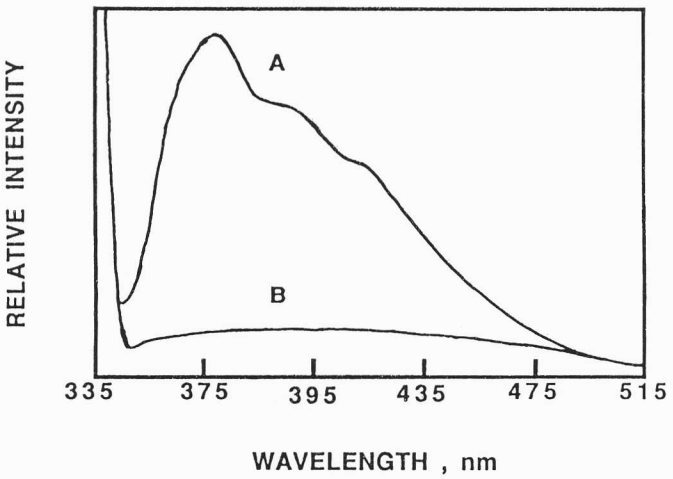


FIG. 1. Fluorescence emission spectra of (A) 8.3×10^{-5} **M** solution of 1,6-dibromo-2-naphthol in methanol, and (B) 5×10^{-3} solution of 1,6-dibromo-2-naphthol in 0.11 **M** functionalized Brij solution. The excitation wavelength was 325 nm and the scan was run between 335 nm and 515 nm at 60 nm/min.

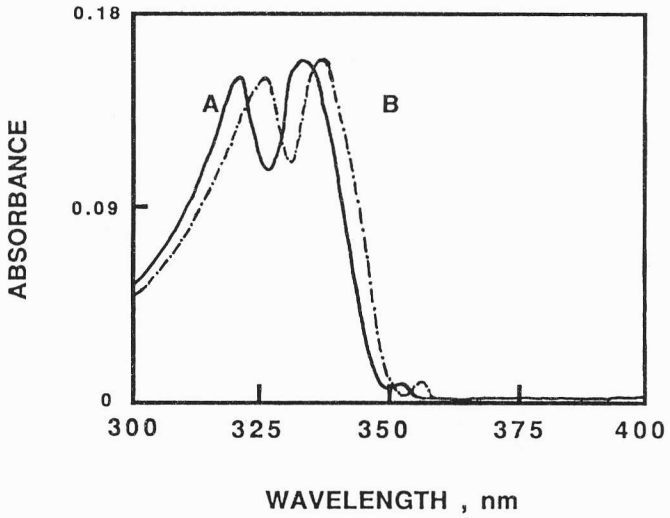


FIG. 2. Absorption spectra of (A) 5.5×10^{-6} **M** solution of 1,6-dibromo-2-naphthol in methanol, and (B) 5.5×10^{-6} **M** solution of 1,6-dibromo-2-naphthol in 0.11 **M** functionalized Brij solution.

Methods. The degree of fluorescent quenching was measured with a Perkin-Elmer LS-5. The recorder (Perkin-Elmer R-100A) full scale was set for 100 intensity units, and the fixed scale of the LS-5 was set to 1.0. Measurements were made of the compounds in pure methanol to determine the fluorescent intensity on the LS-5, with $\lambda_{ex} = 325$ nm and slits of 5 nm (excitation and emission). The emission spectrum of the compound in methanol was compared with the emission spectrum of the compound in brominated Brij. Absorption spectra were obtained with a Hewlett-Packard 8451A diode array spectrophotometer. An empty suprasil quartz cell was used as a reference blank.

Resonance Raman light was measured with a SPEX 1403 Raman double spectrometer (0.85 m, *f*/7.8, Spex Industries, Metuchen, NJ). The wavelength used for excitation was the 3250-Å line of a He-Cd continuous-wave laser (Omnichrome, Chino, CA). The beam was passed through a 20% transmitting 3250-Å filter and reflected to the sample holder accommodating the quartz capillary tube which held the sample. Approximately 3 mW of coherent light irradiated the sample. The Raman light was collimated and focused with quartz lens 90° from incident radiation into the spectrometer. Light was detected by a water-cooled RCA Model C-31034 PMT (RCA Corporation, Lancaster, PA, operated at 1300 V) and the signal amplified and processed by a SPEX DM/A spectrometer controller and data processor. The slits of the monochromator were set at 400/800 800/400 nm. Each compound was scanned four times and smoothed once. Background subtraction was also done.

RESULTS AND DISCUSSION

Table I lists the names and structures of five compounds which were analyzed by MMRRS with the use of UV laser excitation. The resonance Raman spectra of all these compounds were obtained in aqueous solutions containing 0.11 **M** of the functionalized Brij surfactant. No resonance Raman spectra could be obtained for any of these compounds when they were dissolved in neat

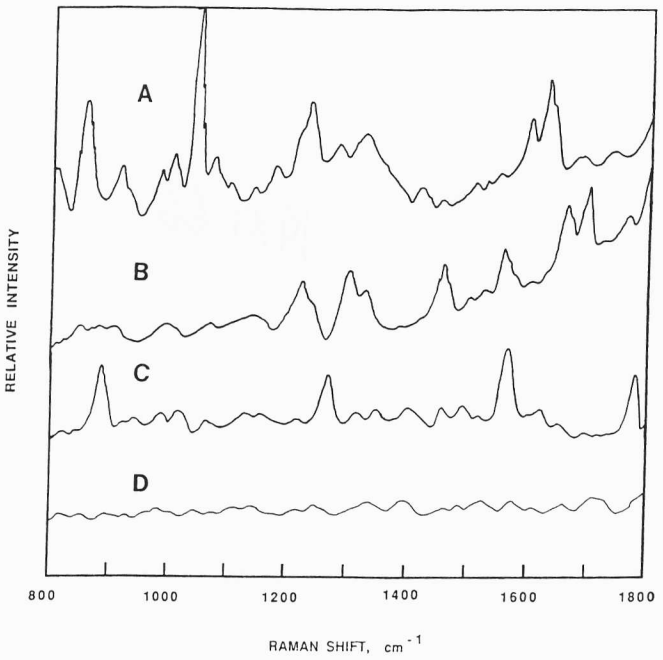


FIG. 3. Resonance Raman spectra of (A) 3-(2-nitrovinyl)-1-phenyl indole; (B) 1,6-dibromo-2-naphthol; (C) vanillin azine; and (D) 0.11 **M** brominated Brij (blank). The concentrations for all were 5×10^{-3} **M**. The wavelength of excitation was 325 nm. Other experimental conditions are described in the text.

organic solvents. Indeed, all that could be seen was a highly fluorescent background. Likewise, these compounds cannot be analyzed in pure water, because of insolubility and/or fluorescence.

Micelles facilitate the dissolution of compounds which have limited solubility in aqueous solutions but which are freely soluble in organic solvents. This is true for all the compounds listed in Table I. Micelles eliminate the need for organic solvents which themselves have interfering Raman lines. Water is a good Raman solvent, and the surfactant is sufficiently dilute so as to produce little or no background signals. Obviously the surfactant should not absorb at the excitation wavelength, as this would produce a resonance enhancement of the background signal.

Micelles can also be used to promote luminescence quenching. Figure 1 shows the fluorescence spectrum (A) of a solution of 1,6-dibromo-2-naphthol in methanol. The analogous spectrum (B) is shown for a solution of the same compound in the 0.11 **M** functionalized Brij in water. In organic solvents the fluorescence would completely obliterate the resonance Raman signal, but the aqueous surfactant allows the spectrum to be obtained with relative ease (see Fig. 3).

The beneficial effects of the micelles used in this study include factors other than solvation and luminescence quenching. Micelles can alter the absorption wavelength and the extinction coefficient of many compounds.[16] Figure 2 shows one example of this behavior for the absorption spectra of 1,6-dibromo-2-naphthol in methanol and the 0.11 **M** functionalized Brij. In this case, the micelle produced a 5-nm red shift of the absorption band towards the UV laser line (325 nm), thereby enhancing the resonance effect.

Other beneficial micellar properties include the ability to stabilize transient species and prevent photodegradation.[14,17] Micelles can modify fluorescence depolarization behavior and also affect the apparent pKas of ionizable solutes.

Figure 3 shows three examples of Raman spectra which are obtained with the use of functionalized micelles as well as the surfactant blank. Detailed spectra are easily obtained by this method. Note, for example, the resonantly enhanced signals at 875, 1260, and 1560 cm^{-1} for vanillin azine (3C). These bands are also found for vanillin azine in the infrared spectrum and the regular Raman spectrum of a solid sample.

As previously mentioned, the luminescence completely obliterated the resonance Raman scattered light when the compounds were analyzed without the Brij, even at analyte concentrations of 10^{-5} **M** or less. It is apparent from this study that specially functionalized micelles allow one to obtain resonance Raman spectra of a variety of highly luminescent compounds using UV laser excitation. Not only does this technique appear to be widely applicable, but it is relatively simple and inexpensive compared with other methods.

ACKNOWLEDGMENT

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