# An Amphiphilic Hemicyanine Dye Employed as a Sensitive Probe of Water in Reverse AOT Micelles

Martin Hof[†]

*Physical Chemistry, Charles University, Cz-12840 Praha 2, Czech Republic*

Panagiotis Lianos*[,‡]

*Engineering Science Department, University of Patras, 26500 Patras, Greece*

Andre Laschewsky[§]

*Department de Chimie, Universite Catholique de Louvain, B-1348 Louvain-la-Neuve, Belgium*

*Received September 18, 1996. In Final Form: February 12, 1997*[⊗]

When the amphiphilic hemicyanine dye *N*-*n*-heptyl-4-(2-(4-(dimethylamino)phenyl)ethenyl)pyridinium bromide is solubilized in reverse AOT micelles, it forms an adduct which extensively modifies absorption and fluorescence spectra of the dye. The formation of an adduct is very sensitive to the presence of water. Thus the water content of AOT micelles can be accurately monitored by the photophysical behavior of the dye. In particular the red edge excitation shift can be used as a handy and accurate index to determine the water content.

## Introduction

A considerable amount of work has been published on the characterization of the state of water molecules in reverse micelles.[1−5] Among several other spectroscopic methods, fluorescence and absorption spectroscopies represent a powerful tool to define the physical properties of the solubilized water molecules. The validity of the information gained by fluorescence and absorption spectroscopies depends on three factors: the location of the probe must be known, the probe concentration must be minimal, and the probe must be sensitive to changes in the physical properties of the solubilized water. Though a lot of information has been already obtained by the use of various dyes,[4] we believe that the hemicyanine dye *N*-*n*-heptyl-4-(2-(4-(dimethylamino)phenyl)ethenyl)pyridinium bromide (H7HC, for chemical formula see Figure 1) represents an outstanding probe for the characterization of the state of water in AOT reverse micelles, in terms of the above given criteria. The reason is that this dye exhibits a large red edge excitation shift (REES) depending on the state of the water in the reverse micelles. The REES is the difference between the fluorescence emission maxima when the dye is excited at different positions (i.e. wavelength) of the absorption spectrum.[6] The REES is measured in a simple routine way, just by recording absorption and fluorescence maxima. The REES strongly depends on the structure of the water−oil interface reflecting both the polarity and viscosity gradient at the interface. Therefore, it monitors in a very accurate manner the water content in reverse micelles and, generally, without microemulsions.

## Materials and Methods

Solvents and AOT (Fluka) were of the best quality available. H7HC was synthesized and purified as previously described.[7,8] Measurements were made at ambient temperature with a Perkin-Elmer Lambda 15 absorption spectrophotometer and with a home-assembled spectrofluorometer using Oriel parts.

## Results

H7HC (5 $\mu$M) was incorporated in reverse micelles in cyclohexane formed by AOT (0.2 M). Water has been added to a final concentration of 6.1 M. Absorption and fluorescence spectra were recorded and found structureless. The absorption maximum of H7HC in AOT reverse micelles is shifted from 441 nm (no water added) to 461 nm (6.1 M water), as seen in Figure 1. In this and the following figures, data points are connected to facilitate discussion. The fluorescence emission in the AOT system depended strongly on the excitation wavelength. When excited at the blue edge of the absorption ($\lambda_{ex} = 390$ nm), the maximum of the fluorescence emission changed within a very large wavelength range, i.e., from 506 to 599 nm, due to water addition (Figure 2). The red edge excited maxima ($\lambda_{ex} = 560$ nm) was shifted from 582 to 607 nm (Figure 2). The REES, i.e., the difference between the above curves, decreased with increasing water from 75.5 to 7.5 nm, as seen in Figure 3. In all the above three figures, the point at zero water concentration was not determined experimentally but its position is easy to find by an obvious extrapolation.

## Discussion

The understanding of the spectroscopic behavior of H7HC in AOT reverse micelles requires some knowledge about its solvatochromic behavior. Though the absorption and fluorescence maxima of H7HC in solvents of different polarity do not follow strictly the unique symmetrical and linear solvatochromic correlation found for related hemicyanines,[9,10] it can be concluded that increasing solvent polarity leads to a blue shift in absorption and a red shift

† E-mail: hof@prfdec.natur.cuni.cz.
‡ E-mail: lianos@ upatras.gr.
§ E-mail: laschewsky@cico.ucl.ac.be.
⊗ Abstract published in *Advance ACS Abstracts,* March 15, 1997.

(1) Zulauf, M.; Eicke, H.-F. *J. Phys.Chem.* **1979**, *83*, 480.
(2) Wong, M.; Thomas, J. K.; Nowak, T. *J. Am.Chem.Soc.* **1977**, *99*, 4730.
(3) Almgren, M.; Johannsson, R.; Eriksson, J. C. *J. Phys.Chem.* **1993**, *97*, 8590.
(4) Correa, N. M.; Biasutti, M. A.; Silber, J. J. *J. Colloid Interface Sci.* **1995**, *172*, 71 and references therein.
(5) Christopher, D. J.; Yarwood, J.; Belton, P. S.; Hills, B. P. *J. Colloid Interface Sci.* **1992**, *152*, 465 and references therein.
(6) Galley, W. C.; Purkey, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *67*, 1116.
(7) Lunkenheimer, K.; Laschewsky, A. *Prog. Colloid Polym. Sci.* **1992**, *89*, 239.
(8) Papoutsi, D.; Bekiari; Stathatos, E.; Lianos, P.; Laschewsky, A. *Langmuir* **1995**, *11*, 4355.

S0743-7463(96)00907-9 CCC: $14.00 

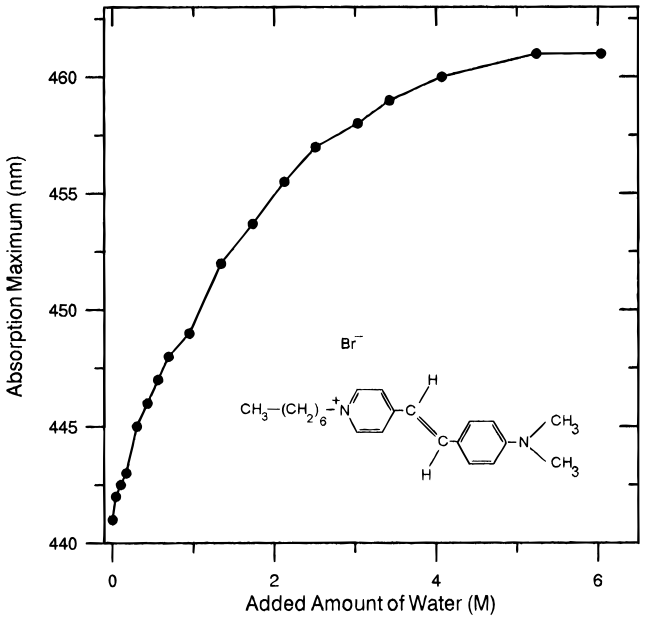


**Figure 1.** Variation of the H7HC (5 $\mu$M) absorption maxima in AOT (0.2 M) reverse micelles as a function of the amount of added water. Insert: chemical structure of H7HC.

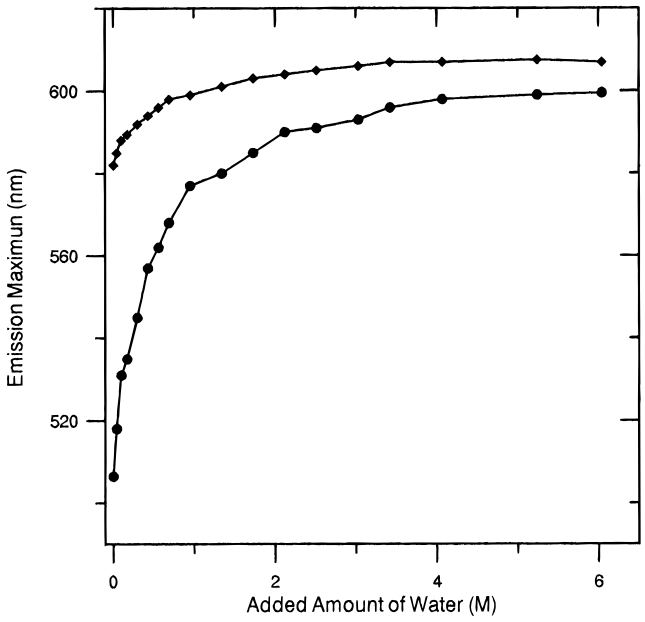


**Figure 2.** Variation of the H7HC (5 $\mu$M) fluorescence maxima in AOT (0.2 M) reverse micelles as a function of the amount of added water: (●) excitation at 390 nm; (◆) excitation at 560 nm.

in fluorescence. The fluorescence and absorption maxima in water (607 and 451 nm, respectively; dielectric constant $\epsilon = 78$) and tetrahydrofuran (589 and 465 nm, respectively; $\epsilon = 3$) may serve as an example. Cyclohexane solutions deviate from this trend: the absorption maximum appears most blue-shifted (446 nm). Moreover, shifting the excitation wavelength to the red end causes a progressive red shift of the emission wavelength (19 nm) and, finally, a red edge excited maximum of 593 nm. Generally, the observation of a REES might indicate a solvent relaxation process occurring on the same time scale as the fluorescence lifetime, finally leading to a relaxed state of minimum free energy.[6] However, such an explanation

(9) Ephardt, H.; Fromherz, P. *J. Phys.Chem.* **1989**, *93*, 7717; **1993**, *97*, 4540.
(10) Fromherz, P. *J. Phys. Chem.* **1995**, *99*, 7188.

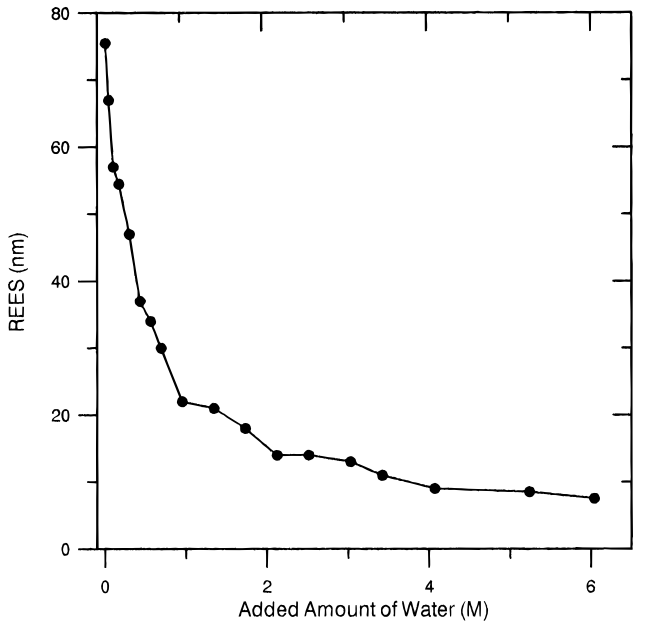


**Figure 3.** Variation of the H7HC (5 $\mu$M) red edge excitation shift in AOT (0.2 M) reverse micelles as a function of the amount of added water.

for the behavior of cyclohexane solutions should be excluded, simply by the fact that solvent relaxation does not take place in nonpolar solvents like cyclohexane. An alternative explanation for the observed REES are interactions between hemicyanine molecules. Such an interaction might be understood in terms of a ground state equilibrium, between H7HC aggregates consisting of different aggregation numbers. This interpretation is supported by a blue-shift in absorption and the appearance of a second absorption peak at 390 nm, when the concentration is raised to 50 $\mu$M.[11] A blue shift in the absorption spectrum due to aggregation has been found for several related hemicyanines and has been assigned to a repulsive interaction between the individual chromophores leading to "H-aggregates".[12−14] Of course, the amphiphilic nature of the dye provides the driving force for the formation of aggregates.

When H7HC was incorporated in the cyclohexane/AOT solution before water addition, we observed a huge REES of 75.5 nm (Figure 3). The appearance of REES might be due to solvent relaxation processes or to dye aggregation. However, both reasons are excluded here: A value of 75.5 nm is too large for a regular solvent relaxation phenomenon, and at the given surfactant (0.2 M) and dye concentrations (5 $\mu$M) the presence of two H7HC molecules in one micelle is most unlikely.

The chemical structure of the dye and AOT leads to the assumption that the positively charged chromophore is located close to the negatively charged AOT headgroup and its aliphatic chain aligns with those of the surfactant molecules. The suggested probe location is supported by results obtained for various normal micelles.[15] The fluorescence and absorption maxima in these systems are strongly red shifted in comparison to the values obtained in solvents. Following the arguments by Loew[16] and

(11) Stathatos, E.; Lianos, P.; Laschewsky, A. *Langmuir*, in press.
(12) Song, Q.; Evans, C. E.; Bohn, P. W. *J. Phys. Chem.* **1993**, *97*, 13736; **1993**, *97*, 12302.
(13) Evans, C. E.; Bohn, P. W. *J. Am. Chem. Soc.* **1993**, *115*, 3306.
(14) Hall, R. A.; Thistlethwaite, P. J.; Grieser, F.; Kimizuka, N.; Kunitake, T. *J. Phys. Chem.* **1993**, *97*,11974.
(15) Unpublished results.
(16) Loew, L. M.; Simpson, L.; Hassner, A.; Alexanian, V. *J. Am. Chem. Soc.* **1979**, *101*, 5439.

Fromherz,[9] this indicates that the positive charge at the pyridinium ring shifts, upon excitation, toward an environment of increased polarity, sensed by the aniline ring. This finding suggests that the aliphatic end of H7HC is located in the hydrophobic domain of the amphiphilic system, whereas the aniline ring is directed toward the bulk water. Moreover, it can be deduced that an increase in the red shift of the fluorescence and absorption maxima indicates an increasing polarity gradient sensed by H7HC.[9,16]

Though we know that the cyclohexane solution of 0.2 M AOT contains small traces of water (≤0.1 M, as indicated by the solvent manufacturer), the attractive interaction between the positively charged pyridinium ring and the negatively charged sulfonate group is, if at all, only to a minor extent intermediated by water molecules. The position of the absorption maximum at 441 nm is beyond the values for regular solvents (Figure 1). Since then the low dye concentration rules out the possibility of H7HC aggregation, we suggest the formation of an ionic adduct with spectral properties different from those of a solvated H7HC molecule. The extremely large REES indicates that a reorientation of the AOT headgroup after excitation leads to an extremely strong stabilization of a relaxed state of minimum free energy. Since excitation shifts the positive charge from the pyridinium to the aniline ring, an energetically unfavorable Franck−Condon state is created. The negative sulfonate group is located at the neutral pyridinium ring, and the AOT headgoup has to reorient to get the negative charge close to the aniline ring. This "ionic relaxation" process occurs apparently on the same time scale as the fluorescence lifetime and finally leads to a strong energetic stabilization by forming the ionic adduct. **For the present system the suggested adduct formation seems to be the most reasonable explanation of the observed spectral behavior.**

It has been demonstrated by several techniques that water in AOT reverse micelles stays up to a certain water/surfactant ratio "structured".[1,17] The term "structured" means that the water molecules strongly interact with the AOT polar headgroup and the $Na^+$ counterion. For the given concentration of 0.2 M AOT, all water molecules up to a concentration of approximately 2 M are supposed to be structured. Further water addition leads to the formation of a water pool. Thus at a concentration of approximately 4 M, a water pool with normal water properties is already formed. It is interesting in this respect that the plateau of the curves in both Figures 1 and 2 is reached at water concentrations, above 2 M. The hemicyanine dye H7HC thus offers four spectroscopic observables for the investigation of the state of the micellar system: absorption maximum (Figure 1), the blue and red excited fluorescence emissions (Figure 2), and the REES (Figure 3).

The H7HC ground state adduct exhibits a strongly blue-shifted absorption (441 nm). Water addition increases the absorption wavelength up to a saturation value of 461 nm reached at approximately 5 M water concentration. Increasing the amount of structured water up to a concentration of 2 M leads to a change of 14 nm, whereas the red shift above 2 M is much less effective. Apparently, the red shift caused by additional structured water is larger than the one due to water pool formation. The shift of the absorption to the range observed in solvents by addition of water indicates that the hydration of the AOT headgroup and of the positively charged chromophore weakens the attractive interaction.

As already pointed out, the blue and red excited emissions prior to water addition show a huge difference (75.5 nm). **Addition of water leads to a dramatic decrease of the REES** so that at 1 M water it is reduced to about 20 nm. Similar behavior was found for the blue-excited emission: The emission wavelength increased from 506 to 581 nm due to addition of 1 M water. We conclude that approximately half of the maximum structured water (2 M) is necessary to hydrate both components to such an extent that the formation of an ionic adduct becomes less favored. Increasing the amount of structured water leads to a less efficient change, and at 2 M we determined a REES of 14 nm, which is characteristic of solvent relaxation processes of micellar H7HC microenvironments.[15]

Increasing the water concentration above 2 M, and thus forming a water pool, changes the spectroscopic parameters to a much smaller extent. The further decrease in the REES indicates that a growing water pool lowers the viscosity of the water molecules in the close vicinity of the aniline ring. The further blue shift of absorption and red edge excited fluorescence maximum indicates that a growing water pool enlarges the polarity gradient at the interface sensed by H7HC. Thus, the environment of the aniline ring appears to be in closer contact with the water pool than the pyridinium ring, which is a further confirmation of the suggested H7HC localization.

## Conclusions

The formation of an ionic ground and excited state adduct between the sulfonate group of AOT and the pyridinium ring makes H7HC an outstanding dye for the characterization of the state of the water in AOT reverse micelles. The most impressive observable is the blue edge excited fluorescence which is extremely sensitive to the smallest amounts of "structured" water. Its change of 93 nm within the investigated range exceeds the water-induced changes of spectral parameters of other dyes.[4] Moreover, H7HC gives information about both types of water and has the additional advantage of using very low dye concentrations (5 $\mu$M).

**Acknowledgment.** This work was financed by the Human Capital and Mobility Project CHRX-CT-0273. M.H. is especially thankful to the European Commission that allowed him by the above project to visit the hosting laboratory in Patras.

LA9609074

(17) Baglioni, P.; Nakamura, H.; Kevan, L. *J. Phys. Chem.* **1991**, *95*, 3856 and references therein.