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Probing solute distribution and acid-base behaviour in water-in-oil microemulsions by fluorescence techniques☆

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Abstract

The distribution and acid-base behaviour of the four solutes harmine, chromotropic acid (4,5-dihydroxynaphthalene-2,7-disulfonate, disodium salt), 2-naphthol and 5,10,15,20-tetrakis [4-trimethylammonium)phenyl]-21*H*,23*H*-porphine tetra-*p*-tosylate (TTMP) have been studied in water-in-oil (w/o) microemulsions using fluorescence and absorption spectroscopy. Carbon tetrachloride is a quencher of fluorescence of these compounds, and studies using this as oil phase in microemulsions show that chromotropic acid is located in the water domain, TTMP at the surfactant-water interface, while the distribution of harmine or 2-naphthol depends on the degree of protonation. Detailed studies have been made on harmine. In water/AOT/cyclohexane microemulsions the cationic form is observed up to much higher apparent pH than in aqueous solutions. An important factor is shown to be the compartmentalisation of hydroxide ions between water pools. Similar effects are observed with the other probes, and it is suggested that compartmentalisation of hydrogen or hydroxide ions is a major effect in many acid-base reactions in microemulsions. The validity of the concept of pH in microemulsions under these conditions is questioned. Fluorescence lifetime measurements are also shown to provide information on the dynamics of the processes, and demonstrate the importance of diffusion of solutes from organic solvent to the microemulsion pool. A comparison is made of the behaviour of harmine in water/AOT/cyclohexane and water/lecithin/cyclohexane microemulsions.

Keywords: Water AOT cyclohexane; Carbon tetrachloride; Harmine; Microemulsion

1. Introduction

Water-in-oil (w/o) microemulsions are thermodynamically stable systems consisting of nanometer sized individual domains of water (sometimes called water pools or droplets), which are separated by a monolayer of amphiphile from an oil continuous region. Their properties have been extensively reviewed [1–7]. One particularly im-

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portant property is the possibility of varying the size of the water pool over a wide range by changing the water/amphiphile mole ratio (ω_0). Microemulsions find uses in areas as diverse as microreactors for chemical or enzymatic catalysis [4,8–10], matrices for the formation of nanoparticles or monodisperse polymers [11,12] and model systems for biological membranes [4]. For many of these applications, knowledge of the microscopic distribution of solutes is important.

Various techniques have been applied to the study of solute distribution, including NMR spectroscopy, small angle X-ray and neutron scattering, fluorescence and various other photophysical methods [4,6]. In general, with small solute molecules three distinct solubilisation sites can been considered, the water pool, the bulk organic liquid, and the interface. If large, or highly charged, solutes are present, they may also change the microstructure of aggregates or even induce formation of small aggregates around the solute [4]. In cases where acid-base reactions of solute may occur, the dominant site for the solute and its conjugate acid or base may be different. However, it is found, in general, that microemulsions appear to exert a 'buffering' effect, and that the apparent pH inside a water droplet is different from that of the solution added [13,14]. Various methods have been used to try to assess the acidity of the microemulsion water droplets [15–19]. Potentiometric measurements on buffered solutions in water/AOT (sodium(bis-(2-ethylhexyl)sulfosuccinate)/heptane solutions at high ω_0 values have shown similar electrical response to that shown by the pure aqueous solutions [15]. However, such measurements are not possible at low ω_0 values, and the lack of understanding of the effect of interfacial interactions between the electrodes and microemulsion on observed potentials makes interpretation of these observations highly speculative. An alternative approach has been to measure acid-base equilibria of suitable indicators spectrophotometrically, and to calculate local pH, assuming the applicability of the Henderson-Hasselbach equation [15–17]. However, this method has been criticised [4], since micellar water has different properties from bulk water, and the pK of the indicator in such media cannot be measured independently. Another technique that has been used in the case of phosphate buffers to obtain an empirical acidity scale is based on the differences between values of the ^{31}P NMR chemical shift in pure water and microemulsions, assuming that the pK value of the phosphate is the same in both the systems [16,18]. Support for such an approach in the case of phosphate buffers in water/lecithin/deuterobenzene microemulsions is given by the observation of a single ^{31}P NMR signal [20]. However, this method is only applicable where an appropriate buffer, such as phosphate, is present at a sufficiently high concentration such that a uniform distribution is observed in all the droplets. An additional approach has been to determine the water acidity kinetically in terms of proton transfer reactions of certain fluorescent probes [19]. This method is attractive, although it requires both suitable probes, whose properties may be different from other solutes of interest, and information on their localisation sites.

In this paper we will address some points concerning acid-base behaviour of solutes in microemulsions. In particular we will consider three questions:

How do we or can we define pH in microemulsions?

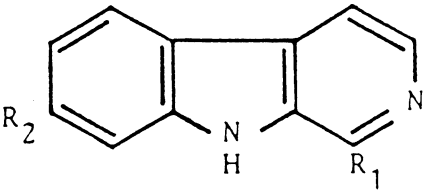
How important is compartmentalisation of H + or OH- ions in the acid-base behaviour of solutes in microemulsions?

How is the diffusion or distribution of the probe affected by the ω_0 value of the microemulsion?

For this, we will study the acid-base equilibria of a number of fluorescent probes. Particular emphasis will be given to a group of compounds, the β-carbolines. We have been interested in the photophysical behaviour of these compounds [21–23]. Both synthetic and naturally occurring β-carboline derivatives are known, and they show photosensitizing activity towards a variety of systems, including bacteria, fungi, viruses, etc [22]. In addition, they are fluorescent products of tryptophan produced in human lenses [24], and may act as markers of metabolic changes. However, for such applications, it is important to know their localisation in biological media. To obtain a deeper understanding on their distribution in such systems, a study has been undertaken of their photophysical behaviour in water/AOT/cyclohex-

ane microemulsions [22]. In aqueous media, the β-carbolines undergo complex acid-base equilibria in both ground and excited states to produce cationic, neutral and (in the lowest excited singlet state) zwitterionic forms [21–23]. Structures and equilibria are shown in Fig. 1. In contrast, in nonprotic organic solvents only the neutral form is observed. The ground and excited state pK_a values are different [25], thus providing a further valuable tool for probing distribution and acid-base behaviour in micro-heterogeneous systems. In water/AOT/cyclohexane microemulsions, it was found [22], that the three compounds norharmane, harmane and harmine appear to be located predominantly at the surfactant/water interface, but that on decreasing pH there is an increase in partitioning towards the water domain. In dynamic fluorescence studies, four kinetically distinguishable species are observed in the excited state, neutral species in cyclohexane, neutral species in droplets, cation and zwitterion. More detailed studies were made on harmine [22], and these will be described in Section 3.

Water-in-oil (w/o) microemulsions can also be formed using carbon tetrachloride as the organic solvent [26–30], This has an advantage for photophysical studies as it is a good excited state quencher [31], and if luminescence methods are



(1)HARMINE	R_1=CH_3	R_2=OCH_3	pK_a=8.0	$pK_a(S_1)$=12.9
(2)HARMANE	R_1=CH_3	R_2=H	pK_a=7.7	$pK_a(S_1)$=12.8
(3)NORHARMANE	R_1=H	R_2=H	pK_a=7.2	$pK_a(S_1)$=13.0

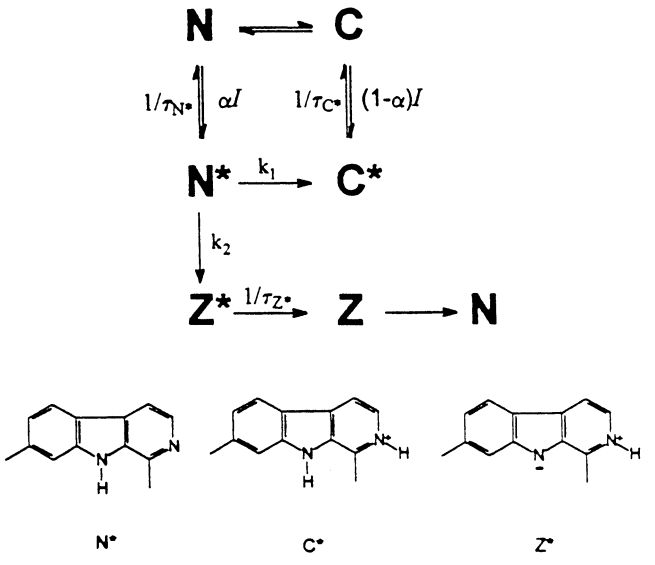


Fig. 1. Structures and acid-base equilibria of β-carbolines.

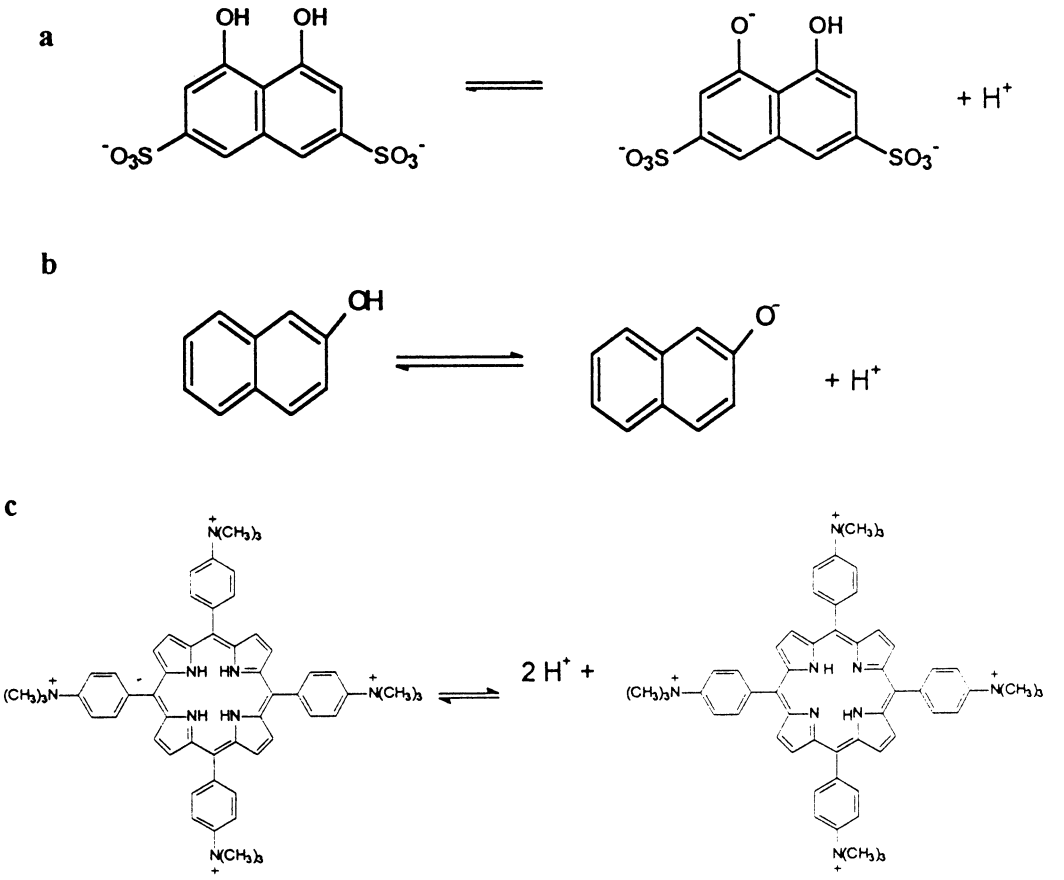


Fig. 2. Structures and acid-base equilibria of (a) chromotropic acid; (b) 2-naphthol; (c) TTMP.

used any fluorescence must arise from solute either in the aqueous domain or the interface.

We have also been interested in w/o microemulsions using lecithin as amphiphile. These have been studied by a variety of spectroscopic and scattering techniques [32–34], and may act as good models for biological cells. In addition, under certain conditions, lecithin forms highly viscoelastic 'microemulsion gels' [35].

In this paper, we will compare the behaviour of harmine in water/AOT/cyclohexane, water/lecithin/cyclohexane and water/AOT/CCl^4 microemulsions. In addition, we will also report the results of a fluorescence study of the distribution and acid-base behaviour in w/o microemulsions of three other probes, 2-naphthol, chromotropic acid and the cationic porphyrin TTMP (5,10,15,20-tetrakis[4 - (trimethylammonium)phenyl] - 21*H*,23*H*-porphine tetra-*p*-tosylate salt). These three compounds (Fig. 2) were chosen, as they are likely to have dominant distribution in organic phase, aqueous domain and interface, respectively.

2. Experimental

The surfactant AOT was purified as described earlier [22]. Chromotropic acid was recrystallised from ethanol–water. 2-Naphthol was recrystallised from hot water after treatment with decolourising charcoal [36]. The lecithin used in this study was a gift from Dr A. Khan, and is from

the same batch as used in [32]. Carbon tetrachloride was purified by washing with 0.1% solution of KOH in ethanol, the ethanol removed by standing over alumina, the solution dried over anhydrous calcium chloride, and finally fractionally distilled from anhydrous potassium carbonate. Millipore or bidistilled water was used in the preparation of microemulsions. Other reagents were of the purest grade commercially available and were used as purchased.

UV/visible absorption and fluorescence spectra were measured on Shimadzu UV-2100 spectrophotometer and SPEX Fluorolog 111 spectrofluorimeters respectively. Further details of the apparatus and methods are given in [22]. Fluorescence decays were obtained using time-correlated single photon counting, as described previously [21–23,37].

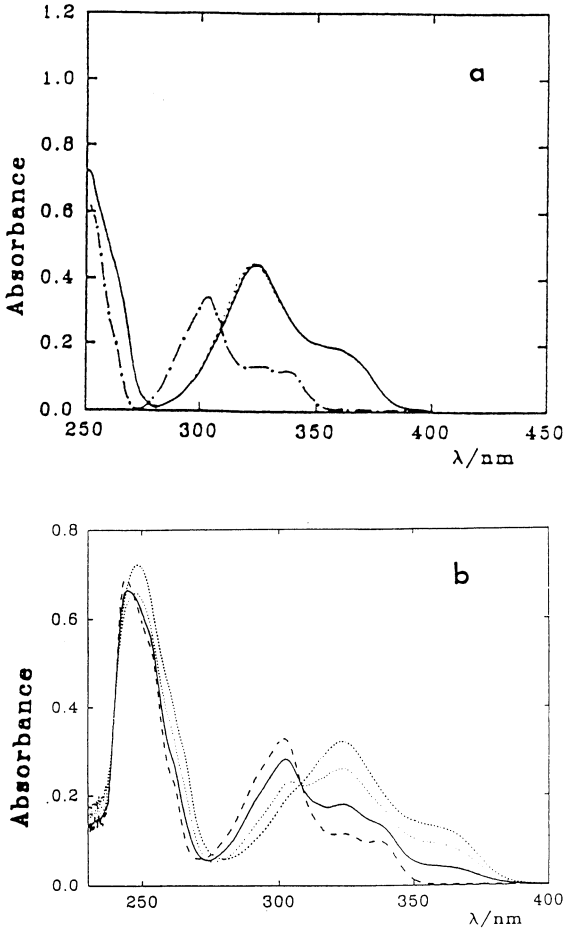


Fig. 3. Absorption spectra of harmine in water/AOT/cyclohexane microemulsions: (a) $\omega_0 = 5$ at pH 6.4 (-), 12.4 (- -) and 12.8 (-.-); (b) $\omega_0 = 12.5$ at pH values 5.5 and 12.4. For details see [22].

3. Results

3.1. Harmine

UV/visible absorption spectra were run of solutions of harmine in water/AOT(0.1 M)/cyclohexane microemulsions at $\omega_0 = 5$ and various pH values (Fig. 3; note, the pH value is of the unbuffered aqueous solutions added). Between pH 6.8 and 12.4 only, the absorption spectrum of the cationic form is observed, while above pH 12.8, the characteristic absorption of the neutral form is also seen. The pK_a of harmine in water is 8.0, so that dissociation of the harmine cation is occurring at much higher apparent pH than in aqueous solution. Similar spectral changes are observed in the fluorescence spectra (Fig. 4), but the decrease in cation emission and increase in neutral form emission occurs at slightly lower apparent pH (10.3–12.0). In contrast to what is observed in aqueous solution [23], no significant emission is observed from the zwitterion, suggesting that harmine is either predominantly at the interface, or may partition to the bulk organic solvent. Partitioning studies between octanol and water [22] and solubility measurements [38] show that the neutral form only has a low solubility in the aqueous phase. The behaviour of harmine in these microemulsions has also been studied at various ω_0 values [22]. Representative absorption and emission spectra at $\omega_0 = 12.5$ are shown in Figs. 3 and 4, and good isosbestic points are observed. At this ω_0 value a weak zwitterion emission is also observed. Fluorescence lifetimes also give information on acid-base behaviour and distribution, and decays have been studied for $\omega_0 = 12.5$ looking at emissions at 350, 410 and 500 nm, wavelengths corresponding predominantly to emission from excited neutral, cation and zwitterion, respectively, [22]. Using the emission spectra, and observed kinetic decays at these three wave-

Fig. 4. Fluorescence spectra of harmine in microemulsions (a) water/AOT/cyclohexane microemulsions, $\omega_0 = 5$ at pH 10.3 (. .), 12.0 (- . . -) and 12.8 (- . -); (b) water/AOT/cyclohexane microemulsions, $\omega_0 = 12.5$ at various pH values between 5.5 and 12.4; (c) water/AOT/CCl4 microemulsions at $\omega_0 = 5$.

lengths, analysis has been made in terms of four species, neutral in microemulsion droplets, neutral in cyclohexane, cation and zwitterion [22]. In these studies, all the four species were observed over the whole apparent pH range studied, showing that there must be partitioning in both ground and excited states between the aqueous domain and the bulk cyclohexane. The lifetime of the neutral form in cyclohexane in these microemulsions, decreases with increasing ω_0, and from analysis of the data it was shown that this diffuses into the droplets with rates close to diffusion control [22].

If harmine is located predominantly at the amphiphile/water interface, the observation of cation at much higher apparent pH values than in water may be due, in part, to changes in its pK_a. However, this is unlikely to be the whole story, as although the ground and excited state pK_a values differ by 5 pH units in pure water, the changes in ground and excited state acid-base equilibria seen in microemulsions only appear to differ by 1–2 pH units. It is difficult to see how an interface could affect these properties so dramatically. Under the unbuffered conditions used in this study the compartmentalisation of hydroxide ions between microemulsion droplets must also be important. Following the ideas initially advanced by Tachiya [39] and Infelta [40], and subsequently developed by Almgren [41], we have used a Poisson distribution and calculated the fraction of microemulsion droplets containing between 0 and 4 hydroxide ions [22]. These are illustrated in Fig. 5, and give what, on first sight, is an unexpected result; up to fairly high apparent pH values the number of droplets containing hydroxide ions is very small. The previously reported buffering effect of microemulsions [13,14] can thus be seen to result, at least in part, from the compartmentalisation effects of hydroxide ions between the nm sized water domains. Similar results have been presented for the partitioning of hydrogen ions [42,43]. Under these conditions, it is not clear that pH has any real thermodynamic or kinetic significance in microemulsions.

Both the structure and size of microemulsion droplets can be affected by the presence of a cosurfactant. This has consequences on the degree of compartmentalisation. Solutions of harmine in water ($\omega_0 = 5$)/AOT (0.1 M)/cyclohexane were studied in the presence of various concentrations of 2-ethylhexanol. In both the absorption and fluorescence spectra, the contribution of the neu-

tral form of harmine increased with increasing concentration of cosurfactant. Representative fluorescence spectra at apparent pH 11.37 and different 2-ethylhexanol concentrations are shown in Fig. 6. These clearly indicate a decrease in the emission due to the cationic form at 403 nm on increasing cosurfactant concentration, and are in agreement with the increasing size of the water droplets, and, consequently, the increasing of the probability that the same droplet will have both hydroxide ion and harmine.

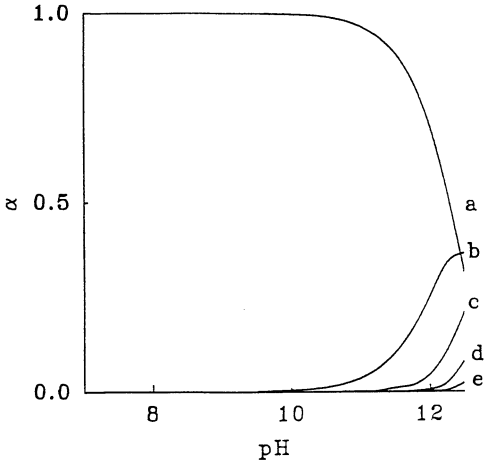


Fig. 5. Poisson distribution of number of hydroxide ions per microemulsion droplet as a function of pH for the system water ($\omega_0 = 12.5$)/AOT (0.1 M)/cyclohexane for (a) 0, (b) 1, (c) 2, (d) 3, (e) 4 hydroxide ions per droplet. Reprinted with permission from A.P. Varela, M. da Graça Miguel, A.L. Maçanita, H.D. Burrows and R.S. Becker, J. Phys. Chem. (1995) (99) 16093. Copyright (1995) American Chemical Society.

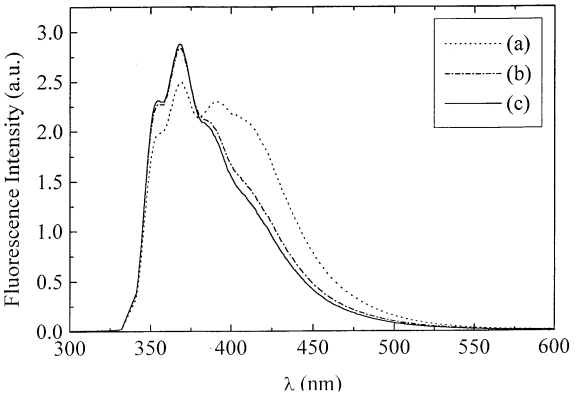


Fig. 6. Fluorescence spectra of harmine in water (ω_0 5)/AOT (0.1 M)/cyclohexane microemulsions, pH 11.37, in the presence of 2-ethylhexanol (a) 0.2 M; (b) 0.4 M; (c) 0.6 M.

Carbon tetrachloride is found to quench harmine fluorescence. Absorption and fluorescence spectra were run on harmine in H_2O/AOT (0.1 M)/CCl_4 microemulsions, with $\omega_0 = 5$. A typical fluorescence spectrum is shown in Fig. 4(c), and again shows that emission comes just from cation in the water domain. The pH of the water pools was increased by adding aqueous ammonia, and under these conditions it was possible to see the fluorescence of the neutral form at 367 nm. This indicates that some of the neutral harmine must be located at the interface, since neutral harmine has a low solubility in water, and the fluorescence of neutral harmine in CCl_4 is quenched. However, it was not possible to get any quantitative information, as with time, marked changes were observed in the emission spectrum, and new bands appeared at longer wavelengths, possibly due to photodegradation [43]. Photodegradation of β-carbolines in chlorinated hydrocarbons has been reported earlier [44], and these results confirm that some fraction of the harmine must be in the organic solvent. Combination of this result with the observation of fluorescence from the neutral form of harmine at the interface is in agreement with the idea already presented [22] of a dynamic partitioning of harmine between the droplet and the organic solvent.

The behaviour of harmine was also studied in water/lecithin (0.05 M)/cyclohexane microemulsions for ω_0 between 12.5 and 17.5. This range was limited by the stability of these microemulsions. In both absorption and fluorescence spectra, the cationic form of harmine was observed up to higher apparent pH values than in pure water. For example, in Fig. 7 fluorescence spectra are shown for microemulsions at pH 11.75 and ω_0 12.5 and 17.5, where emissions from both the cationic and neutral forms are observed. As with water/AOT/cyclohexane microemulsions, the relative contribution of the neutral form increases with ω_0. Fluorescence decays of harmine at 350, 410 and 500 nm were obtained in these microe-

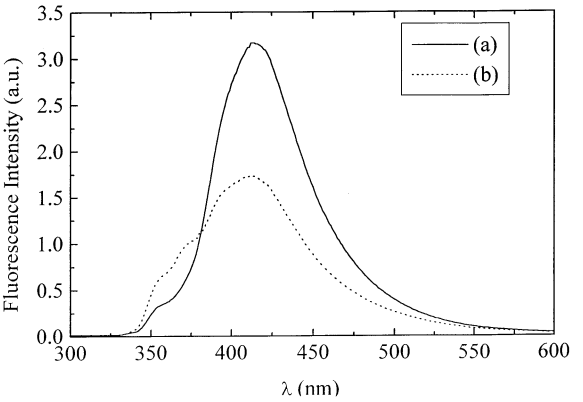


Fig. 7. Fluorescence spectra of harmine in water/lecithin (0.05 M)/cyclohexane microemulsions at pH 11.75 for (a) ω_0 12.5; (b) ω_0 17.5.

mulsions at various pH values between 5.3 and 12.5. Again these were analysed in terms of four components. For the specific case of ω_0 17.5, pH 11.92, the decay components were attributed to neutral in water pool ($\tau_1 = 0.67 \pm 0.11$ ns), neutral in cyclohexane ($\tau_2 = 4.67 \pm 0.28$ ns), cation ($\tau_3 = 6.67 \pm 1.17$ ns) and zwitterion ($\tau_4 = 18.5 \pm 1.5$ ns). The zwitterion contribution was fairly small, and appeared to be markedly less than in water/AOT/cyclohexane microemulsions. On decreasing the pH at constant ω_0, the contribution of the cation relative to the neutral form increased, while the lifetime of the neutral form in both water pool and cyclohexane decreased. These decreases in τ_1 and τ_2 parallel the behaviour in water/AOT/cyclohexane microemulsions, and suggest that neutral excited harmine can diffuse from cyclohexane into the water pool, where it is quenched by reaction with protons and/or water. For constant pH, the relative contribution of the neutral form decreased with decreasing ω_0, in agreement with what is observed in the fluorescence spectra.

3.2. Chromotropic acid

Well-defined changes are observed in both absorption and fluorescence spectra on varying pH of aqueous solutions of chromotropic acid. Typical spectra are shown in Fig. 8. From these spectral changes, values of $pK_a = 5.65$ and $pK_a^* = 2.19$ were determined. The values for the ground state are in reasonable agreement with the literature data [45].

Carbon tetrachloride was found to quench the fluorescence of both the chromotropic acid and its conjugate base. Attempts to study chromotropic acid in water/AOT/CCl_4 microemulsions by fluorescence were unsuccessful due to phase separation. However, chromotropic acid was soluble in water/AOT (0.1 M)/carbon tetrachloride-isooctane (50% (v/v)) up to $\omega_0 = 6$. A reasonably intense band around 430 nm was observed in the fluorescence spectra in this medium, and confirms that chromotropic acid is located predominantly inside the water domain.

Absorption and fluorescence spectra were run for chromotropic acid in water/AOT (0.1 M)/cyclohexane microemulsions at various pH values for ω_0 between 2.5 and 40. Typical data are illustrated in Fig. 9. At the lowest pH value studied (2.5) for $\omega_0 = 2.5$, the major contribution to the absorption spectrum came from the conjugate base form. However, with increasing ω_0, significant contributions were also observed from the acid. These results are consistent with the acid-base equilibria of chromotropic acid being dependent on the compartmentalisation of H^+ between the water pools. At low ω_0 values, there are few protons in each water pool, and equilibrium lies on the side of chromotropic acid conjugate base. As ω_0 increases, more pools contain both protons and probe, and between ω_0 10 and 40 it is possible to see equilibria between the acid and conjugate base forms over the apparent pH range 2–7.

Fluorescence spectra were also recorded for chromotropic acid in water/AOT (0.1 M)/cyclohexane microemulsions (Fig. 9). At the lowest ω_0-value (2.5), the spectra appeared to show emission from both acid and conjugate base forms. However, this result contradicts what is observed in absorption spectra. A more probable explanation is that there is complexing between chromotropic acid and AOT head group, as has been suggested for related compounds [19]. Above $\omega_0 = 10$, the dominant emission is at around 430 nm, and is attributed to the conjugate base. More detailed studies on this system are in progress and will be reported in due course.

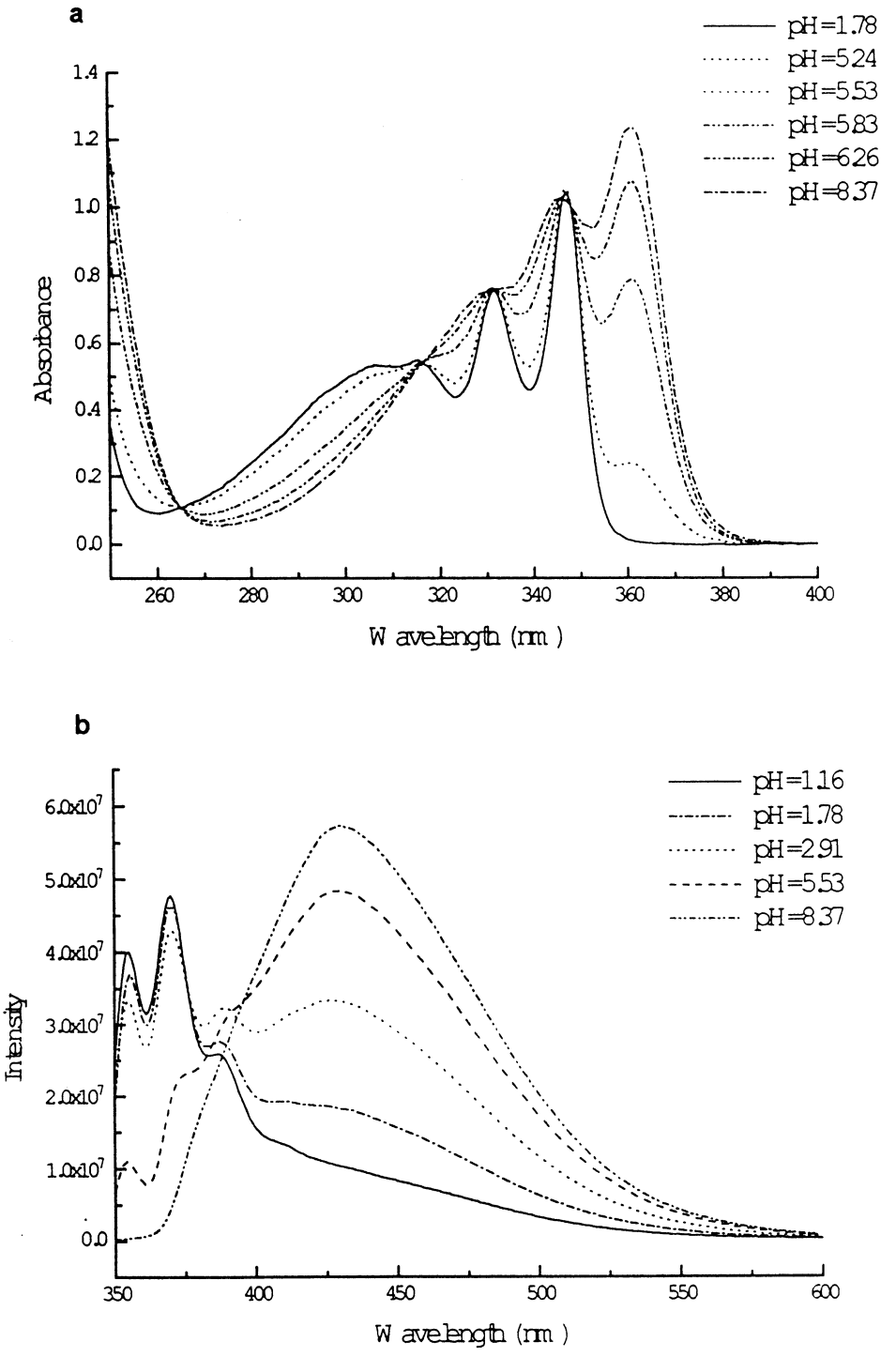


Fig. 8. (a) Absorption and (b) fluorescence spectra of aqueous solutions of chromotropic acid at various pH values.

3.3. 2-Naphthol

The behaviour of 2-naphthol in H_2O/AOT/heptane microemulsions has been reported earlier [19]. From differences between absorption or fluorescence spectra in this medium and pure heptane it was suggested that the solute is located predominantly at the interface in the microemulsion. Surprisingly this molecule did not undergo deprotonation in its excited state, even though its low pK_a^* value (2.8 [46]) would favour this process. This was interpreted in terms of complexing the naphthol to the AOT sulfonate headgroup [19]. However, it is difficult to see what the driving force is for such an interaction.

We have studied the behaviour of 2-naphthol in water/AOT(0.1 M)/CCl_4 at various pH values with ω_0 5–10 using both absorption and fluorescence. Studies in ethanol solution show that carbon tetrachloride quenches both the neutral and ionic forms of 2-naphthol with rates close to diffusion control [43], so that any fluorescence can only come from the probe in the water pool or interface. Up to apparent pH 13 there is no sign

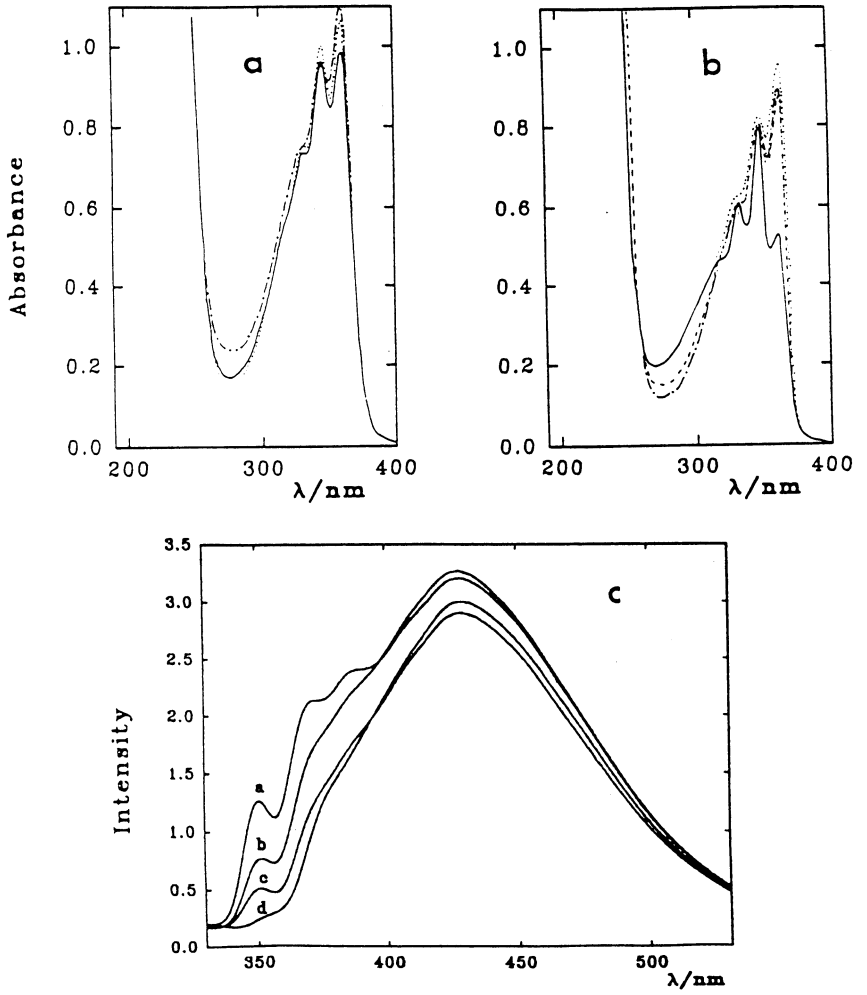


Fig. 9. Absorption spectra of aqueous solutions of chromotropic acid in water/AOT/cyclohexane microemulsions at various pH values (2.1–7.0) at ω_0 (a) 5; (b) 15; (c) fluorescence spectra of aqueous solutions of chromotropic acid in water/AOT/cyclohexane microemulsions at $\omega_0 = 10$ at various pH values between 2.0 and 6.1.

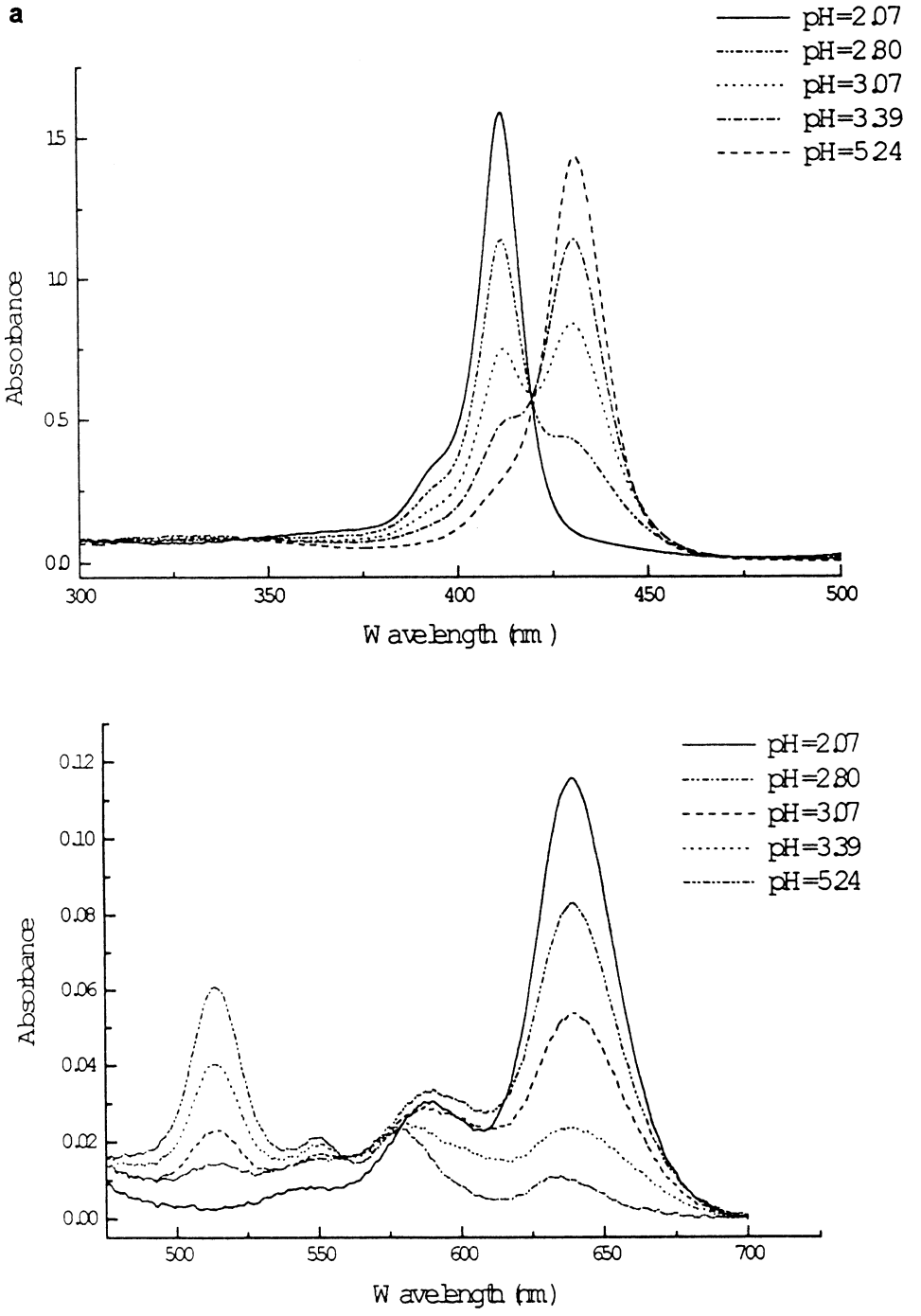


Fig. 10. (a) Absorption spectra of Soret and Q band region of aqueous solutions of TTMP in water at various pH values; (b) spectrophotometric titration curves for absorption changes at 411.8 nm (triangles) and 430.5 nm (squares and circles) for aqueous solutions of TTMP.

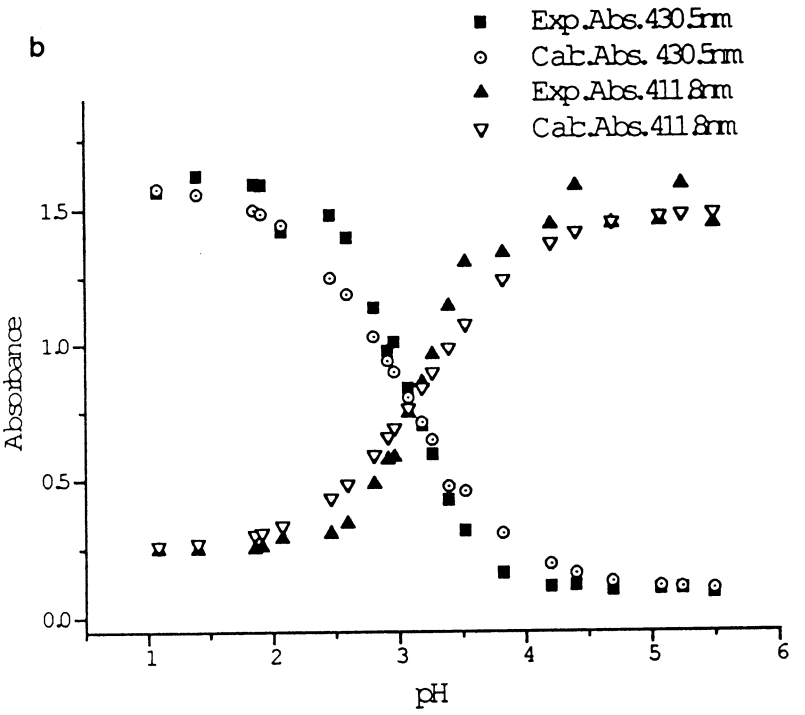


Fig. 10. (*Continued*)

of any fluorescence in these microemulsions, showing that the excited 2-naphthol is completely quenched. It must either be in the organic phase, or have ready access to it during its lifetime. If, as suggested [19], it is complexed by the AOT then the complex must be nonfluorescent. However, sulfonates are not known to be good quenchers of fluorescence of aromatic compounds. We feel that the most plausible explanation for our observations is that most of the 2-naphthol is in the carbon tetrachloride. At apparent pH 13.15, $\omega_0 =$ 10, a very weak fluorescence around 420 nm is observed. This is attributed to the 2-naphtholate anion, and must arise from the small percentage of water pools, which have both probe and hydroxide ions.

3.4. TTMP

Absorption spectral studies on aqueous solutions of TTMP show spectral changes in the pH range 2–5. Spectral changes in the 350–500 nm region (Soret band), together with a titration curve are shown in Fig. 10. From this, a value of $pK_a = 3.11$ was obtained. Changes are also observed in the 500–700 nm region (Q band), and the change in spectrum from four to two bands consistent with a change in symmetry [47] due the double proton transfer reaction shown in Fig. 2. Changes in fluorescence spectra are also observed over this pH range, and are also shown in Fig. 11. From these a value of $pK_a^* = 2.93$ was obtained. Solubility measurements and partitioning studies of TTMP between water and carbon tetrachloride show that it is completely insoluble in CCl_4, and partitions both above and below the pK_a to the aqueous phase.

Preliminary studies have been made of the porphyrin in water/AOT/CCl_4 microemulsions up to $\omega_0 = 6$ [43]. These show that TTMP only exists as the free base in these systems. However, slight differences are observed in both absorption and fluorescence spectra, compared with pure water, and it is probable that this positively charged compound is located at the AOT-water interface. It is worth noting that the molecule has a discotic

shape, and its size is comparable to the radius of the water pool. From X-ray diffraction (XRD) measurements, the diameter of 5,10,15,20 tetrakis(phenyl)-21*H*,23*H*-porphine diacid is about 1.5 nm [48], while if we calculate the diameter of a water droplet for $\omega_0 = 6$ using data for water/AOT/decane microemulsions [49], we get a value 1.44 nm. Under these conditions, it is very probable that we are in the suggested situation [4] where the aggregates are formed round the porphyrin, and are stabilised by electrostatic interactions.

4. Discussion

It has long been known [13–15] that when aqueous solutions of a certain pH are added to produce w/o microemulsions, the apparent pH of the resultant water domains is different from the value of the original aqueous solution. In some cases, buffers are added to try to avoid this problem. However, the microemulsion properties of water/surfactant/oil systems may be altered by the presence of buffer, and for many applications it is desirable to prepare buffer-free solutions.

The main reasons proposed for this effect of microemulsion can be divided into effects of microemulsion structure and interfaces on p*K* values or on the autoprotolysis of water, possibilities of pH gradients in microemulsions and distribution effects of solutes, hydrogen and hydroxide ions. In this study, we have looked at the behaviour of four probes, which can be located in the water microdomain, interface or oil, depending on ionisation. In all cases the apparent pH of solutions is found to differ from that of the added solution. Fluorescence studies using harmine, which shows different acid-base equilibria in ground and excited states, suggest that the dominant effect is not a change in pK_a when the probe is at the interface. Instead it is suggested that compartmentalisation of hydroxide and solute ions between the water pools is important. Similarly, it is shown that at pH < 7, compartmentalisation of hydrogen ions is also important. In these circumstances, attempts to define 'apparent pH scales' are of limited use. However, the buffering effect of microemulsions, caused by this compartmentalisation, does have one very important practical consequence. Under these conditions, the majority

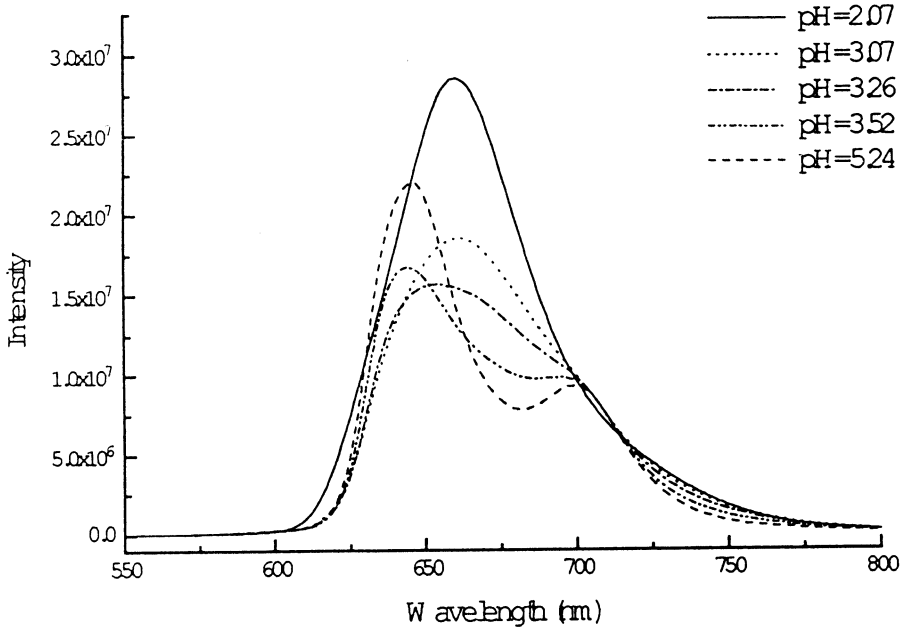


Fig. 11. Fluorescence spectra for aqueous solutions of TTMP at various pH values.

of water pools will be close to neutrality so that for any enzymic or other process which would normally occur in aqueous solution around pH 7, it is not necessary to add any extra component if the reaction is carried out in w/o microemulsion droplets.

Fluorescence lifetime measurements on harmine in water/AOT/cyclohexane microemulsions also show another important effect in these systems; that of diffusion of solutes between organic solvent and the water microdomains. This process can be rapid, with rates that depend upon diffusion control.

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