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The use of Nile Red to monitor the aggregation behavior in ternary surfactant–water–organic solvent systems

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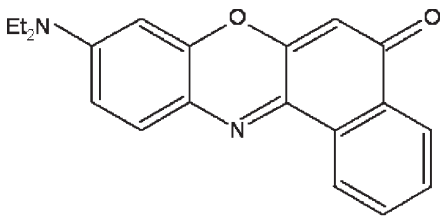
ABSTRACT: Ternary systems of surfactants, water and organic solvents were studied by monitoring the steady-state fluorescence of the versatile solvatochromic probe Nile Red. We found not only that Nile Red can be used throughout the whole isotropic regions in the phase diagram, but also that subtle changes in the aggregation state of the surfactant can be monitored. The formation of inverted micelles in *n*-hexane could be followed upon the addition of small amounts of water, in addition to the formation of normal micelles in water and water–organic solvent mixtures. In aqueous $C_{12}EO_4$ solutions the temperature-dependent micelle-to-vesicle-to-inverted micelle transition was visualized by Nile Red fluorescence. Finally, the incorporation of solvent into the micellar interior could also be monitored using Nile Red as the probe. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: Nile Red; ternary phase diagram; organic solvent; surfactant; micelle; inverted micelle; aggregation

INTRODUCTION

Ternary mixtures of surfactants, water and organic solvents exhibit complex phase behavior. These mixtures of in many cases different kinds of amphiphiles (ionic, non-ionic, soap) are often employed in laundry and other types of detergents. Our interest lies in the aggregation state of the surfactant in the optical isotropic regions of the complex phase diagram.

We found the solvatochromic fluorescent probe Nile Red to be highly efficient in its capability to identify different aggregate morphologies formed by amphiphile mixtures. Nile Red[1] has been used previously in ternary surfactant systems.[2,3] Nile Red is soluble over a very wide range of solvents and shows a large bathochromic absorbance shift with increasing solvent polarity (108 nm from *n*-hexane to water),[4] which is linear with that of the long-wavelength absorption band of the popular $E_T(30)$ probe.[5]



Nile Red

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The $E_T(30)$ value of a solvent is the energy of the intramolecular charge transfer transition of 2,6-diphenyl-4-(2,4,6-triphenyl-1-pyridinium) phenolate, which depends strongly on the polarity of the medium.[6] Nile Red in its ground state has low polarity, whereas in the excited state the molecules undergo an intramolecular transfer of an electron from the donor (dialkylamino group) to the acceptor group which is accompanied by a twist between the donor and the acceptor moieties. This twisted intramolecular charge transfer (TICT) process gives a highly polar state,[5] for which the activation barrier decreases linearly with increasing $E_T(30)$.[7–9] Consequently, the excited state with a large dipole is stabilized in more polar solvents. The large solvatochromic shift makes it possible to excite probe molecules selectively in different environments. In micelles Nile Red is situated in the interface. However, some molecules are facing water, whereas others are located more towards the hydrophobic tails of the amphiphiles. In inverted micelles there are two interfaces, the surfactant headgroup region facing a water pool and the surfactant tail region facing the apolar solvent. Because of the large polarity-dependent absorbance shift, Nile Red in a more polar or apolar environment can be selectively excited.[8] These properties of the probe lead to an excitation-dependent emission maximum (λ_{max}) when different microdomains (e.g. micelles, bilayers or inverted micelles) are present. If there is no aggregation of the surfactant molecules, for example below the critical micelle concentration (CMC) or in a solvent in which surfactant molecules are randomly mixed,[10] no excitation-dependent emission is found.

Since Nile Red is poorly soluble in water, there is a large preference to partition into micelles or other aggregates which offer hydrophobic binding sites. Furthermore, probe molecules have a general tendency to be located at the surface of micelles owing to the large surface-to-volume ratio.[11]

In the present study, we examined in detail the emission maximum of Nile Red (λ_{max}), determined accurately with a log-normal fit, in ternary surfactant–water–organic solvent mixtures. The results allowed us to identify both large and small, subtle changes in the organization of the surfactants in these solutions and to determine the phase behavior.

EXPERIMENTAL

Nile Red was obtained from ACROS (Landsmeer, The Netherlands). A 2.5 mM Nile Red stock solution was made in ethanol and diluted 2000-fold in the surfactant systems. Non-ionic detergent Neodol 1–5 (C_xEO_y, x on average 11, y on average 5) was obtained from Shell Chemicals, penta ethylene glycol mono-*n*-dodecyl ether ($C_{10}EO_5$) from Bachem (Switzerland), tetraethylene glycol mono-*n*-dodecyl ether ($C_{12}EO_4$) from Nikko Chemicals (Tokyo, Japan). LAS-acid (linear alkylbenzenesulfonic acid) from Lever Brothers (Port Sunlight, UK), isostearic acid (Prisorine 3509) from Uniqema (Gouda, The Netherlands) and monoethanolamine (MEA) from BASF. All surfactants used were of technical grade except $C_{10}EO_5$ and $C_{12}EO_4$, which were of the highest purity available, and all other chemicals were of analytical grade. The surfactant mixture consisted of non-ionic Neodol 1–5, LAS-acid and isostearic acid (molar ratio 37:38:25) neutralized with a 10 mol% excess of MEA. MEA–LAS was made by mixing equal molar amounts of MEA and LAS acid. Surfactants were mixed with water and/or organic solvents to the desired ratio (w/w) and stirred until a homogeneous isotropic solution was obtained.

Nile Red fluorescence was measured on an SPF-500c spectrofluorimeter (SLM Aminco) at 25 °C using an excitation wavelength between 490 and 590 nm. Fluorescent emission was measured from 550 to 700 nm at 5 nm intervals. The Nile Red emission maximum (λ_{max}) was calculated using a log-normal fit.[12] In Fig. 1, emission spectra of Nile Red in water, methanol and *tert*-butanol are given. A 5 nm step size for the emission was found to be sufficient to obtain a resolution of several tenths of a nanometre after a log-normal fit. The width of the fitted peak was carefully monitored to make sure that the Nile Red signal is derived from one population. A broadening of the peak could mean that multiple populations were measured and a deconvolution of the peak is necessary. Surface tension was measured using a drop volume tensiometer (Lauda, TVT1).

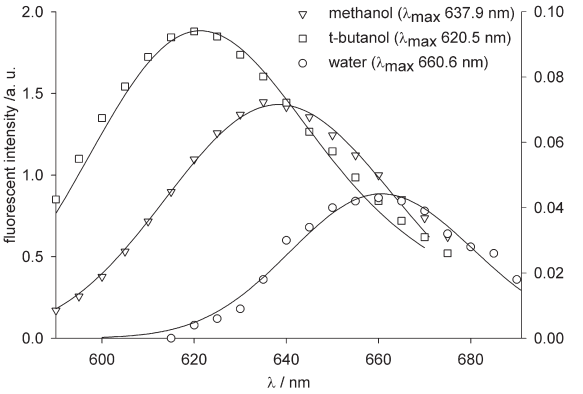

Figure 1. Nile Red fluorescence in methanol, *tert*-butanol and water excited at 550 nm. A four-parameter log-normal fit was used to determine the emission maximum. The intensity of Nile Red in water is very low, therefore the scale was increased (right *y*-axis)

RESULTS AND DISCUSSION

We found Nile Red to be highly effective for measuring the local polarity throughout the ternary phase diagram of surfactants, water and organic solvents. Furthermore, we found that Nile Red could be used to monitor subtle changes in the organization of the surfactant in the solvent systems that we explored.

When a surfactant is dissolved in an apolar medium such as *n*-hexane, it is likely that inverted micelles are formed with their hydrophobic tails directed towards the solvent. This requires, however, hydration of the headgroups of the surfactant molecules in the core of the aggregate. Nile Red can partition either into the interface with water or into the interface between the hydrophobic tails and the apolar solvent. By changing the excitation wavelength, the different probe environments can be selectively excited.[8] It is unlikely that inverted micelles are formed in the complete absence of water.

We measured the excitation-dependent Nile Red fluorescence of a 10% (w/w) Neodol 1–5 solution in *n*-hexane on adding small amounts of water [Fig. 2(a)]. Appreciable changes in λ_{max} were found after the addition of 0.05–1% (w/w) water. A strong increase in polarity (increase in Nile Red λ_{max}) was seen at high excitation wavelengths, which is indicative of the formation of inverted micelles. Hydrated surfactant headgroups will form a region of high local polarity, the 'water pool' of an inverted micelle. This shift in the emission peak coincides with a strong increase in fluorescent intensity at 590 nm. Nile Red, located close to the aqueous core of the inverted micelles, is selectively excited at 590 nm. A slight increase in polarity was found at an excitation wavelength of 490 nm, between 0.05 and 0.5% water, indicative of the penetration of some water into the *n*-hexane (hydrated surfactant molecules not in inverted micelles). Probably 0.05–0.5% water is not sufficient to

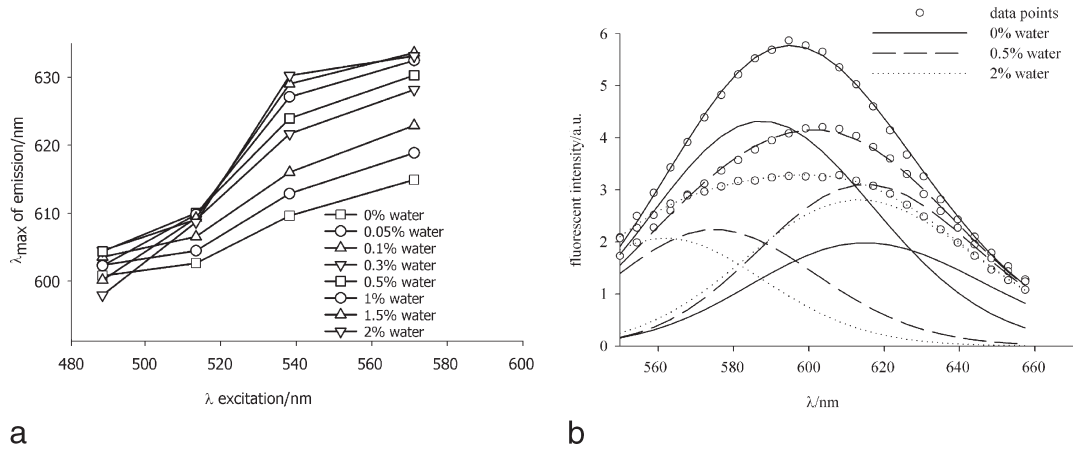

Figure 2. (a) Excitation-dependent emission of Nile Red in 10% (w/w) Neodol 1–5 in *n*-hexane. Influence of water on the formation of inverted micelles. (b) Deconvolution of the spectra excited at 490 nm. Each set of data points is deconvoluted into two peaks representing the polar and apolar interface. The sum of these two is drawn through the corresponding data points

assemble all the surfactant molecules into inverted micelles. With a further increase in the water content, the emission at 490 nm decreases again. This indicates further structuring of the surfactant molecules into inverted micelles. The general polarity of the solvent will decrease in the organized solution because there will be fewer randomly dissolved surfactant molecules and a smaller contribution of their polar headgroups to the general polarity. This is indeed observed at 2% of water [Fig. 2(a)]. On the polar side there is hardly any influence on increasing the water concentration from 1 to 2%. Indeed, 2% water is about the maximum allowed before a phase separation takes place. If no inverted micelles were formed in the absence of water, we would expect the 0% water line to be flat, because of the random dissolution of the surfactant molecules. However, the observed significant slope is probably due to water which is already present in the Neodol 1–5 solution (0.2% as determined by Karl Fisher titration), leading to some organization of surfactant molecules.

The original spectra resulting from excitation at 490 and 520 nm can be deconvoluted into two spectra originating from Nile Red residing at the two interfaces of the inverted micelles, a polar interface and an apolar interface [Fig. 2(b)]. At 0% water the polarities of the two interfaces are only slightly different since formation of inverted micelles hardly occurs under these conditions. On addition of water, clearly two interfaces are present, a polar interface at an almost fixed position and an apolar interface undergoing a hypsochromic shift with increasing amounts of water, due to increased structuring of the surfactant molecules as discussed above. When the samples are excited at 550 or 590 nm, the emission spectra are only derived from the polar interface.

Nile Red could also be effectively used to measure the CMC of a surfactant solution in water and other polar solvents by following the maximum of the Nile Red emission peak (λ_{max}) as a function of the surfactant concentration (Fig. 3). The equivalence point reflects

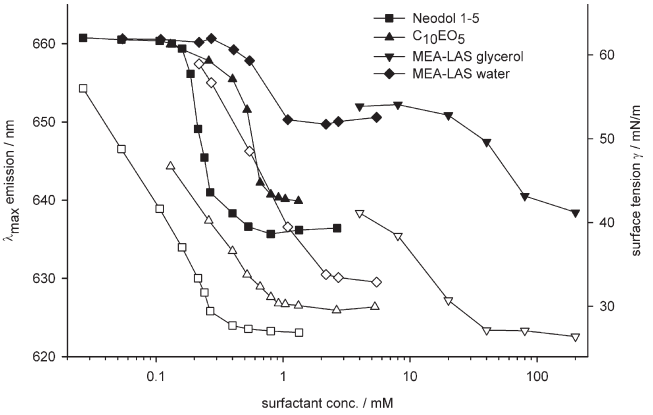

Figure 3. CMC of $C_{10}EO_5$ and Neodol 1–5 in water and of MEA-LAS in glycerol and in water measured using the Nile Red (NR) emission wavelength maximum (λ_{max}, closed symbols; NR was excited at 550 nm) and using surface tension measurement (γ, open symbols)

the CMC, and coincides with a strong increase in the fluorescence intensity (not shown), as was demonstrated previously.[13] The advantage of using the λ_{max} instead of the fluorescence intensity is that the λ_{max} can also be used in turbid solutions without making a correction for the absorbance and in non-aqueous polar solvents in which Nile Red gives a hight intensity by itself, as demonstrated for glycerol. The CMCs of $C_{10}EO_5$, Neodol 1–5 and MEA-LAS were compared with the results obtained by surface tension measurements. Since Nile Red is hardly soluble in water, it is most likely effectively shielded by surfactant molecules due to the hydrophobic effect,[14] leading to a lower CMC value. This effect is well known for other hydrophobic fluorescent probes, such as pyrene, as discussed by Capek in a recent review.[15] Low concentrations of Nile Red (micromolar range) do not influence the CMC as measured by surface tension (data not shown). This means that the shielding of Nile Red by surfactant molecules lowers the Nile Red λ_{max} and the apparent CMC. However, it does not affect the CMC as measured by surface tension. In a non-aqueous

solvent hydrophobic effects do not play a role, as can be seen from the CMC measurements of MEA–LAS in glycerol, where the surface tension overlaps with the Nile Red data (Fig. 3). Obviously, the formation of micelles in glycerol (and also in other polar organic solvents) is less cooperative, which shows up as a faint curve for both λ_{max} and for the surface tension. Following a shift in λ_{max} instead of the Nile Red intensity has the advantage that it can be used both in polar organic solvents and in water. As soon as the CMC is reached, Nile Red will bind in the interfacial region of the micelle, and the locally decreased polarity is expressed by an emission peak at lower wavelength.

Because a fraction of the Nile Red will be located more to the outside and some more towards the core of the micelle, a small difference in emission maximum can be found when the probe is excited with different wavelengths [Fig. 4(a)]. Owing to the large polarity-dependent absorbance shift of Nile Red, the probe which resides more towards the core of a micelle will be preferentially exited at lower wavelength and emit at a lower wavelength. By contrast, the probe residing more towards the outside of the micelle (facing solvent) will be preferentially excited and emit at higher wavelength. The contribution of the different probe positions to the fluorescent signal will therefore change slightly with the excitation wavelength, which results in an overall shift of λ_{max}. Different probe positions have also been reported for the slightly different 2-hydroxy-Nile Red, as demonstrated by selective quenching of probe molecules at the water interface.[16]

When surfactants are dispersed in methanol, there is no sign of surfactant aggregation and the surfactant molecules are randomly mixed.[10] This is reflected in the excitation wavelength-independent emission of Nile Red (490–590 nm) [Fig. 4(b)]. On mixing a 10% (w/w) surfactant-mix solution in methanol with a 10% (w/w) surfactant-mix solution in water, a different behavior of the excitation-dependent fluorescence of Nile Red was observed [Fig. 4(a)]. We find that up to 40% (w/w) methanol the micelles stayed intact, as indicated by the excitation-dependent Nile Red emission (although the slope decreases). This change in slope is most likely due to the incorporation of methanol into the core of the micelles or a decrease in aggregation number of the micelles with unchanged geometry[17] and to the decreased polarity of the solvent. Incorporation of methanol into the core of the micelle shows up particularly at low excitation wavelength (e.g. 490 nm) as an increase in the polarity (increase in λ_{max}, methanol is more polar than the hydrocarbon surfactant tails). Nile Red molecules that report more from the surface of the micelles show a decrease in polarity on addition of methanol. The interfacial region seems not to be affected by methanol as long as the micelles persist. In methanol the Nile Red fluorescence is excitation-independent and just reports the polarity of the solution containing randomly dissolved

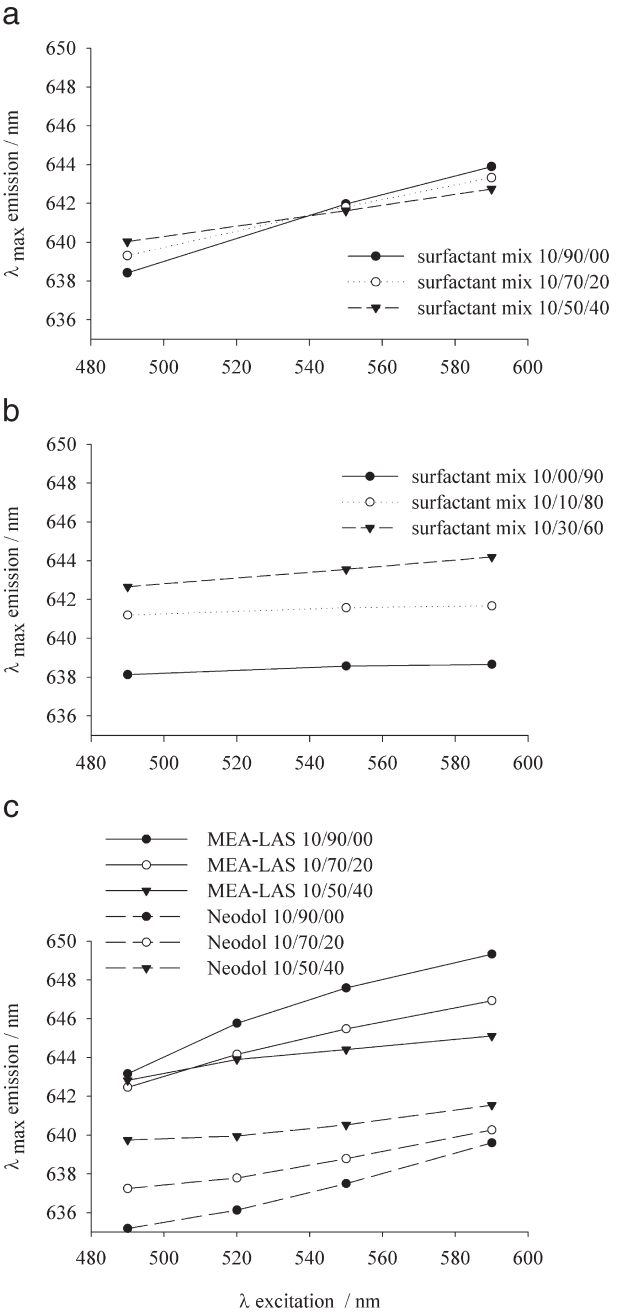

Figure 4. Excitation-dependent fluorescence of Nile Red in a surfactant mixture (a, b) and in a non-ionic (Neodol 1–5) and an ionic (MEA-LAS) surfactant (c). Effect of methanol on 10% surfactant in water (a, c) and effect of water on 10% surfactant in methanol (b) (surfactant:water:solvent, w/w/w)

surfactant molecules. The addition of a surfactant-mix in water to a surfactant-mix in methanol will in this case lead to in increase in polarity, as can be seen by an upshift of the horizontal lines [Fig. 4(b)]. Between 60 and 40% methanol micelles are gradually formed, as indicated by the deviation from the almost linear relationship when two solvents of different polarity are mixed[9] (M. C. A. Stuart *et al.*, in preparation).

The previous experiments were performed with a surfactant mixture with a rather broad composition. The

individual components were found to give similar results, although the position of the probe and the general polarity of the surfactant solutions differ. The ionic LAS has a much higher general polarity than the non-ionic Neodol 1–5 [Fig. 4(c)]. As expected, in the ionic LAS the location of Nile Red is more towards the outside of the micelles, which can be seen on addition of methanol to micelles formed in water. At low excitation wavelength the Nile Red emission maximum is not influenced. At high excitation wavelength, however, Nile Red senses the addition of methanol as a decrease in polarity.

In micelles formed from the non-ionic Neodol 1–5, the response of Nile Red fluorescence to the addition of methanol is completely the opposite. In the Neodol 1–5 micelles Nile Red is likely buried between the short ethylene oxide chains. This environment is less polar than that at the micellar surface of the ionic LAS aggregates. Methanol penetrates into the core of the micelles, leading to an increased local polarity, as can be seen by an increase in λ_{max} at 490 nm excitation. The interfacial region of hydrated ethylene oxide groups in these micelles is hardly influenced by the addition of methanol.

In addition to measurements in micelles, Nile Red can also be used to monitor the phase behavior, as is demonstrated with $C_{12}EO_4$, which forms in water micelles (L_1) below 20 °C, between 20 and 50 °C the surfactant is lamellar (L_α) arranged into vesicles (niosomes) and above 65 °C a micro-emulsion is formed of inverted micelles (L_2).[18] The Nile Red λ_{max} is the highest in micelles at low temperature and lowest in inverted micelles at high temperature [Fig. 5(a)]. In micelles some water will penetrate between the headgroups of the surfactant molecules, which is reflected by a higher polarity experienced by Nile Red. In a bilayer the surfactant molecules are better packed and water is more expelled from the interface, reflected by a lower Nile Red λ_{max}. In inverted micelles Nile Red has the opportunity to migrate to the hydrophobic tails which are now pointing outwards, leading to an even lower λ_{max}. The formation of an L_2 phase in water above 65 °C was checked by the Nile Red signal in a 95% (w/w) $C_{12}EO_4$ sample in water [see the phase diagram Fig. 5(b)] and a 9% $C_{12}EO_4$–1% H_2O in *n*-hexane dispersion. In both cases the Nile Red signal is identical [bars in Fig. 5(a)] with the signal in water above 65 °C. From the original spectra with their best fit to determine λ_{max} [Fig. 5(c)] it is clear that there is an overall shift in polarity. The intensity decreases with increase in temperature as a result of the hypsochromic absorbance shift. Because the samples are only excited at 550 nm the apolar interface is not excited. This makes Nile Red useful for the determination of the phase behavior and for the vesicle-to-micelle transition, without making corrections for the turbidity.[19]

In sum, we have shown that both large (formation of inverted and normal micelles) and small (increased structuring of surfactant in apolar solvent, incorporation

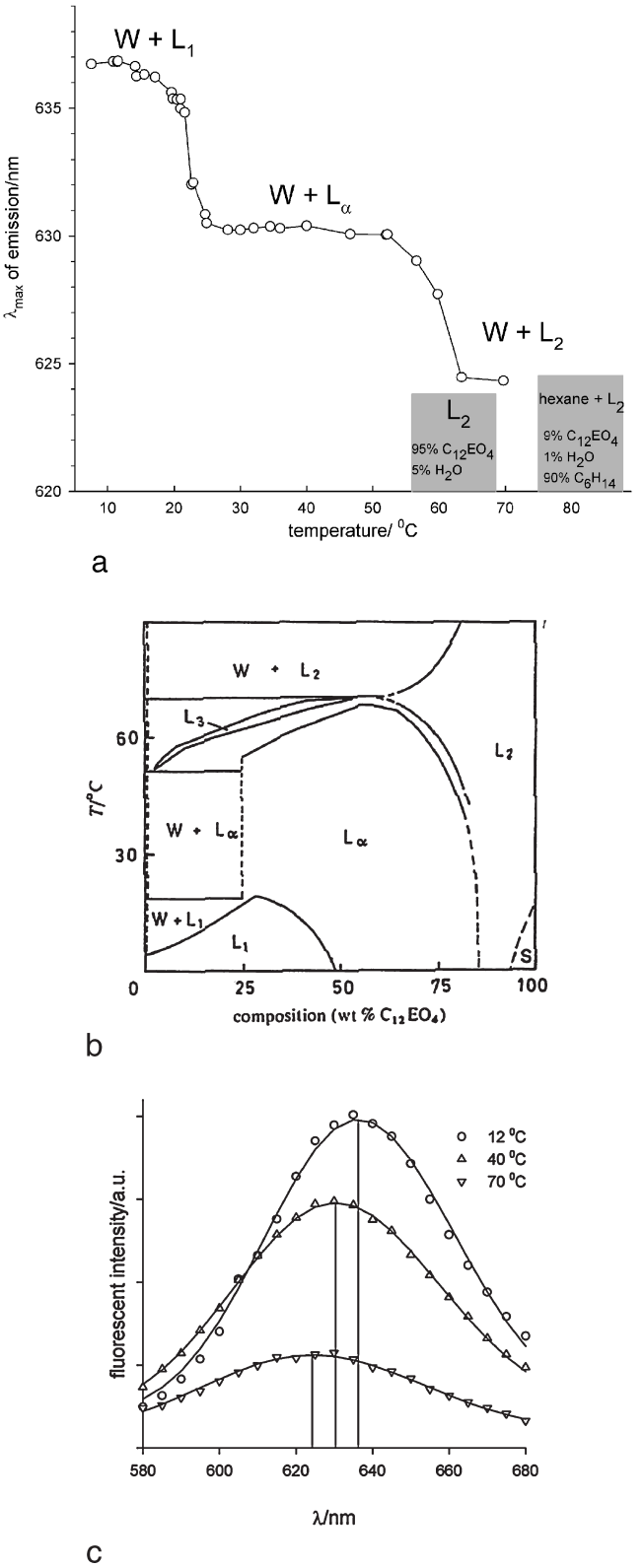

Figure 5. (a) Temperature-dependent phase behavior of 1% (w/w) $C_{12}EO_4$ measured by Nile Red fluorescence (λ_{max}) (line). L_2 phase of 95% $C_{12}EO_4$ in water and 9% $C_{12}EO_4$–1% water in *n*-hexane (bars). (b) Phase diagram of $C_{12}EO_4$. Reproduced by permission of the Royal Society of Chemistry.[18] (c) Fluorescent emission spectra of 1% (w/w) $C_{12}EO_4$ in water, excited at 550 nm, at 12, 40 and 70 °C with their calculated maximum (drop lines)

of solvent into micellar core) changes in the organization of surfactant both in water and in polar organic solvents can be successfully monitored using Nile Red as a solvatochromic**,** fluorescent probe. These properties of the probe exemplify the unique versatility of Nile Red in characterizing surfactant self-assembly processes and phase behavior. Of course, the probe molecule may affect the aggregation of the amphiphilic molecules, but the low concentrations of Nile Red will ensure that these effects will be of minor importance. A great advantage is the use of the emission maximum, λ_{max}, which makes it possible to employ turbid solutions.

Further studies of more complicated surfactant systems will be reported in due course (M. C. A. Stuart, J. C. van de Pas and J. B. F. N. Engberts in preparation).

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