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pH Responsive Aggregation States of Chiral Polymerizable Amphiphiles from L Tyrosine and L Phenyl Alanine in Water

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Abstract

Sodium salts of maleamic acid derivatives of lauryl ester of tyrosine (MTNa) and phenyl alanine (MPNa) in water, exhibited strong pH responsive behaviors of viscosity and specific conductivity that originate from concentration and pH dependence of their aggregation states. The aggregates were characterized by a novel spin-probe-partitioning electron paramagnetic resonance (SPPEPR) method and Dynamic Light Scattering (DLS). Results of high precision fitting of the second harmonic EPR spectra of the small spin probe di-tert-butyl nitroxide (DTBN) in these aggregates together with viscosity, conductivity, and DLS showed that at pH ~ 7.54, MTNa formed micelles, MPNa vesicles and MTNa exhibited a pH-induced micelle to vesicle transition as pH was lowered toward 6. MTNa, at pH ~ 7.54, formed small micelles at low concentrations that transformed to long worm like micelles for concentrations ~ 0.05M accompanied by a 30-fold increase in solution viscosity. The hydrodynamic radii from DLS confirmed the presence of small micellar aggregates of radius ~ 2 nm in MTNa at pH ~ 7.54 at the lower concentrations; coexisting micelles (~ 2 nm) and vesicles (~ 50 nm) at pH near 6.5 and vesicles (radii ~ 70 nm) at pH near 6 and large vesicles (85 nm) in MPNa at pH ~ 7.60. Both of MTNa and MPNa precipitated upon reduction of pH below 6 and below 7 respectively. The rate of transfer of DTBN between the aqueous phase and the aggregate was calculated from the high field Lorentzian linewidths of the EPR spectra. The activation energy for the transfer determined from the temperature dependence of the rate of transfer is 12.7 kJ per mole for MTNa vesicles (pH ~ 6) and 20.6 ± 1.3 kJ per mole for MPNa (pH ~ 7.60). The pH-induced transformations were reversible.

Introduction

Reversible transformations in aggregated structures from micelles to vesicles in response to external stimuli like pH and temperature are crucial properties for the design of smart materials^{1, 2}. The recently synthesized new polymerisable amphiphiles: maleamic acid derivatives of lauryl ester of tyrosine (MTNa) and phenyl alanine (MPNa) form aggregates with just such properties. Furthermore, these amphiphiles are significant for at least two more reasons: one for the desirable characteristics of non toxicity and biodegradability, and two, for the scope to form nanoparticles of polymeric amphiphiles with potential in applications such as drug delivery. The use of maleamic acid derivatives in design of polymeric nanoparticles was recently established³.

Investigations on the aggregated structures varying between micelles of MTNa and vesicles of MPNa in water at pH 7.57±.03 were recently reported⁴. Visible changes were observed in

solutions of MTNa and MPNa on reducing the pH. MTNa transformed from clear to bluish translucent solution on lowering the pH from 7.54 to 6.50, and turbid at $6.00 > \text{pH} > 5.30$. MPNa solutions became turbid upon a small reduction in pH and eventually precipitated at $\text{pH} < 7.00$. These preliminary observations prompted further investigations of MTNa and MPNa aggregates in solutions.

Viscosity and conductivity measurements revealed the occurrences of major changes in aggregation states in response to concentration and pH variations. Detailed characterization of the aggregates was then undertaken using a novel EPR approach, complemented by Dynamic Light Scattering. EPR of nitroxide spin probes solubilized in aggregates such as micelles and vesicles is a well-known widely used method to determine properties of the local environment of the probe within the aggregate-water interface^{5, 6}. Fluid micellar or liquid bilayer environments permit fast motion of the spin probe leading to motional narrowing of its triplet EPR spectra and narrow well-resolved lines. Measured properties derived from line fitting of the spectra typically include polarity and microviscosity (from the rotational correlation times)⁷. The polarity of any medium is the volume fraction of OH dipoles in that medium^{8, 9}. In the headgroup region of amphiphilic molecular aggregate-water interfaces, the main contribution to polarity is from the interfacial water. Micelles and bilayers in general exhibit differences in line shapes and properties derived thereof^{6, 10–12}. These differences do not always lend themselves to conclusive interpretations in terms of the structure. With the proper choice of spin probe and high precision in line fitting and together with one or more complementary techniques, it is possible to distinguish clearly whether a particular aggregate is a bilayer or micelle.

In this work, a novel application of a spin-probe-partitioning electron paramagnetic resonance (SPPEPR) yielded information on the forms and physicochemical properties of the aggregates in MTNa and MPNa solutions. Hydrodynamic radii of the aggregates were measured by Dynamic Light Scattering (DLS). The SPPEPR, DLS, Viscosity, and Conductivity experiments were mutually consistent and together showed that MTNa and MPNa formed different types of aggregates and responded differently to pH. MTNa exhibited a pH-induced transformation from micelles (at pH 7.54) to vesicles (at pH 6). DLS measurements supported the conclusions from the SPPEPR while yielding the hydrodynamic radii of the aggregates and further showed the coexistence of micelles and vesicles in MTNa at pH 6.5.

Materials and Methods

Materials

The maleamic acid derivatives of lauryl esters of tyrosine (MTH) and phenyl alanine (MPH) were synthesized as reported earlier⁴ and used in the measurements after conversion into sodium salt as detailed below. The spin probe, DTBN, was obtained from Sigma-Aldrich and used as received.

Sample Preparations

Conductivity, Viscosity, and DLS

Milli-Q deionized water or fresh doubly distilled was used as the aqueous solvent. The solutions of MTH and MPH were prepared in equimolar NaOH solution to achieve stoichiometric neutralization resulting in their respective sodium salt solutions. The sample solutions were stirred for 4 hours at 50 °C to ensure complete solubilization. The pH of these solutions was ~7.50. Samples for lower pH measurements were obtained by adding required amounts of 1M HCl. The structure of MTNa and MPNa are shown in Fig.1.

EPR

An appropriate amount of stock solution of DTBN in ethanol was taken in a glass vial. The ethanol was then evaporated under N₂ flux to form a thin film of DTBN. The sodium salt solutions of MTNa and MPNa were then added to the DTBN thin film and were stirred for 1 hour to ensure homogeneous distribution of DTBN. The DTBN concentration was low enough to prevent interference from spin exchange effects¹³.

Experimental Methods

Conductivity and Viscosity

Specific conductivity (κ) and relative viscosity (η_{rel}) were measured for selected concentrations of MTNa solutions at pH 6.50 and 6.00. The solutions were thermostated at 28 ± 0.1 °C for 45 minutes. Specific conductivity was measured with EUTECH instrument conductivity meter (model con 510). A dip type cell of cell constant 1.00 cm^{-1} was used. The uncertainty of the measurement was within 0.10%. κ was measured for MTNa solutions in presence of the electrolyte, KCl. The individual conductivities of 1 mM KCl and MTNa only solutions were also measured and the sum of these two values is referred to as the additive conductivity. The difference between the additive conductivity and the specific conductivity of MTNa solutions in presence of KCl, denoted by Δk , was calculated.

The viscosity measurements were performed using a 3.0 ml Ostwald Capillary Viscometer. The flow time for water was 62 s. The flow time at each concentration was measured at least three times to check that the difference between flow times was not greater than 1 s and in most cases it was not greater than 0.5 s. The reported relative viscosity is the average of these three values.

Spin-probe-partitioning electron paramagnetic resonance (SPPEPR)

DTBN, is a small nitroxide spin probe that partitions between the aggregate and the aqueous phase. The observed EPR spectrum is a superposition of the two isotropic triplets of DTBN in the aggregate and DTBN in water, referred to in this work as the “aggregate” and the “water or aqueous phase” signals or lines, respectively. The three absorption lines of a triplet are referred to as the high, center, and low field lines, according to their field positions. Second harmonic of the EPR absorption spectra, which is naturally better resolved, was measured.

EPR spectra were measured at X-band using a Bruker ESP 300 spectrometer interfaced with a Bruker computer and equipped with a Bruker variable temperature unit (model B-VT-2000). Experimental details may be found in published work^{11, 14, 15}. The second harmonic SPPEPR spectra, were analyzed as described previously¹¹. Details of the software and fitting method, developed at CSU Northridge, to fit the spectra may be found in published work^{11, 12}. In cases where the two triplets could be well resolved, the EPR parameters derived from the fit were the field positions of the three EPR lines of each of the aggregate and water triplets, Lorentzian and Gaussian EPR line widths and heights of these lines. The hyperfine coupling constant, A, in each environment is one half of the field separation between the high and low field lines.

The rate of DTBN transfer from water to aggregate, k_w , was calculated using,

$$k_w = \frac{\sqrt{3}}{2} \gamma \delta B_w \quad (1)$$

with the peak-to-peak transfer broadening δB_w of the water EPR line given by,

$$\delta B_w = \Delta B_{pp}^L(\text{water-aggregate}) - \Delta B_{pp}^L(\text{water}), \quad (2)$$

where $\Delta B_{pp}^L(\text{water-aggregate})$ and $\Delta B_{pp}^L(\text{water})$ are the peak-to-peak Lorentzian linewidths of the high field EPR lines of DTBN in the aqueous phase of the sample and in pure water respectively¹². Reference to Fig. 2, for example, may serve as a visual guide. $\Delta B_{pp}^L(\text{water-aggregate})$ and $\Delta B_{pp}^L(\text{water})$ are the peak-to-peak Lorentzian linewidths of the high field EPR lines in Fig. 2b and 2c. $\gamma = 1.76 \times 10^7 \text{ s}^{-1}\text{G}^{-1}$ is the magnetogyric ratio.

The aggregate and water lines overlap closely when the transfer rate between the two environments is fast compared to $\gamma\Delta B$, where ΔB is the field separation between the lines. In such situations, where resolution of the two lines was not possible, a single average value of the hyperfine splitting was derived.

Dynamic Light Scattering

DLS measurements were carried out on DynaPro Nanostar Model WDPN06 (Wyatt Technologies), equipped with GaAs laser (120mW) operating at a nominal wavelength of 658 nm. The scattered light was collected at 90° by solid state Single Photon Counting Module (SPCM) detector. The sampling time was set to an optimum value to obtain a fully decaying intensity correlation function (ICF), which was typically 10 seconds. The ICF's were single exponential decays with baselines that were unity within the precision of the measurements. The exponential fit to ICF yielded translational diffusion coefficient (D_t) of the particles in the sample. The hydrodynamic radius (R_h) of the sample was then derived from D_t using the Stokes-Einstein equation¹⁶. All sample solutions were filtered through 0.2 μm Whatman nylon syringe filters. The temperature of the sample solutions was controlled by an internal Peltier effect heat pump with an accuracy of $\pm 0.01^\circ\text{C}$.

Results

The visible changes in the appearance of the solutions of MTNa and MPNa at a concentration of 0.1M guided the selection of the pH parameters for detailed investigations. Accordingly, pH 7.54, 6.50 and 6.00 were selected for MTNa. Measurements at pH < 7.00 were not possible for MPNa as the solution became turbid and precipitated within a few minutes of lowering the pH slightly below 7.00.

Relative Viscosity

The relative viscosity (η_{rel}) of MTNa solutions at pH 7.54, 6.50 and 6.00 are presented in Table 1. The weak concentration dependence of η_{rel} at pH 6.50 and 6.00 is in sharp contrast to its 30-fold increase at pH 7.54 at 0.05M. Instances of such increase in viscosity have been found to be a consequence of transformation of small micelles to long cylindrical or worm like micelles¹⁷⁻¹⁹. Absence of a strong increase at the lower pH indicates no major structural changes.

Conductivity

The results on $\Delta\kappa$ (see Methods) are presented in Table 2. The difference $\Delta\kappa$ represents the concentration of ions available for conduction and gives information on the rigidity of aggregated structures, generally associated with bilayers^{4, 20}.

Particularly significant are the order of magnitude changes in $\Delta\kappa$ with reduction in pH at each of the concentrations. Larger $\Delta\kappa$ means reduced availability of free ions for conduction. pH-induced micelle to bilayer transformation can lead to such changes because

of the increased entrapment of KCl inside bilayers as opposed to that in micelles and the subsequent reduced number of free ions²¹. This inference, in combination with η_{rel} changes, suggests formation of bilayers or vesicles in MTNa at pH 6.50 and 6.00. The changes in η_{rel} and $\Delta\kappa$ are reproducible and reversible with change in pH.

From the pH titration experiments, the degree of neutralization (α) = $C_{\text{A}^-}/C_{\text{HA}}$, representing here the conversion of salt to acid, was calculated at pH 6.5 to be 0.35 and 0.68²². C_{A^-} is the concentration of MTNa and C_{HA} that of the acid. This means that at pH 6.50, MTNa is a mixture of 35 % COOH and 65 % COONa. On lowering the pH to 6.30, the composition changes to 68 % COOH and 32 % COONa. The increase in COOH in the mixtures on lowering the pH possibly brings about changes in the aggregation state and hence the drastic changes in the observed properties²³.

EPR and DLS

SPPEPR and DLS experiments were conducted on each of the solutions of MTNa and MPNa at various concentrations in the range 25 to 300 mM and various temperatures between 20 and 50 °C and at different pH in the range 6 to 7.60.

MTNa and MPNa at pH 7.57±0.03

A representative second harmonic SPPEPR spectrum of DTBN in MPNa at pH 7.60 is presented in Fig. 2a. The asymmetry in the spectrum, visible in all of the high, low and center field lines indicates that the probe experiences at least two types of environments. Two triplets are expected, as DTBN is soluble in both water and the aggregate. A least-squares-fit to the complete spectrum of six lines, where each was a sum of Gaussian and Lorentzian lineshapes was performed. The spectrum was described by two symmetric triplets. The resolved signals and the residuals of the fit are displayed in Fig. 2 b and c and 2d respectively. The hyperfine coupling constants denoted by A_{AP} and A_{WP} of the two signals are defined as marked and were derived from the fits. A_{WP} for the signal in Fig. 2c is 17.16 G. It is the value measured for pure water; thus confirming that this signal is from the DTBN in the aqueous phase.

In contrast, the spectrum obtained in 100 mM MTNa at pH 7.54, shown in Fig. 3a, is symmetric. Here the aggregate and water signals appear to overlap. Apparently the transfer rate of DTBN between the MTNa aggregate and the aqueous phase is faster than in MPNa; and rapid enough to preclude observation of the individual aggregate and water resonances. The data were fit to a three line spectrum. The fit, shown in Fig. 3b, yielded the hyperfine coupling constant, A_{AT} , for DTBN in the MTNa solution. The faster transfer rate in MTNa than in MPNa as evidenced by the symmetric lines suggests a less compact, more fluid aggregate that permits easier flow of DTBN between the two regions.

The temperature and concentration dependences of the hyperfine coupling constants in the aggregates of MPNa and MTNa at pH ~ 7.50 are presented in Fig. 4 and Fig. 5 respectively. These values are less than 16.0 G in MPNa and greater than 16.4 G in MTNa. Typically the higher the molecular packing lower is the interfacial water content and hence polarity. The lower values of A_{AP} as compared to A_{AT} indicate therefore closer packing in MPNa. Furthermore for the probe DTBN, numerical values less than 16 G for A_{AP} are values typical of bilayer vesicles and the value of 16.4 and greater for A_{AT} for MTNa are micelle-like^{6, 10–12}.

The decrease in the values of A_{AT} with increase in concentration is about four times the decrease of A_{AP} . The more pronounced effect in MTNa is an argument in favor of micelles. Decrease with concentration is in general due to increase in partitioning of DTBN in to

aggregates. However because the water and micelle lines appear together as a single line in micelles; as the DTBN fraction in the micelle increases, the intensity of the micelle line increases, resulting in the field position of the combined peaks to be weighted more toward the micelle peak position. In vesicles, the aggregate and water lines being separated to begin with do not experience as much of an effect in the field positions.

The temperature dependence of A_{AT} in MTNa shows that with rise in temperature, the portion of DTBN in the lower polarity region, namely the aggregate interface, increases. Later in this paper, the activation energies for the transfer are presented.

From a consideration of the transfer rate effects on the line shapes and the polarities of MTNa and MPNa it is most likely that MTNa forms micelles and MPNa aggregates as vesicles at pH 7.57 ± 0.03 . Just the observation by itself of a symmetric three line spectrum is indicative of micelles because DTBN in vesicles generally exhibits six lines. On a cautionary note, occurrence of six lines does not unambiguously mean the presence of vesicles because micelles of some surfactants with large headgroups can and do prevent fast transfer of DTBN between water and interface with consequently, a six line SPPEPR spectrum¹².

Direct evidence for the forms of the aggregates was obtained from Dynamic Light Scattering (DLS). As shown in Fig. 6a, the hydrodynamic radii of particles in 25 mM MTNa solutions were distributed around 2 nm; a size that is typical of lauryl surfactant micelles²⁴; whereas large aggregates of 85 nm radii characteristic of vesicles was found in 25 mM MPNa (Fig. 6b) and about 100 nm in 100 mM MPNa. The small peak at a radius of a few nm indicates the presence of a low concentration of micelles; however the aggregates are predominantly (95 %) vesicles. EPR signals in MPNa are also dominated by vesicles. This is clear from the excellence of fit obtained to two triplets, one of which is due to water (as determined from the value of 17.16 for A_{WP} (Fig. 2c) which is that of water) and the other is due to vesicles.

MTNa at pH < 7

MTNa solutions take on a bluish hue as pH is lowered to about 6, indicative of particles of sizes known for vesicles. The DTBN EPR spectra in MTNa at pH 6 are asymmetric, markedly different in profile from that at pH 7.54 and qualitatively similar to that of MPNa. The second harmonic SPPEPR spectrum of DTBN in 100 mM MTNa at pH ~ 6 is shown in Fig. 7. A structure different from that at pH 7.54 and a slower DTBN transfer rate that brings about an asymmetric lineshape similar to that of MPNa at pH 7.60 is suggested. Fit of the spectrum to a superposition of two triplets yields the resolved groups of lines shown in Fig. 7b and c. The value of the hyperfine coupling constant, A_{WT} , for the triplet in Fig. 7c is again that for water as in Fig. 2c for MPNa. The hyperfine coupling constant, A_{AT} , for DTBN in the MTNa aggregates at pH 6, obtained from the fit is about 16.4 G, less than that at pH 7.54. The change from a three line spectrum to a six line spectrum signifies transition from micelles to vesicles. The comparisons between MTNa and MPNa and between different pH are presented graphically in Fig. 8.

DLS data on MTNa at pH values < 7, presented in Fig. 9 a and b confirm the transformation of micelles at pH 7.54 to vesicles at pH ~ 6 through a coexistence pH region in between of micelles and vesicles. A mixture of small aggregates of micellar size (radii 2 nm) and large particles of vesicle like dimensions (radii 50 nm) were present at pH ~ 6.5. Further reduction in pH toward 6 resulted in a collection of just the larger aggregates of size 70 nm.

Increasing the pH back to 7.54 after reduction to 6, reproduced the formerly observed results at the higher pH. The transformations were therefore concluded to be reversible.

The peak-to-peak transfer broadening δB_W of the water EPR line was calculated using the fit values, in eq. 2, of ΔB_{pp}^L (water-aggregate) and ΔB_{pp}^L (water) of the peak-to-peak Lorentzian linewidths of the high field EPR lines of DTBN in the aqueous phase of the sample and in pure water respectively, for MPNa vesicles at pH ~ 7.60 and MTNa vesicles at pH 6 at each of the various temperatures of measurement. The DTBN transfer rate, k_W from the aqueous phase to the aggregate is then given by eq. 1. An Arrhenius temperature dependence of the form,

$$k_W = k_{W0} e^{-\frac{E_A}{RT}} \quad (3)$$

was found to describe k_W vs. $1/T$. The activation energies, E_A , obtained from exponential fits to the data, shown in Fig. 10, were 20.6 ± 1.3 kJ per mole, averaged over the values for three different concentrations, for MPNa and 12.7 kJ per mole for 100 mM MTNa. The larger barrier for DTBN transfer from water to aggregate in MPNa than in MTNa is consistent with the slower transfer rate that was deduced from the observation of the presence of two triplets in MPNa. The presence of the probe in the aggregate-water increases with temperature as may be deduced from the observed increase in the rate of water to aggregate transfer with increase in temperature, for the MTNa and MPNa vesicles. This is again consistent with the conclusion derived from the observed decrease with temperature of the polarity in MTNa at pH ~ 7.54 where the aggregates are micelles.

Discussion

The relationship between the size of the hydrophobic portion and the size of the hydrophilic headgroup of an amphiphile is known to be responsible for the structure of the aggregate formed by the self-assembly of the amphiphiles in water^{25,26}. In earlier work on micelles of Lauryl esters of *L*-tyrosine (LET) and *L*-phenyl alanine (LEP), the effect of molecular architecture on aggregate properties and on the conformation of the molecules within the aggregate was investigated²⁷. The observed differences were found to originate from the presence in LEP and absence in LET of the phenolic OH. The structural differences between MTNa and MPNa aggregates and the pH response of MTNa can be accounted for in a similar manner. MPNa, at pH ~ 7.60 , with the two hydrophobic parts, the aromatic ring and the hydrophobic tail on either sides of the ionic headgroup may be viewed as a double hydrophobic chain molecule. Bilayer formation by such molecules is not surprising; examples include lipids, and twin tailed nonionic as well as some ionic surfactants^{28–30}.

The headgroup acquires non-ionic character and decreased polarity in the acid form of MPNa that forms upon slight reduction in pH, impacting its solubility and causing precipitation. In MTNa, the phenolic OH confers some hydrophilic character to the phenolic part of the hydrocarbon portion, causing it to point into the interface and becoming part of the headgroup as was found in LET micelles²⁷. The resulting structures are micelles because of the increased hydrophilic and headgroup volumes in the molecule. In the acid form, obtained on lowering pH, the hydrophilic character is reduced by protonation at the carboxyl ionic site causing transformation into large aggregates whose sizes indicate that they are vesicles. The pKa of the phenolic OH is ~ 10 . Therefore, at the pH conditions in this work, the degree of dissociation or neutralization of phenolic OH is not an issue.

The numerical value of the interface polarity reported by a spin probe depends on its location within the interface. Polarities reported by a spin probe for micellar interfaces are in general always greater than that found for bilayers. The number of molecules per unit area in a bilayer interface is always greater than that in a micelle interface. Accordingly and supported by previous experimental observations the starting rationale adopted to view the EPR results was: closer molecular packing in bilayers as opposed to that in micelles leads to

(i) lower interface water concentration and hence lower polarity; and (ii) slower molecular transfer rates between water and interface; because of which the SPPEPR of DTBN in vesicles may be expected to show both of water and interface triplets¹¹. Polarity values and EPR line profiles alone are not sufficient to draw equivocal conclusions on the structure; but they do give a good indication whether the numbers and line profiles are micelle-like or bilayer-like and thus of what the structure could be. A case can be made for micelles when only three lines are present. On the other hand micellar aggregates of all surfactants do not all necessarily show six lines, that is, a six line DTBN SPPEPR spectrum is not exclusive to vesicles. Conclusive evidence can be obtained when combined with a particle sizing technique like DLS.

The hyperfine coupling constants determined with DTBN are higher than 16.3 G for a large variety of micelles and lower than 16.0 G in bilayers. The polarities found for MPNa are in the bilayer range, whereas values in MTNa at pH 6 are higher. The OH groups of the molecules in the aggregate also contribute to polarity. In the acid form there is an additional OH in MTNa. This additional OH contributes to an increase in polarity while the increased packing upon bilayer formation and the consequent reduction in interfacial water lower polarity. This may account for the polarity not being lowered down to about that in MPNa vesicles or other bilayers.

The advantage of employing spin probes that partition between the aggregate and the aqueous phase is the ability to derive the activation energy for the transfer of a molecule from the aqueous region to the aggregate interface.

The main emphasis of this work is the pH stimulus response, because pH changes produced interesting and remarkable structural changes, and also because pH-induced phenomena find more applications and interest in various fields such as food and medicinal chemistry, polymers and interfaces etc. In practical applications, induced property changes are of consequence. The results of this work show that a combination of concentration and pH stimulate drastic changes in the solution transport properties of viscosity and conductivity.

Conclusions

Aggregate formation by MTNa and MPNa and the pH response of their properties and structures were investigated by complementary applications of Viscosity, Specific Conductivity, spin-probe-partitioning EPR and Dynamic Light Scattering techniques. Interpretations of the results of each of these methods in terms of the aggregation states are consistent with each other. Thus there is compelling evidence for vesicle formation by MPNa and micelle formation by MTNa at pH > 7 and micelle to vesicle transformation, in MTNa, upon reduction of pH. The observed structural difference between ionic MTNa and MPNa aggregates in water was attributed to the phenolic OH in MTNa and its absence in MPNa. The pH response of MTNa is due to the protonation of the carboxyl group at the lower pH that induces non-ionic character in MTNa. Thus the scientific bases for the pH responses of the properties of MTNa and MPNa solutions originate in their molecular architectures. The reversible responses of aggregate structures of MTNa and MPNa and their solution properties to the external stimulus of pH predicate the potential of these surfactants as smart materials.

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References

1. Alarcon CDH, Pennadam S, Alexander C. Stimuli Responsive Polymers for Biomedical Applications. *Chem. Soc. Rev.* 2005; 34:276–285. [PubMed: 15726163]
2. Peppas NA, Hilt JZ, Khademhosseini A, Langer R. Hydrogels in Biology and Medicine: From Molecular Principles to Bionanotechnology. *Adv. Mater.* 18(11):1345–1360.
3. Gaspar LJM, Baskar G. Glycine Derived Polymerizable Cosurfactant in the Synthesis of Functionalized Poly(butyl acrylate) Nanolatexes. *Biomacromolecules.* 2006; 7(4):1318–1322. [PubMed: 16602755]
4. Angayarkanny S, Vijay R, Baskar G, Mandal AB. Formation of Self-Aggregated Structures of Different Types in Water of Chiral Polymerizable Amphiphiles from L-Tyrosine and L-Phenylalanine. *Langmuir.* 2012; 28(25):9378–9386. [PubMed: 22686136]
5. Ranganathan R, Peric M, Medina R, Garcia U, Bales BL, Almgren M. Size, Hydration, and Shape of SDS/Heptane Micelles Investigated by TRFQ and ESR. *Langmuir.* 2001; 17:6765–6770.
6. Alves M, Bales BL, Peric M. Effect of lysophosphatidylcholine on the surface hydration of phospholipid vesicles. *Biochim. Biophys. Acta, Biomembr.* 2008; 1778(2):414–422.
7. Singh JS, Miller J, Ranganathan R. Physicochemical characterization of phospholipid solubilized mixed micelles and a hydrodynamic model of interfacial fluorescence quenching. *J. Phys. Chem. B.* 2007; 111(31):9317–9324. [PubMed: 17629323]
8. Bales BL, Messina L, Vidal A, Peric M, Nascimento OR. Precision Relative Aggregation Number Determinations of SDS Micelles Using a Spin Probe. A Model of Micelle Surface Hydration. *J. Phys. Chem. B.* 1998; 102:10347–10358.
9. Bales BL, Ranganathan R, Griffiths PC. Characterization of Mixed Micelles of SDS and a Sugar Based Nonionic Surfactant as a Variable Reaction Medium. *J. Phys. Chem. B.* 2001; 105(31):7465–7473.
10. Alves M, Peric M. An EPR study of the interfacial properties of phosphatidylcholine vesicles with different lipid chain lengths. *Biophys. Chem.* 2006; 122(1):66–73. [PubMed: 16517048]
11. Peric M, Alves M, Bales BL. Precision parameters from spin-probe studies of membranes using a partitioning technique. Application to two model membrane vesicles. *Biochim. Biophys. Acta, Biomembr.* 2005; 1669(2):116–124.
12. Peric M, Alves M, Bales BL. Combining precision spin-probe partitioning with time-resolved fluorescence quenching to study micelles: Application to micelles of pure lysomyristoylphosphatidylcholine (LMPC) and LMPC mixed with sodium dodecyl sulfate. *Chem. Phys. Lipids.* 2006; 142(1,2):1–13. [PubMed: 16569402]
13. Bales BL, Peric M. EPR Line Shifts and Line Shape Changes Due to Spin Exchange of Nitroxide Free Radicals in liquids. *J. Phys. Chem. B.* 1997; 101:8707–8716.
14. Plachy WZ, Windrem DA. A Gas-Permeable ESR Sample Tube. *J. Magn. Reson.* 1977; 27:237–239.
15. Hyde JS, Sczaniecki PB, Froncisz W. Alternatives to field modulation in electron spin resonance spectroscopy. *J. Chem. Soc., Faraday Trans. 1.* 1989; 85:3901–3912.
16. Wyatt PJ. Light scattering and the absolute characterization of macromolecules. *Analytica Chimica Acta.* 1993; 272:1–40.
17. Angelescu D, Khan A, Caldararu H. Viscoelastic Properties of Sodium Dodecyl Sulfate with Aluminum Salt in Aqueous Solution. *Langmuir.* 2003; 19(22):9155–9161.
18. Pal OR, Gaikar VG, Joshi JV, Goyal PS, Aswal VK. Small-Angle Neutron Scattering Studies of Mixed Cetyl Trimethylammonium Bromide, α -Butyl Benzene Sulfonate Solutions. *Langmuir.* 2002; 18(18):6764–6768.
19. Singh J, Unlu Z, Ranganathan R, Griffiths PC. Aggregate properties of sodium deoxycholate and dimyristoylphosphatidylcholine mixed micelles. *J. Phys. Chem. B.* 2008; 112(13):3997–4008. [PubMed: 18335917]

20. Horbaschek K, Hoffmann H, Thunig C. Formation and Properties of Lamellar Phases in Systems of Cationic Surfactants and Hydroxy-Naphthoate. *J. Colloid Interface Sci.* 1998; 206(2):439–456. [PubMed: 9756656]
21. Wolf C, Bressel K, Drechsler M, Gradzielski M. Comparison of Vesicle Formation in Zwitterionic and Catanionic Mixtures of Hydrocarbon and Fluorocarbon Surfactants: Phase Behavior and Structural Progression. *Langmuir.* 2009; 25(19):11358–11366. [PubMed: 19746944]
22. Tan JS, Gaspar SP. Dilute Solution Behavior of Polyelectrolytes. Potentiometric Titration Studies on Chain Conformation. *Macromolecules.* 1973; 6(5):741–746.
23. Fukuda H, Goto A, Yoshioka H, Goto R, Morigaki K, Walde P. Electron Spin Resonance Study of the pH-Induced Transformation of Micelles to Vesicles in an Aqueous Oleic Acid/Oleate System. *Langmuir.* 2001; 17(14):4223–4231.
24. Almgren, M.; Swarup, S. The Size of Sodium Dodecyl Sulfate Micelles with Various Additives: A Fluorescence Quenching Study. In: Lindman, K. L. M. a. B., editor. *Surfactants in Solution*. Vol. 1. Plenum; New York: 1984. p. 613
25. Stigter D. Micelle formation by ionic surfactants. II. Specificity of head groups, micelle structure. *J. Phys. Chem.* 1974; 78(24):2480–2485.
26. Wennerstrom H, Lindman B. Micelles. *Physical chemistry of surfactant association*. *Phys. Rep.* 1979; 52(1):1–86.
27. Vijay R, Singh J, Baskar G, Ranganathan R. Amphiphilic Lauryl Ester Derivatives from Aromatic Amino Acids: Significance of Chemical Architecture in Aqueous Aggregation Properties. *J. Phys. Chem. B.* 2009; 113(42):13959–13970. [PubMed: 19778004]
28. Ma G, Barlow DJ, Lawrence MJ, Heenan RK, Timmins P. Small-Angle Neutron-Scattering Studies of Nonionic Surfactant Vesicles. *J. Phys. Chem. B.* 2000; 104(39):9081–9085.
29. Kato K, Walde P, Koine N, Ichikawa S, Ishikawa T, Nagahama R, Ishihara T, Tsujii T, Shudou M, Omokawa Y, Kuroiwa T. Temperature-Sensitive Nonionic Vesicles Prepared from Span 80 (Sorbitan Monooleate). *Langmuir.* 2008; 24(19):10762–10770. [PubMed: 18720959]
30. Kunitake T, Okahata Y. A totally synthetic bilayer membrane. *J. Am. Chem. Soc.* 1977; 99(11): 3860–3861.

Figure 1.
Schematic of the structure of sodium salts of (a) MTNa and (b) MPNa

Figure 2.

(a) Second harmonic SPPEPR spectrum of DTBN in 100 mM MPNa at pH 7.60 at 50°C. The spectrum was resolved by a fit to a superposition of two triplets. The resolved spectrum of DTBN originating from (b) the aggregate and (c) aqueous phase. (d) Residual of the fit. A_{WP} and A_{AP} are one half the distances between the outer lines of the EPR signal from the aqueous and aggregate phases, respectively.

Figure 3.

(a) Second harmonic SPPEPR spectrum of DTBN in 100 mM MTNa at pH 7.54 at 50 °C. (b) Fit to the experimental spectrum in (a). (c) Residual of the fit. The hyperfine splitting constant A_{AT} is one half the distance between the outer lines of the EPR signal.

Figure 4. Temperature dependence of the hyperfine splitting constant of DTBN in MPNa aggregates at pH ~ 7.60 for three concentrations.

Figure 5. Temperature dependence of the hyperfine splitting constant of DTBN in MTNa aggregates at pH 7.54 for three concentrations.

Figure 6. Dynamic Light Scattering at 28 °C in 25 mM (a) MTNa at pH 7.54 and (b) MPNa at pH 7.60. Peak positions give the hydrodynamic radii of aggregates.

Figure 7.

(a) Second harmonic SPPEPR spectrum of DTBN in 100 mM MTNa at pH ~ 6 at 50°C. The spectrum was resolved by a fit to a superposition of two triplets. The resolved spectrum of DTBN originating from (b) the aggregate and (c) aqueous phase. (d) Residual of the fit. A_{WT} and A_{AT} are one half the distances between the outer lines of the EPR signal from the aqueous and aggregate phases, respectively.

Figure 8. Comparison of the hyperfine splitting constants between MTNa and MPNa at pH 7.60 and between pH ~ 6 and 7.54 in MTNa.

Figure 9. Hydrodynamic radii of aggregates from DLS at 28 °C in 25 mM (a) MTNa at pH ~ 6.5 and (b) MTNa at pH ~ 6.

Figure 10.

Temperature dependence of the water to aggregate transfer rate of DTBN. The lines are exponential fits to the form in eq. 3.

Table 1

Relative viscosity (η_{rel}) of MTNA solutions in water at different concentrations at pH 6.50 and 6.00. Temp. 28 $\pm$ 0.1 ° C

[MTNa] M	MTNa (g/ml)	η_{rel}		
		pH 7.54 ^(a)	pH 6.50	pH 6.00
1.00×10^{-4}	4.64×10^{-5}	1.02	1.05	1.02
5.00×10^{-4}	2.32×10^{-4}	1.02	1.05	1.02
1.00×10^{-3}	4.64×10^{-4}	1.02	1.05	1.02
5.00×10^{-3}	2.32×10^{-3}	1.04	1.05	1.02
1.00×10^{-2}	4.64×10^{-3}	1.05	1.12	1.02
3.00×10^{-2}	1.39×10^{-2}	1.67	1.30	1.09
5.00×10^{-2}	2.32×10^{-2}	33.27	2.47	1.32

^(a) values measured previously and presented here for direct comparison⁴

Table 2

Conductivity data on MTNa solutions at pH 7.54, 6.50, and 6.00 in water at 28 ± 0.1 °C. Additive conductivity κ is the sum of the measured conductivity of a 1 mM KCl solution and that of MTNa in absence of KCl. $\Delta\kappa$ is the difference between the additive conductivity and the measured conductivity of MTNa in presence of 1 mM KCl.

[MTNa] M	Additive conductivity κ ($\mu\text{S}/\text{cm}$)			$\Delta\kappa$ ($\mu\text{S}/\text{cm}$)		
	pH, 7.54 ^(b)	pH, 6.50	pH, 6.00	pH, 7.54 ^(a)	pH, 6.50	pH, 6.00
1.00×10^{-2}	604.4	727	1133	3.3	9.3	24.3
3.00×10^{-2}	1502.4	1785	2819	4.3	18.3	90.3
5.00×10^{-2}	2490.4	2769	4199	10.3	100.3	120.3

^(a) values measured previously and presented here for direct comparison⁴