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The Conundrum of pH in Water Nanodroplets: Sensing pH in Reverse Micelle Water Pools

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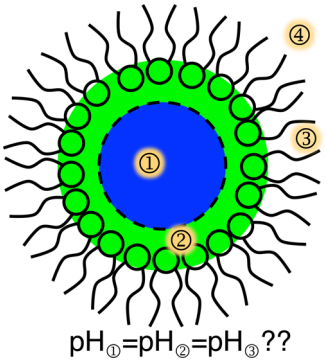
CONSPECTUS

In aqueous environments, acidity is arguably the most important property dictating the chemical, physical, and biological processes that can occur. However, in a variety of environments where the minuscule size limits the number of water molecules, the conventional macroscopic description of pH is no longer valid. This situation arises for any and all nanoscopically confined water including cavities in minerals, porous solids, zeolites, atmospheric aerosols, enzyme active sites, membrane channels, and biological cells and organelles.



To understand pH in these confined spaces, we have explored reverse micelles as a model system that confines water to nanoscale droplets. At the appropriate concentrations, reverse micelles form in ternary or higher order solutions of nonpolar solvent, polar solvent (usually water), and amphipathic molecules, usually surfactants or lipids. Measuring the acidity, or local density of protons, commonly known as pH, of these nanoscopic water pools in reverse micelles is challenging. First, because the volume of the water in these reverse micelles is so minute, we cannot probe its proton concentration using traditional pH meters. Second, the traditional concept of pH breaks down in a nanosystem that includes fewer than 10^7 water molecules. Third, the interpretation of results from studies attempting to measure acidity or pH in these environments is nontrivial because the conditions fall outside the accepted IUPAC definition for pH.

Researchers have developed experimental methods to measure acidity indirectly using various spectroscopic probe molecules. Most measurements of intramicellar pH have employed optical spectroscopy of organic probe molecules containing at least one labile proton coupled to electronic transitions to track pH changes in the environment. These indirect measurements of the pH reflect the local environment sensed by the probe and are complicated by the probe location within the sample and how that location affects properties such as pK_a. Thus, interpretation of the measurement in the highly heterogeneous reverse micellar environment can be challenging. Organic pH probes can often produce ambiguous acidity measurements, because the probes can readily associate with or penetrate the micellar interface. Protonation can also dramatically change the polarity of the probe and shift the probe's location within the system. As a result, researchers have developed highly charged pH-sensitive probes such as hydroxypyrene trisulfonate, vanadate or phosphate that reside in the water pool both before and after protonation. For inorganic probes researchers have used multinuclear NMR spectroscopy to directly measure conditions in the water droplet.

Regardless of the probe and method employed, reverse micellar studies include many implicit assumptions. All reported pH measurements comprise averages of molecular ensembles rather than the response of a single molecule. Experiments also represent averages of the dynamic reverse micelles over the time of the experiments. Thus the experiments report results from an average molecular position, pK_a, ionic strength, viscosity, etc. Although the exact meaning of pH in nanosized waterpools challenges scientific intuition and experimental data are non-trivial to interpret, continued experimental studies are critical to improve understanding of these nanoscopic water pools. Experimental data will allow theorists the tools to develop the models that further explore the meaning of pH in nanosized environments.

I. Introduction

In aqueous environments, acidity is arguably the most important property dictating details of chemistry that can occur. As the size of systems decreases, the number of water molecules becomes limited to a very small proportion and the conventional macroscopic description no longer

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applies. In these nanoscale systems, the traditional introductory chemistry definition of pH based on the hydronium ion concentration, $-\log[H_3O^+]$,[1,2] is no longer valid. Furthermore, the current definition recommended by IUPAC, $pH \approx pa_{H^+} = -\log[a_{H^+}]$ (where $a_{H^+} = m_H\gamma_H/m^\circ$, γ_H is the proton activity constant, m_H is the proton molality, and m° is the standard molality), applies only between pH = 2 and 12 and at ionic strengths <0.1 mol L^{-1}.[3] These relationships are associated with bulk macroscopic phenomena where sufficient numbers of molecules are present to define the relevant concentrations and activities and at ionic strengths where the solute's concentration approximates its activity. The effective proton concentration in confined media is not readily calculated and the concept of pH based on proton activity is also ill-defined. Yet the relative acidity or basicity in a nanoscale droplet of water can have critical importance for chemistry occurring there.[4] Therefore, searching for ways to assess local pH, researchers simply measure the pH in nanoscale systems using pH-sensitive probes, often ignoring the fact that the proton density or local pH in these environments cannot be computed as in normal aqueous bulk solution. Various experimental approaches have characterized nanoscale water using probes with spectroscopic signatures observable by UV−vis spectroscopy, fluorescence spectroscopy, and NMR spectroscopy. Here we describe issues surrounding the concept of acidity and pH in the water pools of reverse micelles with fewer than 10^7 water molecules. We also summarize our studies probing pH and its effects on these systems and reactions in confined spaces.

II. Definition of pH in Bulk Solution

Acid ionization reactions, for example,

$$HA + H_2O \rightleftharpoons A^- + H_3O^+ \quad (1)$$

and concepts associated with pH, defined more than a century ago by Sørensen[1] as $pH = -\log[H^+]$, comprise some of the most fundamental concepts taught in introductory chemistry classes.[2] Understanding these concepts for aqueous systems and recognizing that water self-dissociation is included when HA is H_2O is especially crucial to life sciences. The acid equilibria, given in eq 1, are established in aqueous solution and depend on the equilibrium constant and acid ionization, $K_a = [A^-][H_3O^+]/[HA]$; stated differently the pK_a ($= -\log K_a$) is defined as the pH where $[A^-] = [HA]$.

In a general form of the Henderson−Hasselbach equation,

$$pH = pK_a + \log \frac{[\text{conjugate base}]}{[\text{acid}]} \quad (2)$$

the pH can be derived based on the pK_a of an acid and the concentration of the acid and its conjugate base.[2] However, all these relationships are based on concentrations and thus associated with bulk macroscopic phenomena where sufficient numbers of molecules are present to define the relevant concentrations and at ionic strength where the solute's concentration approximates the solute's activity. The fact that many systems of interest to chemists and life scientists have activity constants far from one is often ignored. The Hammett, H_o, acidity function extends the aqueous pH range into the negative pH regime, permitting characterization of studies in highly acidic solutions.[5] These acidity scales have been established using indicators and allow pH determination in nonaqueous environments, for example, polar non-hydrogen-bond-donor solvents such as dimethylsulfoxide.[5] Although these acidity scales are widely accepted and used in applications outside the IUPAC range (pH 2−12 and ionic strength <0.1 mol L^{-1}), the actual acidity and the pH obtained from the indicator are not always the same; furthermore interpretation remains nontrivial.[2]

The distribution of an acid and its conjugate base can be illustrated using "speciation diagrams". The top panel of Figure 1 shows the speciation diagram for the simplest case of the reaction in eq 1, graphically representing the proportion of acid in the HA form versus the A^- form. The position of the equilibrium shown at the top of Figure 1, also expressed as the pK_a value, depends on the stabilization of each component, HA and A^-, and is generally an intrinsic value. However, nonaqueous media can lead to differential stabilization for each component, thus affecting pK_a through the environment. For molecules with multiple protonation states or side reactions, the speciation diagram becomes much more complicated as shown for vanadate at the bottom of Figure 1. Speciation chemists generally work in the absence of CO_2 gas and at high ionic strengths affording exquisite control of the equilibria in isolation from all other factors;[2,6,7] however, these equilibria can be measured in more complex systems, for example, in the presence of CO_2 gas, yielding information about more natural systems.[4]

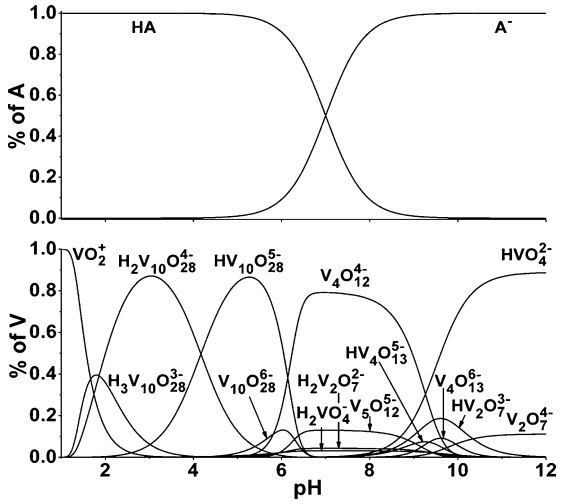

FIGURE 1. Speciation profile for (top) a model monoprotic acid with $pK_a = 7$ and (bottom) 50 mM $NaVO_3$ and 0.15 M NaCl describing the relative concentration of each vanadium species at a given pH. This diagram was calculated using the HySS program and formation constants given in the literature.[6,7]

III. Reverse Micelles, a Confined Environment Model

There exist a plethora of natural and artificial environments that confine water to submicrometer proportions. Biology presents many examples especially in cellular organelles. Additionally, cavities in minerals, porous solids, zeolites, and aerosols can limit water to nanoscopic proportions. One effective way to confine water is in a reverse micelle (RM) structure, represented in Figure 2. At the appropriate ratios of surfactant, organic solvent, and water, RMs form spontaneously when amphiphilic molecules self-assemble in nonpolar solvents.[8] The parameter $w_0 = [H_2O]/[\text{surfactant}]$ commonly characterizes the RM size; when RMs are spherical, w_0 is directly proportional to micelle radius. RMs form in solutions under many different conditions, especially as polar, nonpolar, and amphiphilic species vary.[8] By far the most commonly used surfactant for forming RMs is Aerosol OT (AOT, sodium dioctylsulfosuccinate),[8] but RMs also form from surfactants with cationic headgroups, for example, cetyltrimethlyammonium bromide (CTAB), nonionic surfactants, for example, Triton X-100, Brij, or Igepals, and zwitterionic surfactants, for example, phosphatidylcholines. In some cases, a cosurfactant is required for RMs formation.

A wide range of experimental and theoretical studies have explored many different RM properties. Studies utilizing solvation dynamics, time-resolved IR spectroscopy, neutron scattering, and molecular dynamics simulations have shown that dynamics of intramicellar water slows in comparison with bulk water.[9−11] Additional studies have shown

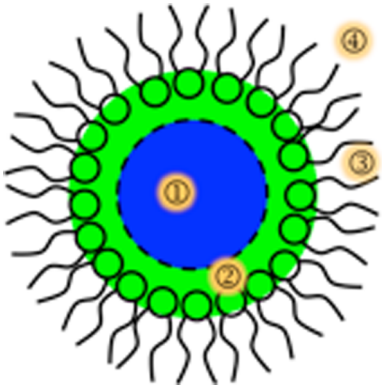

FIGURE 2. Schematic representation of a reverse micelle. Domains in water containing reverse micelles; ① intramicellar water pool with bulk water character; ② aqueous interfacial region. Charged particles, including H^+ and OH^- migrate to the interfacial layer causing the local pH at the interface to differ from the apparent pH in the reverse micellar core; ③ surfactant layer; ④ nonpolar phase.

both enhancement and inhibition of chemical reactions as well as enzyme-catalyzed reactions occurring in RMs.[12] Generally, the effects observed are modest, which contrasts the more dramatic effects (e.g., 10^8-fold increase) on the proton transfer rates associated with cytochrome *c* at the cellular interface.[13]

IV. pH in the Confined Environments of Reverse Micelles, a Statement of the Problem

Three issues emerge regarding the concept of acidity and measurement of pH in nanodroplets. The fundamental issue concerning measurement of acidity is that pH is a macroscopic property. In small RMs, for example, $w_0 \approx 1.5$ with radius ~1.6 nm, the small number of water molecules, about 30.8, challenges our standard concept of pH. Even in the largest RMs, for example, $w_0 \approx 40$, which contains ~300 000 water molecules, there are too few water molecules to utilize macroscopic definitions. In bulk solution at neutral pH, one H_2O molecule is dissociated in the presence of 10^7 H_2O molecules that remain intact, thus defining the smallest volume needed to apply the conventional definition of pH at neutral pH.[14−17] Additionally, water molecules near or penetrating the interface experience very different environments compared with water molecules in interior water pools. Probing these two different regions may yield very different local pH values. Furthermore, varying methods to examine the systems may yield either an average value or an instantaneous snapshot of a local pH.

Measurement of pH in these environments presents an additional problem. In RMs, the water pool is so minute, its pH cannot be probed using a traditional pH meter because

even the tiniest state-of-the-art microelectrodes are orders of magnitude too large. Even if a "picoscopic" pH electrode existed, inserting it into the RMs could compromise the system by disrupting the interface. Luckily, alternative experimental methods enlisting molecular probes have been developed to measure local pH.[9,14,16−27]

A third issue arises because the complex, heterogeneous environments presented by RMs do not present an aqueous homogeneous low ionic strength environment associated with the standard pH definition. To begin, at any moment in time, RM solutions consist of many different local environments; none of the environments indicated in Figure 2 satisfy the requirements used by IUPAC when defining pH. Even if we could define activity in these minuscule water pools, the local value would depend on the precise probe location in the system. Despite these issues, we and others continue to use the concept of pH outside conditions specified by IUPAC for definition of pH recognizing that the definition may not be straightforward but that the experiments will help characterize the systems.[3]

All molecular pH probes are based on a pH-sensitive reaction, generally measured through a spectroscopic signature. However, all of the approaches enlisting molecular probes to measure pH, including our own work, have limitations. The indirect measurement of the pH near the probe depends on several factors, including the probe's domain, as shown in Figure 2. Interfacial domains (Figure 2, ② and ③) will subject different effects on the probe compared with the water domain (Figure 2, ①). Although some studies have considered probe location in their data analysis, uncharacterized or erroneous assumptions can misguide interpretation of measurements. Probes may move between domains when protonation state changes as preferential solvation in the water pool or interface domain lowers the system's energy. The interfacial domain becomes more important as the size of the RM decreases; that is, the probe increasingly senses the interface in small RMs. Because the probe's pK_a is sensitive to the environment,[28] an additional complication arises from the assumption that pK_a is conserved when pH probes are introduced into the microemulsion. Although the pK_a for a water-pool domain probe may maintain the value observed in bulk water, for a different probe, or even the same probe in a different protonation state, located in the interfacial domain the pK_a can differ significantly. Hence, to determine pH in a confined environment accurately requires consideration of both location and pK_a.

Several other factors can influence the pH measured in a RM system. The dynamic nature of RMs leads to collisions resulting in particle coalescence and division as well as the continual partitioning of water molecules, surfactants, and solutes between the various domains in Figure 2.[8] Most measurements reflect an average over many molecules and over time, so they do not show the snapshot of a single molecule reacting. Instead we measure an ensemble average of local pH, which includes the probe location, the pK_a of the probe, and the dynamic micelle environment. Additional factors that can modify measured acidity include ionic strength, conductivity, viscosity, and polarity, which can contribute and should not be ignored.

V. Acidity and pH in Reverse Micelles

pH-sensitive molecular probes have facilitated the measurement of proton activity in bulk solution[2] and local pH in intramicellar water pools.[9,14−27] Spectroscopic approaches such as UV−vis absorption and fluorescence spectroscopies dominate the methods used to measure pH changes in the solution of interest. Other methods such as NMR spectroscopy provide a description of the thermodynamic state and environment in the vicinity of the probe. Recently, we have demonstrated the effectiveness of ^{51}V NMR spectroscopy of a range of probes to measure the local proton activity in RMs.[4,18−21]

V.A. Oxovanadate Probes of Intramicellar pH. As a phosphate analog, vanadate ($H_2VO_4^-$ and HVO_4^{2-}) has many desirable properties including its application as an environmental sensor.[6] The quadrupolar vanadium-51 nucleus with 7/2 spin, small quadrupole moment (-0.05×10^{-28} m^2), 99.76% abundance, high sensitivity (receptivity relative to ^{1}H = 0.38), and large spectral width exhibits properties that make it straightforward to record spectra down to micromolar concentrations.[6,7] The many oxovanadate species (IUPAC uses the term oxidovanadates for oxovanadates) that form in aqueous solution and the fact they all have at least two protonation states lead the oxovanadates to span a wide range of pK_a values from highly acidic to highly basic, as shown in the speciation diagram at the bottom of Figure 1. The oligomerization and protonation reactions are well characterized in bulk media and very sensitive to pH and specific environment. The simple oxovanadate probes are particularly informative because they provide information about pH through chemical shifts, linewidths, protonation reactions, and condensation reactions. We have enlisted the oxovanadate

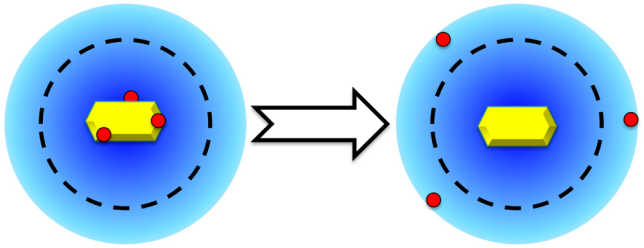

FIGURE 3. A schematic illustrating that when $H_3V_{10}O_{28}^{3-}$ (left side, yellow polygon with red dots) is introduced to the water pool of an AOT or Igepal CO-520 reverse micelle (left side), it loses its protons (red dots) to become $V_{10}O_{28}^{6-}$ (right side, yellow polygon).

speciation chemistry to probe pH and have successfully used it for pH measurements in RMs.[4,18–21]

Using the oxovanadates, we probed water pools in RMs prepared from aqueous solutions with pH ranging from 2 to 13.[4,18–21] At low pH, the major species in aqueous solution is decavanadate, a compact structure with ten vanadium atoms and 28 oxygen atoms. The fully deprotonated form, $V_{10}O_{28}^{6-}$, has dimensions of $5.5 \times 7.5 \times 8.3$ Å^3, which fits well into the RMs.[6,19,20] Using this probe, we demonstrated that even when we use acidic aqueous solutions to make the RMs, the aqueous pool appears essentially neutral or only slightly acidic. When AOT RMs were formed with an acidic aqueous vanadate solution where the $H_3V_{10}O_{28}^{3-}$ ion predominates, the observed ^{51}V NMR chemical shifts demonstrated that the decavanadate species loses two to three protons, as depicted in Figure 3.[7,19,20] Conservation of the ^{51}V NMR linewidths shows that the $V_{10}O_{28}^{6-}$ resides in an aqueous environment, that is, the water pool domain ①. As w_0 approaches 6, the increased linewidths in the spectra indicate reduced mobility for this large oxometalate signifying its interaction with the interface. Complication can arise from the assumption that pK_a is conserved when pH probes reside in the microemulsion environment. Although the pK_a for a probe (such as decavanadate) solvated in the water pool domain may maintain its macroscopic value, the pK_a can differ significantly for a probe located in the interfacial domain, hence in small RMs.[14,17,28] These studies with decavanadate show that the water pool domain ① in RMs approaches neutral pH regardless of the initial pH.

So what is the fate of the H^+ that dissociates from decavanadate species introduced in their protonated forms, $H_3V_{10}O_{28}^{3-}$, $H_2V_{10}O_{28}^{4-}$, or $HV_{10}O_{28}^{5-}$? In RMs formed using the anionic AOT surfactant, the H_3O^+ species are larger than Na^+ counterions that balance the surfactant charge. Thus, most likely H_3O^+ diffuses to the interfacial domain while Na^+ diffuses to the water pool domain to stabilize the $V_{10}O_{28}^{6-}$ species in a fashion similar to chromatographic ion exchange. This creates an acidic interfacial domain and a relatively neutral interior water pool, depicted in Figure 3. This observation is consistent with studies of other water interfaces where H_3O^+ is predicted to reside at the interface.[29] Additionally, we have assumed negligible contribution shifting the $H_3V_{10}O_{28}^{3-}$ deprotonation equilibria because of parameters such as ionic strength, conductivity, viscosity, polarity, and interface effects.

We have extended our studies of decavanadate to RMs formed from neutral Igepal surfactants.[21] Surprisingly, these studies show the same changes in $H_3V_{10}O_{28}^{3-}$ protonation state as we observed for AOT RMs. The ^{51}V NMR spectra clearly show that $H_3V_{10}O_{28}^{3-}$ deprotonates upon introduction to the RMs suggesting that H^+ ions diffuse away from the anion. However, the Igepal CO-520 surfactant has no counterion to exchange with dissociating protons. Assuming negligible effects from decavanadate pK_a changes or effects of other parameters in these systems, both enthalpic and entropic contributions to ΔG would preferentially stabilize the H_3O^+ at the RM interface.[21] Enthalpic stabilization of H_3O^+ at the interface arises from the partial positive charge on the oxygen atom that destabilizes O–H interactions. Entropic stabilization arises from the greater randomization presented by the interface compared with the interior. A plethora of theoretical studies indicate strong preferential location of protons at aqueous interfaces.[29]

Originally we hypothesized that ^{51}V NMR chemical shifts of the oxovanadates could predict pH in the RM water pool. As described above, this works well for acidic solutions introduced into RMs. However, introducing oxovanadate solutions with neutral or alkaline pH into AOT RMs resulted in speciation patterns[18,30] that could not be reproduced in bulk aqueous solution even when pH, temperature, and ionic strength were varied. Because oxovanadate chemistry and the pK_a values of all equilibrating species depend primarily on pH, the speciation observed primarily reflects the system's acidity. However, protonation and oligomerization reactions are also sensitive to factors such as ionic strength, concentration, temperature, and solvent polarity.[6,7] Sometimes these factors cause slight shifts in the speciation observed in RMs, possibly due to pK_a changes.[18] Thus intramicellar vanadate probes sense different environments from bulk aqueous solution. Similar pK_a changes have been reported for other heterogeneous environments such as in aqueous organic solvents mixture and on proteins.[28] For example, Shehatta measured the pK_a

variation of the drug trazodone hydrochloride in water−ethanol mixtures.

Because they are so sensitive to pH, we have used simple oxovanadate probes to examine AOT RMs encapsulating basic solutions from pH 9 through 13.[18,30] In RMs with $w_0 >$ 12 prepared using alkaline vanadate solutions ^{51}V NMR spectroscopy of the probe suggests acidity decreases, that is, pH is moving toward neutral. However, the aqueous vanadium chemistry in this pH range is complex as shown in the speciation diagram at the bottom of Figure 1. Both deprotonation and oligomerization reactions contribute to the chemical shift values and the signal linewidths. The vanadate dimer (also referred to as divanadate), prevalent in this pH range, is particularly sensitive to viscosity and ionic strength, which complicates its application as a pH probe. Related studies using pyrophosphate,[22,23] which is structurally analogous to vanadate dimer, have explored intracellular pH and encountered similar complications. The vanadate study demonstrated that for RMs containing a basic water pool, the pH decreased in contrast to RMs containing acidic solutions where the pH increases upon placement in AOT RMs. For both acidic and basic solutions, the RM environment changes pH toward neutral.

Studies of RMs generally involve mixtures of surfactant, organic solvent, and water equilibrated with CO_2. Accordingly, the pH of the aqueous solution used to prepare RMs in most studies is acidic or near neutral. Interestingly, we have found that atmospheric CO_2 can penetrate the RM solution and lead to pH changes. Given that CO_2 forms H_2CO_3 in aqueous environments which then deprotonates, these results show that CO_2 cannot be ignored in these nanosized systems.[4]

V.B. Other measures of intramicellar pH. Spectroscopy of many molecular probes other than the vanadium oxometalates have been used to explore pH in RMs.[9,14−27] For example, Fujii et al.[23] and Smith and Luisi[22] have used NMR chemical shifts and linewidths in ^{31}P NMR of phosphate and pyrophosphate to gauge proton activity in RMs. The different protonation states for phosphate (pK_a values 2.1, 7.2 and 12.7) and for pyrophosphate (pK_a values 0.85, 1.49, 5.8, 8.2) cover an extensive pH range. The pH-dependent chemical shift variation of the ^{31}P NMR signal of phosphate and pyrophosphate makes them effective methods to determine pH in RM water pools. Indeed, Smith and Luisi developed an indirect acidity scale for water pools in AOT RMs based on ^{31}P NMR chemical shifts.[22,23] Application of this scale to AOT RMs suggested that the pH of the water pool inside the AOT-RM varies little (about 0.3−0.4 pH units) from that of bulk water. The assumption that probe pK_a values changed little is realistic because all the different protonation states of the probes are located in the water pool away from the interface where the physical parameters are less likely to change significantly.[22,23]

A recent study reported interfacial pH changes from T_1 and T_2 measurements of water in RMs.[27] Exploring CTAB RMs, Halliday et al. noted that T_2 values of the H_2O signal were sensitive to pH of the initial solutions from which RMs were formed, whereas T_1 did not vary.[27] This method may be useful for measuring pH changes in these environments in the future and also demonstrating changes in proton transfer rates. Applications of other nuclei such as carbon-13,[24] fluorine-19,[31] and boron-11[31] have been used for studies in RMs, although these have not yet been used to measure intramicellar proton activity.

Most measurements of intramicellar pH have employed optical spectroscopy of organic probe molecules containing at least one labile proton coupled to electronic transitions to track pH changes in the environment.[9,14−27] For example, absorption and excitation spectroscopy of hydroxypyrene trisulfonate (HPTS) reflect the protonation state of the molecule and suggest that the interiors of AOT RMs formed in heptane[15] and isooctane[26] have a buffering effect on the water pool moving the apparent pH toward neutral compared with the solution from which RMs were formed. The high negative charge of HPTS has led researchers to assume it remains in the micellar interior, well solvated by water in the interior of AOT RMs. Although it is a well-known photoacid, with distinct ground and excited state pK_a values, only the ground state pK_a value ∼7.2 has been used to gauge intramicellar pH.[15] Fluorescein is another frequently used pH probe.[17] With four different protonation states (dianion, monoanion, neutral, and cation) spanning a wide pH range, the fluorescein absorption and emission spectra have been used to explore pH in AOT RMs.[17] Fluorescein spectroscopy measurements also support the hypothesis of a buffering environment inside the RMs. Because these probes may be compatible with the interfacial, aqueous, and organic domains in the RM systems, their location is not obvious. Furthermore changing fluorescein protonation states can cause the probe to partition into differing locations in the RMs.[17] Thus observed changes in the spectroscopy can arise from several parameters, which simultaneously mask the true local proton activity.

Organic probe molecules complement inorganic NMR probes in several ways. The ability to use fluorescence for measurements makes organic dyes incredibly sensitive,

with the potential to measure signals from single dye molecules in RMs. Using absorption spectroscopy to measure pH relies on the proportionality of molecular absorbance to concentration, determined through the Beer–Lambert law, $A = \varepsilon bc$. This assumption requires the molar absorptivity, ε, to remain constant as the molecule changes protonation states, which is difficult to assess. Additionally, unlike the fluorescein molecule that has several distinct protonation states and molecular forms, most of the organic probes used possess one single protonation site and thus function only in a small pH range. Therefore, the experimental design must honor the active range of the pH probe, because use outside this range does not provide information on the pH. To circumvent the issue of molecules possessing only a single pK_a, researchers have utilized a range of spectrally active probes to explore the interior of RMs[14,17] El Seoud and co-workers explored the influence of organized surfactant assemblies on molecular probe pK_a values in a wide range of systems.[14,24] Miguel et al. demonstrated that the differing probe molecules partition into various RM domains, Figure 2, depending on the probe's molecular structure.[16] Their work illustrates how the partitioning of the probes among various locations in the heterogeneous RM environment can complicate the interpretation of the results because it is difficult to separate the features that arise from proton activity in the water pool and changes in the other factors in the system.

Experiments probing acidity and basicity in RMs vary significantly. Differences in RM size and composition, for example, varying surfactant and cosurfactant, can be expected to influence the acid/base equilibrium, which complicates comparison between studies.[17] However, differences even exist among measurements on systems with similar composition.[15,25] These differences point to important issues associated with using a molecular probe to measure pH in a confined environment, without extremely careful description of the systems and parameters, such as size and composition, and detailed description of the components. Even though the exact meaning of pH in such limited water pools is not clear, these measurements provide important information, which can facilitate controlled modifications of chemistry occurring in aqueous water pools.

V.C. Theoretical Approaches Related to Intramicellar pH. Two groups have modeled protons inside RMs.[32,33] Karpe and Ruckenstein predicted that the AOT RM inner surface will be more acidic than the core and that the relative pH will vary with w_0.[32] Rodriguez et al. modeled nonionic RMs encapsulating an excess proton.[33] They found that the interfacial domain provides a stable environment for the excess proton and showed that enthalpic and entropic contributions can account for the interfacial solvation of the proton. These simulations imply that the pH of the interface is lower than that of the water pool, in agreement with our experimental observations[19,20] as well as that of others.[27] So far, the theoretical studies have not directly tackled the concept of pH in nanosized systems described in this Account, in part because simulations include only a limited number of water molecules in their calculations. However, a recent approach proposed an absolute standard chemical potential of the proton in any medium would allow determination of pH using electrochemical potentials.[34] Although it has not yet been applied, this approach may have potential for complex media like RMs.

VI. Summary and Conclusions

The fundamental issue concerning measurement of acidity in nanoscale systems is that the pH is a bulk and macroscopic property and that the conventional definition of pH in water droplets with few water molecules is not simple. In RMs, the water pool is so minute its pH cannot be probed using traditional pH meters. Instead, alternative experimental methods enlisting molecular probes have been developed to measure local pH based on a pH-sensitive reaction. However, all of the approaches enlisting molecular probes to measure pH, including our own work, have limitations. Consideration of probe location and pK_a is critical for accurate determination of the pH in a confined environment. Because pH measurements reflect averages both over many molecules and over time, the measurements do not show the snapshot of a single molecule reacting; instead we always measure average pH over an ensemble local environment, which includes the location, the pK_a of the probe, and the dynamic micelle environment and potential influences from ionic strength, conductivity, viscosity, and polarity. The complexities of pH measurement in confined media are endless; still researchers have obtained valuable information about local acidity values in these systems. Of utmost importance, researchers must continue to make experimental measurements, even on complex nanosized systems, and caution should be exercised when interpreting the experimental data.

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BIOGRAPHICAL INFORMATION

Debbie C. Crans was born in Copenhagen, Denmark, in 1955. She studied for the Cand. Scient. degree in biochemistry and chemistry at the H. C. Ørsted Institute, University of Copenhagen, Denmark. In 1980, she went to Harvard University where she earned a Ph.D. in chemistry working on enzyme-catalyzed organic synthesis with G. M. Whitesides. She did postdoctoral studies in enzymology at the University of California, Los Angeles, as an American Heart Fellow working with O. L. Chapman and P. D. Boyer. At Colorado State University, Prof. Crans's studies are in the area of biological, bioinorganic, and bioorganic chemistries. She is interested in coordination chemistry and metals in medicine and recently has been investigating metal complex interactions with interfaces. At Colorado State University, she holds a faculty position in Chemistry and is a member of the Cell and Molecular Biology Program. Prof. Crans is a leader in the field of vanadium chemistry and biochemistry and was honored for her contributions with the First Vanadis award in 2004 and with the Colorado Section ACS award in 2010. She was elected an ACS Fellow in 2009.

Nancy E. Levinger was born in New York, NY, USA, in 1961. She attended Northwestern University where she was a part of the Integrated Science Program. After earning B.A. degrees in Integrated Science and physics, she moved to the University of Colorado where her Ph.D. degree in chemical physics with W. C. Lineberger focused on spectroscopy and dynamics in large cluster ions. As a NSF postdoctoral fellow, she worked on ultrafast electron transfer dynamics with P. F. Barbara at the University of Minnesota. Since joining the faculty at Colorado State University in 1992, her work has focused on structure and dynamics of molecules in confined environments. She has collaborated with people across the world applying many techniques to explore properties of reverse micelles. At Colorado State University, she holds faculty positions in Chemistry and in Electrical Computer Engineering. For her contributions in science and education, she holds the title of University Distinguished Teaching Scholar. She is a fellow of the American Physical Society and AAAS.

FOOTNOTES

The authors declare no competing financial interest.

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