Chemically Induced Dynamic Electron Polarization Studies of a pH-Dependent Free Radical Cage Formed in a Photoinitiator Labeled Poly(methacrylic acid)

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ABSTRACT: Time-resolved electron spin resonance (TR-ESR) has been used to investigate the effect of pH-dependent poly(methacrylic acid) clustering on the radical pair generated from photolysis of a poly(methacrylic acid) acid sample randomly labeled with the common α-hydroxy ketone photoinitiator **1**. The TR-ESR spectra for the ketyl radical show a dramatic change in electron spin polarization mechanism, from a predominantly triplet mechanism (TM) at low pH (below 6) to a predominantly radical pair mechanism (RPM) at high pH (above 7). The ratio of RPM/TM polarization exhibits a sigmoidal behavior as a function of pH which passes through an inflection point at pH 6.9 ± 0.1. The inflection point agrees well with previous estimates for pH-dependent cluster opening in poly(methacrylic acid). These results indicate that the pH-dependent cluster is capable of forming a cage around the geminate radical pair which restricts its motion at low pH. At higher pH, the cluster opens, allowing the ketyl radical to escape the pair. Studies of the benzoyl partner in the radical pair, which is attached covalently to the polymer backbone, indicate differing environments within the cluster even at pH below cluster opening. This study represents the first application of TR-ESR and electron spin polarization (ESP) to the problem of pH-dependent clustering and reveals new features of the pH-dependent poly(methacrylic acid) cluster.

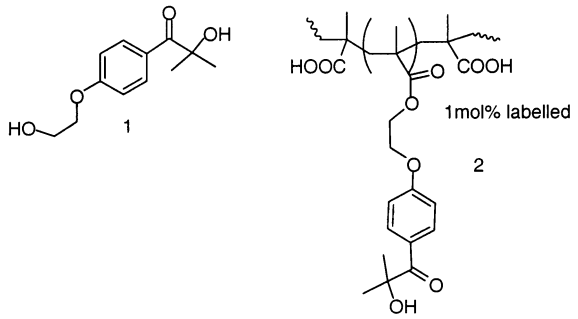
Introduction

In aqueous solutions of poly(methacrylic acid) (PMA) below a critical pH the polymer chain has the curious behavior of clustering around aromatic species adsorbed to the polyion or attached to it. This is evidenced by the large change in the photophysical characteristics of fluorescent dyes reflecting their separation from the aqueous medium[1−3] and the inability of cyclodextrin to complex with binaphthyls clustered within the PMA.[4] Within the cluster, molecular motion is impeded as shown by the large extension of the rotational relaxation time of the attached dye[3] and by the large reduction of the rate of racemization of a binaphthyl atropisomer attached to PMA.[4]

The above phenomena are clearly related to the titration behavior of PMA. The polymer chain does not expand with increasing ionization below a critical change density.[5] Also, the p*K* remains constant over a range of pH after an initial increase before resuming its rise with further increase in pH.[6] This has been interpreted[7] as indicating that within the pH range of the p*K* plateau there is a gradual transition from one to another local conformation with no significant change in the overall expansion of the polyion. Only when this transition is complete does the long-range Coulombic repulsion between the ionic charges with increasing pH lead to the large expansion of the PMA. This is then the point at which any cluster formation around hydrophobic moieties is disrupted.

More recently, we have studied[8] PMA to which small proportions of hydrophobic polymerization photoinitiators were attached. In the pH range for cluster formation, the radicals formed from the photolysis of the photoinitiator would be trapped within the cage of the cluster, precluding polymerization of a monomer dissolved in the surrounding aqueous medium. In this way the observation of photoinitiated polymerization as a function of pH would act as a probe to detect access of the produced radicals to the monomer molecules. This experiment was conducted with acrylamide dissolved in an aqueous solution of the poly(methacrylic acid) **2**, which was labeled to an extent of 1% with the common photoinitiator **1** (Scheme 1).

Scheme 1. Compounds Investigated: 1, HHMP; 2, HHMP−PMA; 1 mol % label



Irradiation of **2** at a series of pHs gave results consistent with the picture of a pH-dependent cage effect in that only at pH values in the range of the expandable coil did significant polymerization of the acrylamide occur with formation of a graft copolymer, with the unexpected benefit of forming an interesting physical hydrogel.[8,9]

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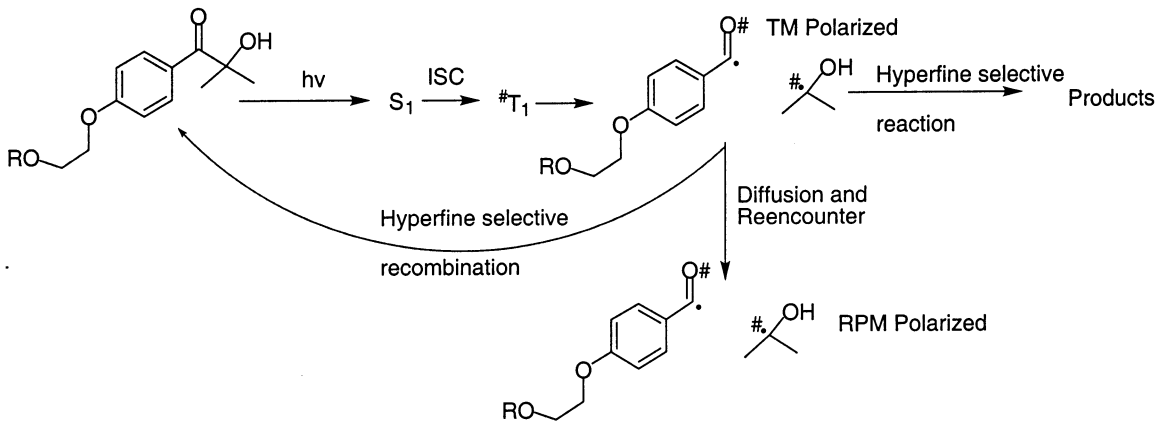
Scheme 2. Photochemistry of 1 (R = H) and 2 (R = PMA)[a]



[a] Photolysis (308 nm) leads after ISC to a spin-polarized triplet which rapidly cleaves with conservation of polarization to the triplet mechanism polarized radical pair. This radical pair is capable of generating radical pair mechanism (RPM) polarization through diffusive excursions and reencounter.

Further insight into this pH-dependent clustering phenomenon could be attained by directly observing the free radicals generated on irradiation of **2** by ESR. Such a direct observation could yield new kinds of insight into the nature of the hydrophobic cluster and specifically its ability to act as a supercage for radical reactions. Consistent with the fact that time-resolved ESR studies of photogenerated radical pairs have been used to elucidate the supramolecular structure of micelles,[10] we have found that this technique can be used for the present work.

It has been well established that photolysis of **1** followed by intersystem crossing leads to cleavage of the carbonyl−alkyl bond from the triplet state and generates a polarized ketyl and benzoyl radical.[11−13] When **1** is covalently bound to PMA, an analogous photochemical primary process is expected except that the resultant benzoyl radical must be attached to the PMA backbone, while the ketyl radical is free to diffuse away. In this study we have observed and investigated the transient polarized ESR signal of the ketyl radical as a function of pH. We have found that the mechanism of polarization changes as a function of pH, in a manner that correlates with the opening of the hydrophobic cluster consistent with earlier studies using other kinds of probes.[2−4,9,10]

Experimental Section

Compound **1** (Ciba Specialty Chemicals) was used as received. Methanol (HPLC grade, Fisher) was used as received. Distilled deionized water was used for buffer solutions. Potassium acetate (Fisher), acetic acid (Fisher), potassium hydrogen phosphate (Aldrich), and potassium dihydrogen phosphate (Aldrich) were used as received.

Poly(methacrylic acid) and **2** were synthesized as follows. The photoinitiable monomer (HHMP−MA) was formed by reacting methacryloyl chloride with 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (HHMP) in methylene chloride with triethylamine. Methylene chloride was purified by adding portions of H_2SO_4, $NaHCO_3$, and water in a separatory funnel and decanting the organic layer. Methylene chloride was dried overnight with $MgSO_4$.Triethylamine was dried with CaH_2, distilled, and stored over NaOH pellets. The reaction was performed with an argon blanket and in a dry ice/acetone bath. The flask was also wrapped with aluminum foil to protect from light. The reaction was left overnight, and the following morning saturated $NaHCO_3$ was added. The ester formed with the primary hydroxyl group, the desired product, was monitored by thin-layer chromatography using hexane:ethyl acetate (3:1) as an eluent and separated by column chromatography using hexane:ethyl acetate (4:1).

HHMP−PMA was formed by reacting HHMP−MA with methacrylic acid in 1,4-dioxane with AIBN at 65 °C for 3 h. Methacrylic acid was freshly distilled under vacuum. 1,4-Dioxane was distilled with $LiAlH_4$. All components were added to an air-free reaction flask. Three cycles of freezing in dry ice/acetone, vacuum, thaw, and argon purge were performed to remove oxygen. Adding methanol and dissolving the polymer formed quenched the reaction. Dialysis was performed using a 10 000 molecular weight cutoff Slide-A-Lyzer from Pierce in a 3000 mL beaker of deionized water. The water was changed daily for 7 days. The product was lyophilized, and the percent photoinitiator was determined by UV spectroscopy using the extinction coefficient for HHMP in methanol.

Time-resolved EPR experiments employed the pulses (308 nm, ca. 10mJ/ pulse, 20 ns) from a Lambda Physik Lektra 50 excimer laser, a Bruker ER 100D X-band EPR spectrometer, and a PAR boxcar averager and signal processor (models 4420 and 4402). Argon-saturated solutions were flowed through a quartz flow cell (∼0.3 mm thick) in the rectangular cavity of the EPR spectrometer. Further details are described elsewhere.[13−15]

Solutions of **2** (0.5 mM in label, 50 mM in PMA according to monomer concentration) in 80% aqueous buffer (acetate for pH below 5.7, phosphate for pH above ($[K^+] = 0.2$ M)) and 20% methanol were used for the TR-ESR experiments. The integration window used in these studies was 400−600 ns after the laser pulse. Solutions of **1** were prepared analogously, both with and without PMA.

Results and Discussion

Photolysis of compound **1**, in analogy to the behavior of many ketones,[11] shows two different mechanisms of chemically induced dynamic electron polarization (CIDEP). The triplet mechanism (TM**)** results from overpopulation of one of the three triplet states on intersystem crossing, an imbalance which is preserved in the α-cleavage step and leads to a net emissive (E) signal for the ketyl radical.[11,16] In the radical pair mechanism (RPM) polarization results from hyperfine selective mixing of triplet and singlet wave functions and produces the E/A pattern for the ketyl radical. The

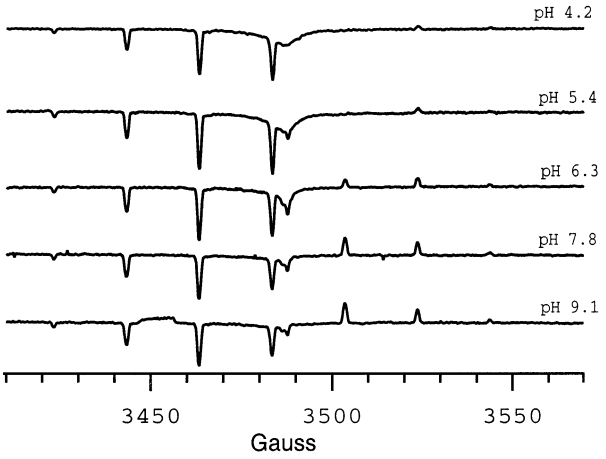


Figure 1. TR-CW-ESR spectra recorded 400−600 ns following laser excitation (308 nm) of HHMP−PMA **2** (0.5 mM HHMP, 50 mM PMA (based on monomer)) at various pH values in argon-saturated aqueous methanol (80% aqueous) buffer solutions at room temperature.

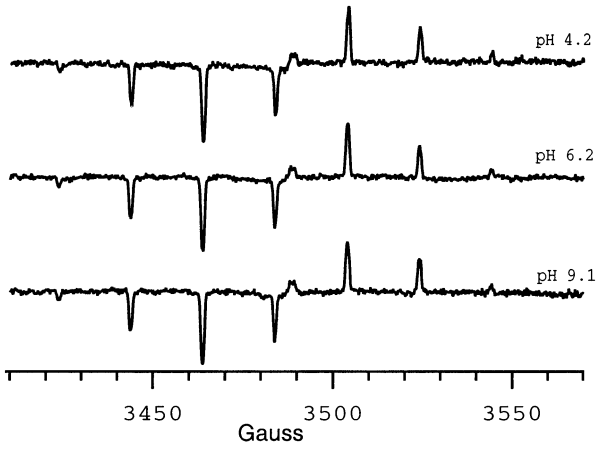


Figure 2. TR-CW-ESR spectra recorded 400−600 ns following laser excitation (308 nm) of HHMP **1** (0.5 mM) at various pH values in argon-saturated aqueous methanol (80% aqueous) buffer solutions at room temperature.

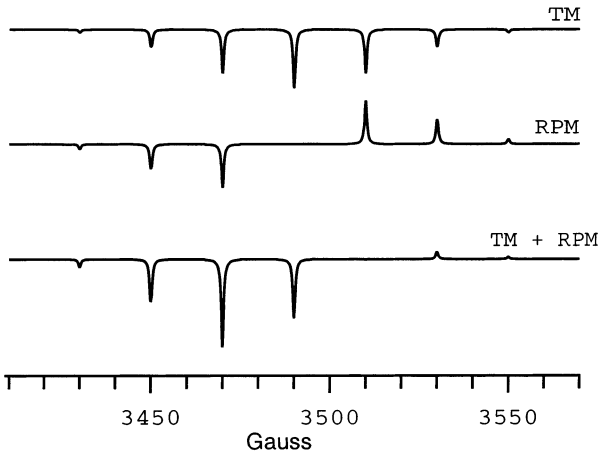


Figure 3. Simulation of TR-CW-ESR spectra for dimethylketyl radical under conditions of triplet mechanism (TM) polarization, radical pair mechanism (RPM) polarization, and the sum of TM and RPM mechanism polarizations (TM + RPM).

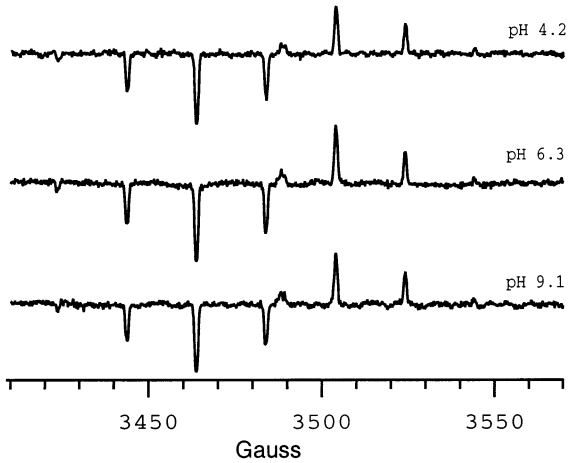


Figure 4. TR-ESR of HHMP (Irgacure 2959 0.5 mM) in the presence of PMA (50 mM in monomer) in 80% aqueous buffer−20% methanol; pH dependence (identical conditions to HHMP−PMA).

experimental results of the CIDEP produced by photolysis of **2** as a function of pH are illustrated in Figure 1. The dimethylketyl radical signal produced by photolysis of **1**, as a model for **2**, consists of a seven-line pattern (A_β = 19.7 G).[13] The benzoyl radical produced by photolysis of **1**, as a model for **2**, appears at 3488 G (see Figure 2). Simulations[17] of the CIDEP spectrum produced as a mix of the TM and RP mechanisms are shown in Figure 3. Both mechanisms are observed for **1** in solution, and in aqueous solution (see Figure 2) the radical pair mechanism is the dominant mechanism.

Figure 1 shows the TR-ESR spectra for **2** in a time window 400−600 ns after the laser flash as a function of pH. Notice at low pH the low and center lines of the ketyl radical show strong emissive signals; however, the high field lines are extremely weak. This spectrum is due to a large amount of triplet mechanism (TM) polarization which is E and which cancels out the positive E/A RPM polarization in the high field lines. At higher pH, the RPM mechanism polarization increases with respect to the TM polarization as can be seen by the increased strength of the absorptive high field lines. Our experimental data for the dimethylketyl radical can be modeled by simulation (Figure 3) using a linear combination of the pure TM and RPM spectra, and from this we can extract a ratio of TM to RPM polarization for each experimental spectrum (the error in our estimate is ±2%). This ratio is plotted vs pH in Figure 4.

From Figure 5, it can be seen that the ratio of RPM/TM polarization for the ketyl radicals is dependent on the pH of the solution. At pH below 5.5, the ratio RPM/TM is 0.9 ± 0.1. As the pH rises, this ratio passes through an inflection point at pH 6.9 ± 0.1. At pH above 9, the value of RPM/TM approaches a constant value of approximately 2.6. The above results are consistent with previous studies on hydrophobically labeled poly(methacrylic acid)[2−4] and specifically with studies using HHMP−PMA as a photoinitiator for acrylamide.[8,9] All results indicate that the hydrophobic cluster around **2** is closed at low pH but opens at higher pH. Furthermore and importantly, the inflection point in our measured ratio of RPM/TM occurs within the pH range where the polymer capsule has been proposed to open (pH ∼ 6.5) as independently judged by previous work in which the capsule and cyclodextrin competed for complexation with a binaphthyl group appended to PMA.[4] Opening of the capsule allows complexation of the cyclodextrin with the appended binaphthyl group, which is revealed by a large induced circular dichroism. In a parallel study

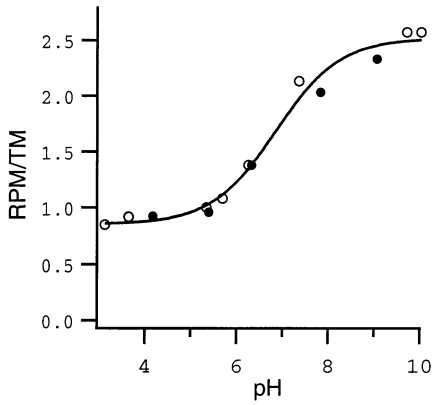


Figure 5. Graph of RPM/TM as a function of pH using two data sets: set a (dark circles) and set b (open circles); fit to a sigmoidal function (line through points). The inflection point of the sigmoid is calculated to be pH 6.9 ± 0.1.

we have studied the pH dependence of the induced circular dichroism associated with the photoinitiator group in **2**, which again signals the opening of the capsule in the identical pH range.[9] These complexation studies,[4,9] conducted in water, additionally demonstrate that the presence of methanol, necessary in the present work to attain the required concentration for measurement sensitivity, does not affect the encapsulation properties of PMA. The latter is consistent with early work in this area demonstrating that far larger proportions of methanol than used here are necessary to preclude the encapsulation properties of PMA.[1]

Control experiments confirm that the pH-dependent change in the polarization mechanism is only present for covalently bound **2**. The TR-ESR spectra of **1** in aqueous methanol in the presence of PMA (see Figure 4) show no change in the ratio of RPM/TM as a function of pH. In this control, the RPM/TM ratio for **1** in aqueous methanol solution in the presence of PMA at pH 6.2 is 2.9 ± 0.1, which is slightly higher than the limiting value for **2** at high pH.[18]

The underlying reason for the pH-dependent change in polarization mechanism has yet to be determined conclusively. However, two possibilities seem likely. One possibility is that the rate of α-cleavage observed for **2** is changed relative to **1** due to a change in the polarity of the medium. This could result in a net decrease in the amount of triplet mechanism. A second possibility is that at low pH the diffusion of the geminate radical pairs is hindered due to encapsulation of the primary geminate radical pair, resulting in a lower amount of radical pair mechanism. It is interesting that a similar uncertainty pertains to the observation of enhanced fluorescence for aromatic probes at low pH in poly(methacrylic acid), which could arise via the exclusion of water from the probe or via a reduction in conformational motion.[4,19]

The hydrophobic cluster formed by PMA in fact is known to change the polarity of the environment around the label, as has been determined previously by Nakashima et al.,[20] who studied pyrene I_1/I_3 ratios, that is, the ratio of the first and third vibrational bands of pyrene fluorescence, which has been shown to be a reliable reporter of the polarity of the pyrene environment. At low pH (below 5), the polarity of the medium surrounding the hydrophobic cluster ($I_1/I_3 \sim 1.4$) was found to resemble ethyl acetate ($I_1/I_3 = 1.45$), whereas at higher pH, the medium surrounding the label becomes more polar ($I_1/I_3 = 1.8$) to a value similar to dimethylformamide.[21]

The photophysics of **1** is known to be dependent on solvent effects. For example in THF, the TR-ESR spectra of **1** are mostly the triplet mechanism.[22] However, in aqueous solution, the radical pair mechanism dominates (see Figure 2). It is possible that the changing environment around the probe molecule (**2**) as a function of pH could be changing the photophysics of **2** and therefore the observed CIDEP mechanisms. However, if this were the case, the change in polarization mechanism would be due to a decrease in the rate of α-cleavage (type 1 reaction), which would result in a net reduction of the triplet mechanism but not necessarily affect the contribution of the RPM polarization. As can be seen from Figure 1, there is very little decrease in the center field line strength as the pH is increased. The strength of center field line is due solely to TM polarization (see Figure 3). The fact that the center field line changes very little with pH suggests that the amount of triplet mechanism does not decrease significantly with higher pH. This result argues against a simple change in polarity as responsible for the change in TR-ESR signal for **2**.

A more likely possibility is that the hydrophobic cluster prevents radicals from undergoing hyperfine selective ISC, thereby reducing the amount of RPM at low pH. In this hypothesis, the hydrophobic cluster does not allow the geminate radical pair to separate prior to recombination, and so there will be less possibility for the spin sorting necessary for radical pair mechanism polarization to be generated. The dimethylketyl radical is only able to escape efficiently when the hydrophobic capsule is opened, and this occurs cooperatively around pH 6. This picture is consistent with the observation that in this pH range the benzoyl radical, which must remain attached to the backbone, initiates grafting of polyacrylamide[8,9] as well as with the racemization kinetics of appended binaphthyl groups and the access of these groups to cyclodextrin encapsulation.[4]

The benzoyl radical spectrum of **2** is observed as a broad signal at 3487 G at low pH (pH 4.18) (see Figure 1). At higher pH, (pH > 6.3), more narrow and structured benzoyl spectra are observed at slightly higher field (3488 G).[23] At intermediate pH, both broad and narrow signals appear in the ESR signal.

Studies on the time-resolved ESR of **1** have shown similar results.[24] In these studies, the photolysis of **1** in the crystalline state gives rise to a broad signal. However, in ethanol solution a sharp signal is observed for the benzoyl radical. Photolysis of **1** adsorbed on cotton results in a mixture of broad and sharp signals which is interpreted[24] as resulting from crystalline and liquid environments for the probe in cotton.

Consistent with these studies,[24] the broad and narrow signals in our spectra (see Figure 1) could be indicative of different environments for the polymeric benzoyl radical. The pH has a pronounced effect on the equilibrium between these two environments. At low pH, a glassy or crystalline-like environment is experienced by the polymeric benzoyl radical. As the pH is increased, it appears as though a more liquid environment arises, as evidenced by the sharper benzoyl signal. It is interesting to note that the broad and sharp signals coexist between pH 5.4 and 6.3. These coexisting signals, which overlap with the region of the titration curve in which p*K* does not vary with pH, may be associated with

the coexistence of tighter and looser clusters indicated by the results of the photophysical and racemization studies referred to above. In the studies of the racemization kinetics of binaphthyl groups appended to PMA, slower racemization than that encountered at both higher and lower pH is found in the same pH region. This led to the suggestion[4,25] that there is some readjustment of the cluster about its hydrophobic captive in this p*K* plateau region, a readjustment that is allowed by the coexisting conformational states.

Conclusion

TR-ESR has been employed for the first time in the study of pH-dependent clustering in PMA and has revealed new features about the nature of the PMA cluster. The change in polarization mechanism as a function of pH agrees well with PMA cluster opening as studied by other methods.[2−4,8,9] CIDEP data for the ketyl radical produced by photolysis of the pendant α-hydroxy ketone photoinitiator appear to indicate that at low pH the PMA cluster is capable of forming a cage around the geminate radical pair of **2**, thereby preventing separation of the primary geminate radical pair. This leads to a large "cage effect" for geminate pair recombination and a significant decrease in the efficiency of **2** as a photoinitiator for polymerization of acrylamide in the bulk aqueous environment. TR-ESR for the benzoyl radical indicates that the cage itself undergoes changes in its rigidity between pH 5.4 and 6.3, even below the pH at which cage opening occurs. These results provide further insight into previous studies on **2**.[4,25]

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