

Hydrogen Bonding in a Host–Guest System: C-Undecylcalix[4]resorcinarene and Water in Benzene

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The behavior of water in a benzene solution of C-undecylcalix[4]resorcinarene (UR) is studied by ¹H 2D NMR exchange spectroscopy and by 1D NMR. The rate constants for exchange of the UR hydroxyl protons, H_a, with water over the temperature range 298–338 K are 0.7–4.6 s^{−1} and those for the exchange of UR hydroxyl protons, H_b, over the temperature range 298–328 K are 1.8–8.1 s^{−1}. Activation energies calculated for these exchange processes are 9.0 ± 0.5 kcal/mol and 10.2 ± 1.0 kcal/mol, respectively. The chemical shift of dissolved water is largely dependent upon the concentration of UR. An upper limit for the critical aggregation concentration of UR is 10^{−5} M as shown by ¹H NMR.

Introduction

C-Undecylcalix[4]resorcinarene (UR), a tetradentate host, is able to extract a wide variety of polyhydroxy guests into nonaqueous solvents such as carbon tetrachloride, chloroform, and benzene.^{1–6} The guest molecules form hydrogen-bonded complexes with UR, and the structure and properties of these complexes depend strongly on the number and spatial arrangement of the hydroxyl groups on the guest.^{2,5,7–9} It is especially important that UR can selectively bind sugars and has the potential to enhance the recovery of interesting and important saccharides.^{8,10} The molecular selectivity of UR is also of potential use in the area of chemical sensing. A selective monolayer-modified electrode based on UR shows selectivity in sugar detection, and a self-assembled monolayer of a derivative of UR deposited on a quartz microbalance is reported to detect various chlorocarbons.^{11,12}

Figure 1 shows the structure of UR. Important features of this structure include a bowl-shaped cavity capable of including a variety of structures, a hydrophilic rim with four hydrogen-bonding regions to interact with guests, a long hydrocarbon tail on methine carbons for rigidity as well as solubility in nonaqueous solvents, and aromatic groups capable of nonbonded interactions with included guests.^{1,2,13,14} There are a number of interesting chemical and physical properties which arise from this structure. Water present in (CDCl₃)₂ solutions of the dihydrate of UR interacts with the hydrogen-binding sites of UR and exhibits very slow exchange between bound and unbound states.^{5,7} Vapor pressure osmometry indicates that UR forms aggregates in benzene which have an average molecular weight¹ of 7066. This value is within 5% of an aggregate size of 6 UR molecules. Aggregation of UR also has been reported in chloroform, and binding of guests by more than one molecule of UR occurs in several systems described in the literature.^{1,8} However, it is also possible to have situations in which more than one guest molecule interacts with a single molecule of UR; in the case of octyl glucopyranose, a 4:1 complex is formed with UR which shows a remarkable level of cooperative bonding (Hill coefficient ≈ 4).⁸

Our research is concerned with the nature of the aggregates formed by UR in benzene, the role of water in determining the structure and dynamics of this aggregate, and the ability of the aggregate to include free radical guests. In this paper, 2D NMR

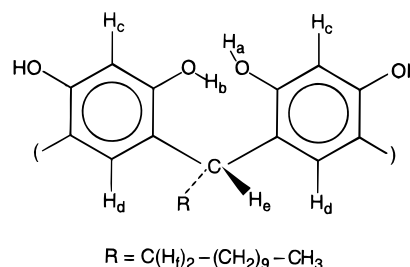


Figure 1. Repeating unit of the cyclic tetramer, UR. Proton designations are indicated.

at 200 MHz is used to explore the exchange between hydroxyl protons of UR with water protons over the temperature range of 298–313 K, NMR at 500 MHz is used to explore the concentration range for aggregate formation by UR, and the overall behavior of water in a benzene solution of UR is explored over a range of concentrations by NMR. A model is proposed for the aggregate of UR in benzene that is based on the molecular weight determined by vapor pressure osmometry, on the results of our experiments, and on published models for hydrogen bond mediated self-assembly of large structures in nonaqueous solvents.¹⁶

Experimental Section

C-Undecylcalix[4]resorcinarene (UR)-dihydrate (Aldrich Chemicals) and benzene-*d*₆ (Isotec) were used as received. Solutions for NMR experiments were degassed on a vacuum line at 0.1 mTorr. Solutions were prepared by sonicating UR in benzene-*d*₆ (Isotec) for 2 min under N₂. ¹H NMR spectra were acquired on a Varian Gemini-200 spectrometer at a frequency of 200 MHz with a 90° pulse width of 38.5 μs. The Gemini is equipped with variable temperature controller and probe.

The ¹H 2D NMR exchange spectroscopy (¹H 2D-EXSY) experiments were carried out using the phase-sensitive pulse sequence, (π/2)_x-τ-(π/2)_x-τ_m-(π/2)_x-acquire. This pulse sequence is identical to the phase-sensitive nuclear Overhauser effect spectroscopy pulse sequence. The experiment consisted of 256 free induction decays (FIDs) (128 for each phase) of 1024 points each collected with an acquisition time of 0.23 s and relaxation delays of 4 s. Each FID consisted of 16 transients. The sweep width in both dimensions was 2200 Hz. The 2D studies were carried out over the temperature range 298–338 K. A mixing

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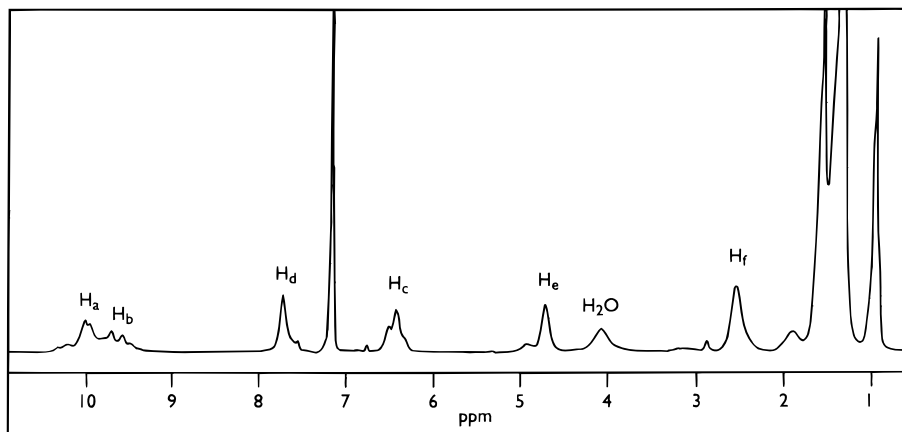


Figure 2. A 200 MHz ^1H NMR spectrum of 5 mM UR in benzene- d_6 . The peak assignments are H_f , 2.54 ppm; H_2O , 4.07 ppm; H_e , 4.70; H_c , (6.41 ppm; H_d , 7.72 ppm; H_b , 9.57 and 9.70 ppm; and H_a , 9.95 and 10.00 ppm.

time (t_m) of 200 ms proved optimal at the temperatures studied. The average T_1 value observed for protons of UR not on the undecyl carbon chain was 0.4 s. The optimum mixing time¹⁸ would be expected to fall between $T_1/2$ and $3T_1/2$, a range of 0.2–0.6 s. Peak intensities were determined by volume integration. The concentration of UR in the 2D experiments was 5 mM in benzene- d_6 .

Three sets of experiments were carried out on a Varian Unity-500 NMR spectrometer with a 5 mm pulse field gradient and indirect detection probe. These experiments were (1) the critical aggregation experiments on UR in benzene, (2) the determination of the proton chemical shifts of UR over the temperature range 298–338 K, and (3) the determination of the chemical shift of water as a function of the concentration of UR.

Results and Discussion

Proton Exchange Studies. The one-dimensional ^1H NMR spectrum of UR in benzene is shown in Figure 2. The spectrum of UR displays peaks in the 9.0–10.0 ppm region, attributed to the hydroxyl protons of UR, and a peak in the region of 4.0 ppm, attributed to dissolved water, for the case that the saturation concentration of water has not been exceeded.

In a regime of slow proton exchange between water and the UR hydroxyl protons, the two regions would be expected to yield cross peaks in a two-dimensional exchange spectroscopy experiment. To explore proton exchange in more detail, proton 2D-EXSY studies of UR in benzene were carried out over the temperature range 298–328 K. The 1D NMR spectrum of UR in benzene displays distinct peaks for hydroxyl protons H_a and H_b (see Figure 1) as assigned previously.^{15,2} The 2D spectra of 5 mM UR are shown in Figure 3. This study was carried out on solutions with no added water. “Indigenous” water was always present since Isotec benzene- d_6 has a water specification of <0.01% H_2O by volume and UR is a dihydrate that is also hygroscopic.²

Rate constants for exchange of protons are calculated from the relative cross peak intensities. The rate equation¹⁹ is $k = \ln\{[1 - (C/D)]/[1 + (C/D)]\}/(-2t_m)$, where C and D indicate cross peak and diagonal peak intensities, respectively. The protons of water form cross peaks with the H_a and the H_b protons of UR in this experiment. The calculation employed the diagonal intensity of the H_a or H_b protons. Over the temperature range 298–328 K, the rate constants for water proton exchange with H_a range from 0.74 to 4.58 s^{-1} and for water proton exchange with H_b range from 1.83 to 8.11 s^{-1} , as shown in Table 1.

TABLE 1: Pseudo First-Order Rate Constant (k) for the Exchange of Protons H_a and H_b with Water Protons as a Function of Temperature

temp, °C	$k(\text{H}_a)$	$k(\text{H}_b)$
25.5	0.74	1.83
35	1.41	3.74
45	2.20	7.34
55	3.09	8.11
65	4.58	nd ^a

^a nd = not determined.

The activation energies for these two exchange processes were calculated from Arrhenius plots of the rate constants over the temperature range 298–328 K. These plots are shown in Figure 4. The data yielded activation energies of $9.0 \pm 0.5 \text{ kcal/mol}$ and $10.2 \pm 1.0 \text{ kcal/mol}$, respectively, for H_a and H_b proton exchange with water. The calculated E_A corresponds to the removal of a hydrogen from an oxygen as the exchange proceeds. The bond dissociation enthalpy of a typical OH bond²⁰ is about 100 kcal/mol.

The results for the OH bonds in the UR–water system indicate a decrease in the OH bond strength by an order of magnitude. It is very likely that cooperative hydrogen bonding plays a role in this behavior. Cooperative hydrogen bonding, in which multiple hydrogen-bonding groups interact simultaneously within a single region, leads to greater OH polarization and stronger hydrogen bonds.¹⁷ It is reported that, in such systems, the covalent OH bonds may be lengthened such that they are nearly indistinguishable from the hydrogen bonds.¹⁷ Such a cooperative effect may involve any hydroxyl groups of one UR, hydrogen-bonded guests, water, or intermolecular hydrogen bonding between UR molecules within an aggregate.

The increased polarity of the OH bonds involved in such cooperativity would account for the far downfield chemical shift of the H_a and H_b protons of UR, 10.0 and 9.6 ppm, respectively, in benzene. The values in chloroform- d are 9.7 and 9.3 ppm. Additional evidence for the effect of hydrogen bonding on the chemical shift of phenolic protons has been presented by Aoyama et al.² in a study of compounds consisting of two joined substituted phenols as a model for UR. Model compounds that are sterically hindered from intramolecular hydrogen bonding show OH chemical shifts, in chloroform, of 5.62 ppm. However, an analogue capable of intramolecular hydrogen bonding shows values of 7.42 ppm for the hydrogen bonded OHs and 5.45 ppm for those OHs not positioned for hydrogen bonding.

Evidence indicates that aggregation is dependent upon these hydroxyl group pairs. An octaacetate analogue of UR does not form aggregates in either benzene or chloroform.² In contrast,

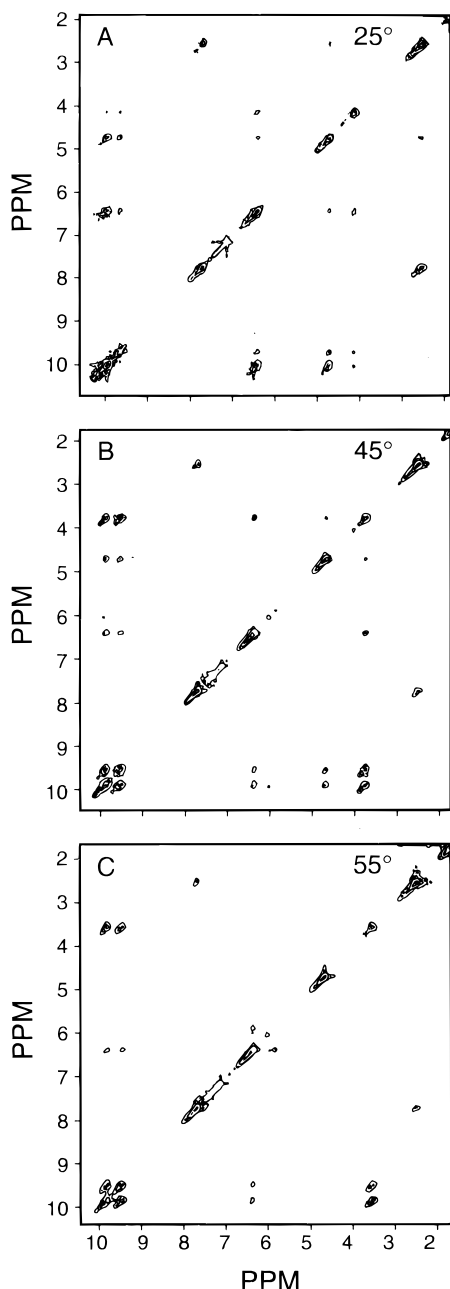


Figure 3. Two-dimensional EXSY spectra of 5 mM UR in benzene at (A) 25 °C, (B) 45 °C, (C) 55 °C. Note that the dissolved water peak moves from 3.59 to 4.1 ppm over this temperature range.

UR forms aggregates, as discussed in the Introduction. In the case of benzene, the average aggregation¹ of 6.2 molecules of UR indicates that the greatest population of aggregates is the hexamer form, presumably in a reverse-micellar configuration in which the hydrophobic tails are directed out into solution and resorcinol tetramers are directed toward each other. Computer modeling using the program Spartan indicates that six molecules of UR can interact in such a way that eight cooperative hydrogen-bonding regions exist in the hexamer. This arrangement resembles a cube with each face formed by the opening of a UR cavity. Each corner is formed by the proximity of two OHs from each of three molecules of UR, resulting in a region of cooperative hydrogen bonding. The presence of a water molecule would increase the cooperativity from six to eight OH groups in one region. Likewise, a guest capable of hydrogen bonding has the potential to increase the cooperativity.

The H_a and H_b protons of UR each appear in the NMR spectrum as a doublet below the temperatures of 318 and 328

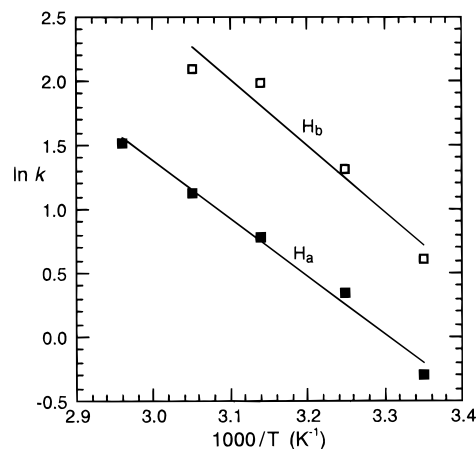


Figure 4. Arrhenius plots, $\ln k$ vs T , for proton exchange of H_a with H_2O and of H_b with H_2O . Linear regression yields $R = 0.99$ and $R = 0.97$, respectively.

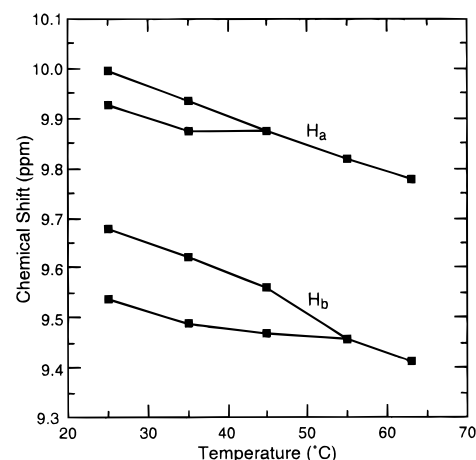


Figure 5. The chemical shifts of H_a and H_b at the temperatures 25, 35, 45, 55, and 63 °C. Note that H_a and H_b appear as doublets below 45 and 55 °C, respectively.

K, respectively. This may be due to an asymmetry in the hydrophilic rim of UR with an associated conformational change that is slow on the NMR time scale at room temperature.²¹ The center of each doublet moves upfield as the temperature of the system is raised above 298 K. After the doublets merge into singlets, they continue to move upfield with increasing temperature. The rate of change in the chemical shift remains constant before and after the doublet to singlet transition as shown in Figure 5. Such a trend in chemical shift occurs only for the hydroxyl protons, H_a and H_b . In contrast, the resorcinol ring proton, H_c , located between the hydroxyl groups, and one of the methylene protons, H_f , undergo a slight upfield shift, displaying only 20% of the change seen for the hydroxyl protons. All other UR peaks maintain a constant chemical shift (within 2%) within the 298–328 K temperature range. The upfield shift could be attributed to a partial breakdown of the hydrogen-bonding cooperativity in the UR aggregate. The resulting decrease in polarization of the UR hydroxyl groups would be expected to alter the chemical shifts.

Critical Aggregation Concentration. Studies were conducted to determine the minimal UR concentration required to maintain a predominance of the hexamer form of UR. It was expected that as the concentration was lowered the populations of different aggregates would shift to favor smaller oligomers and that eventually a monomer species would be detected. Such changes could be followed by monitoring the hydroxyl proton chemical shift.^{22–25} In fact, 1H NMR spectra of UR in the range

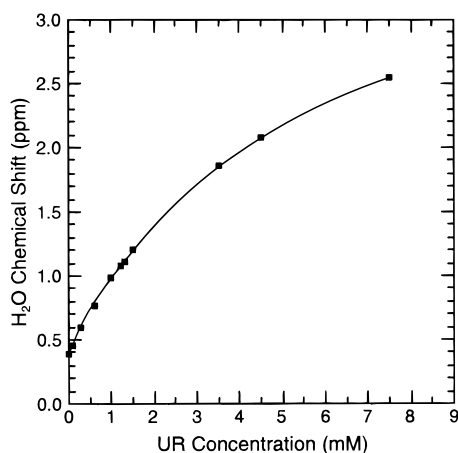


Figure 6. Chemical shift of water in a UR/benzene-*d*₆ solution as a function of UR concentration. NMR data was acquired at 500 MHz.

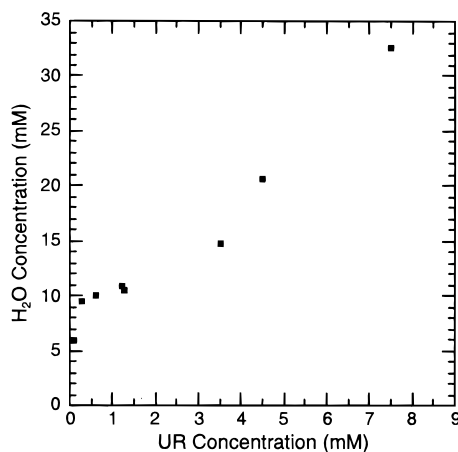


Figure 7. The concentration of water as a function of UR concentration as UR is titrated into benzene-*d*₆. Determined from spectra acquired at 500 MHz.

10^{-5} to 7.5×10^{-3} M displayed no changes in the chemical shifts of UR hydroxyl protons. This is indicative of an extremely low critical aggregation concentration which may result from a strong hydrogen-bonding network within the hexamer form of UR.

¹H NMR of Water in Solution with UR and Benzene.

During the course of these studies, the ¹H NMR peak of water was observed at various chemical shift values. In some cases, it appeared that water might be contributing to the integrated intensity of the methylene peak in the region 1.2–1.6 ppm. Systematic studies were undertaken to probe the dependence of the H₂O peak position on UR concentration and on H₂O concentration in the benzene solutions. Water dissolved in benzene has a chemical shift of 0.4 ppm,²⁶ but such a peak was not observed for most of the UR solutions.

To determine the effect of UR concentration on the chemical shift of H₂O, the position of the water peak was followed by 500 MHz NMR as UR was titrated into benzene-*d*₆. Microliter amounts of concentrated UR were added sequentially to raise the concentration of UR from 0 to 7.5 mM in deuterated benzene. The results are shown in Figure 6. The ¹H NMR chemical shift of water changed systematically from 0.39 to 2.54 ppm. The downfield wing of the water peak began to overlap the methylene peak region of UR when the concentration of UR reached 1.2 mM. At a UR concentration of 2.5 mM, the water peak was completely eclipsed. At 3.5 mM UR, the water peak just moved past the methylene peak of UR. This

change of chemical shift can be attributed to a disruption by UR of the benzene–water microstructure that is responsible for the dramatic 0.39 ppm chemical shift of dissolved water in pure benzene.

The position of the water peak in a 5 mM UR/benzene solution is observed to change for different periods of ultra-sonication (data not shown). After 2 min of sonication, the dissolved water appears in the region 3.5–4 ppm while 10 min of sonication results in a water peak at 1.8 ppm. Future 2D-EXSY studies will probe exchange behavior between the OH protons of UR and the water protons at 1.8 ppm.

The initial concentration of water in benzene-*d*₆ used for the UR titration experiment was estimated to be 5 mM. This value was obtained by measuring the intensity of the water peak relative to the residual proton signal of benzene. The residual proton signal was then calibrated using added UR as an internal standard. The value is consistent with the water specification for Isotec deuterated benzene of <0.01% water by volume (<0.0055 M). As the UR concentration was raised from 0 to 1.3 mM, the concentration of water hovered near 10 mM. In the concentration range 3.5–7.5 mM UR, the water concentration rose from 15 mM to 33 mM. These concentrations are consistent with a contribution of two waters of hydration from each UR. The water concentrations were determined by ¹H NMR peak integrations relative to the methyl peak of UR.

To determine the effect of water concentration on the water chemical shift, water was titrated into a UR solution in which the water peak was coincident with that of the methylene peak of UR. The maximum concentration of water added was 0.1 M. The relative integrated intensity of the methylene region of UR increased with the water additions. That the chemical shift of water did not change sufficiently to move out from under the methylene region of UR indicates that the chemical shift of water is more dependent upon the concentration of UR than on the concentration of water itself. At approximately 50 mM water, saturation of the solution was evident by the appearance of small volumes of water on the sample tube wall. In the NMR spectrum, a peak for undissolved water grew in at approximately 5 ppm. This peak showed no change in chemical shift as it grew in, indicating that only slow exchange is occurring with any solubilized water species.

For comparison, the behavior of the H₂O chemical shift in benzene alone was studied. A ¹H NMR spectrum of Isotec deuterated benzene displays a proton peak for dissolved water at 0.37 ppm. When the water is added to achieve 56 mM water, the room temperature spectrum shows peaks at 5.0, 1.55, and 0.40 ppm with relative intensities of 2 : 0.3 : 1. The solubility of water in benzene²⁷ at 298 K is 33 mM. The peak at 5.0 ppm is assigned to “bulk water” which was observable as small drops of water collected on the inner surface of the NMR tube. The existence of discrete peaks indicates that exchange between the solubilized and bulk water is slow on the NMR time scale.

The literature supports an alternative explanation for the change in water chemical shift with UR concentration. The water peak could result from rapid exchange of water between two environments. It is known that water in the hydrophilic regions of reverse micellar structures in apolar media have properties resembling bulk water² and that the exchange between free and “bulk” water in such a system is fast.²⁸ The observed behavior for a solution of UR and water in benzene is consistent with a micellar type of system in which “bulk water” is rendered soluble as a result of interactions with hydrophilic regions of a hexamer of UR.

Conclusions

The application of 2D-EXSY to the dynamics of proton exchange between UR and water in benzene has yielded rate constants for the exchange processes between the H_a and H_b protons of UR and solubilized water at various temperatures. These rate constants indicate a slow exchange with activation energies for H_a and H_b of 9.0 and 10.2 kcal/mol, respectively. These values are consistent with a cooperative hydrogen-bonding model. The upfield shift of the H_a and H_b proton signals as the temperature is raised from 298 to 338 K is also consistent with a cooperative hydrogen-bonding system. Under these circumstances, a decrease in the extent of hydrogen bonding (an endothermic process) at elevated temperatures leads to a loss in polarization of the hydroxyl groups involved in the interaction resulting in greater shielding of the hydroxyl group protons.

An upper limit for the critical aggregation concentration of UR in benzene has been found to be 10⁻⁵ M. The aggregation observed for UR may, in fact, be a self-assembly process. NMR studies have helped clarify the factors influencing the chemical shift of water in the UR/benzene system. Most notably, the chemical shift of water in a UR/benzene solution is strongly dependent on the concentration of UR. There is little dependence on water concentration until saturation is reached and a peak due to bulk water appears.

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