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Determination of the Purity of Cucurbit[*n*]uril (*n* = 7, 8) Host Samples

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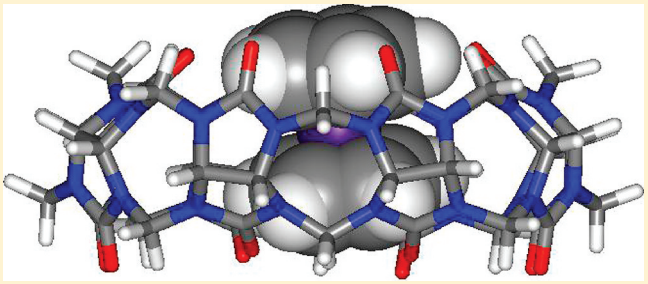
Supporting Information

ABSTRACT: The formation of highly stable inclusion complexes in aqueous solution between the organometallic cobaltocenium cation (Cob^+) and the hosts cucurbit[7]uril (CB7) and cucurbit[8]uril (CB8) was used to develop a simple method, based on UV−vis titrations, to assay the purity of samples of these two hosts. The equilibrium association constant (K) of the Cob^+@CB7 complex had been previously reported by our group as 5.7×10^9 M^{-1} at 25 °C in 50 mM sodium acetate medium. In this work, we determine a K value of 1.9×10^8 M^{-1} at 25 °C in the same medium for the Cob^+@CB8 complex. The high stability of these complexes and their decreased molar absorptivity coefficients (at 261 nm), compared to that for free Cob^+, lead to straightforward titration plots when graphing absorbance versus concentration of added CB7 (or CB8) host, at constant Cob^+ concentration.



In the past decade, the family of cucurbit[*n*]uril (CBn) hosts has received considerable research attention, primarily as a result of the extremely high binding affinities that these hosts can develop with suitable guests.[1] Among all the CBn homologues, CB7 and CB8 (see Figure 1 for structures) are particularly useful due to their relatively large cavity size and facile preparation.[2] CBn samples often contain various impurities, such as water, hydrogen chloride, and ammonium and alkali metal ion salts, typically introduced in the course of their preparation and purification.[3] In addition, CBn samples are relatively hygroscopic and may readily pick up atmospheric moisture.[4] The complete removal of impurities from a CBn sample is often difficult, cumbersome, and time-consuming. Commonly, the impurities are relatively inert in host−guest binding studies, and their removal is not strictly necessary.[5] Therefore, as research on the properties and applications of these hosts keeps burgeoning, it becomes important to develop simple methods to assess the purity of CBn samples, especially when facing quantitative experiments with these hosts. A number of techniques can be used to measure the purity or the effective molecular weight (MW) of these samples, such as NMR, ITC, and TGA, but these methods have either low accuracy (NMR) or lack convenience (ITC, TGA). Herein, we report a simple and convenient method to determine the effective molecular weight (or the degree of purity) of CB7 and CB8 using electronic absorption spectroscopy. The method is based on the fact that both hosts form highly stable complexes with cobaltocenium (Cob^+), an organometallic cation that consists of two cyclopentadienyl anions coordinated to a Co(III) ion. Cobaltocenium is commercially available in pure form as its hexafluorophosphate salt, relatively cheap, and easier to handle (not hygroscopic), which makes it an excellent guest for this analytical purpose.

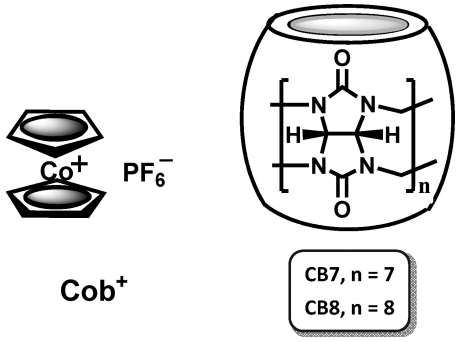


Figure 1. Structures of cobaltocenium and the CB7 and CB8 hosts.

Previous work in our group has shown that Cob^+ forms a highly stable inclusion complex with CB7.[6] The corresponding equilibrium association constant (K) between Cob^+ and CB7 was determined as 5.7×10^9 M^{-1} in 50 mM sodium acetate aqueous solution, using a competition method with a reference guest (1,6-diammoniumhexane).[7] The medium composition was dictated by previous comprehensive work, published by Isaacs and co-workers,[8] in which 50 mM sodium acetate was selected as the medium of choice to determine a large number of K values with CB6, CB7, and CB8. The well-known dependence of CBn binding affinities on the ionic composition of the solution clearly indicates that maintaining a constant solution composition is important to achieve a set of internally consistent K values.[9]

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The high binding affinity between cobaltocenium and CB7 prompted us to investigate the binding interactions between this guest and CB8, as many CB7 guests can also form stable inclusion complexes with CB8.[8] The formation of an inclusion complex between CB8 and Cob^+ was clearly evident from ^1H NMR spectroscopic data (Figure 2). Upon addition of the CB8

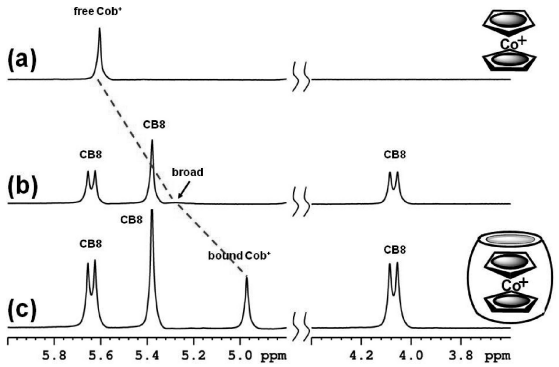

Figure 2. ^1H NMR spectra (500 MHz, D_2O) of 0.2 mM Cob^+ (a) in the absence and in the presence of (b) 0.5 equiv of CB8 and (c) 1.0 equiv of CB8.

host, the singlet resonance for the cobaltocenium protons gradually shifted upfield up to a maximum complexation-induced shift of ~0.7 ppm, which is reached when complexation is saturated with 1.0 equiv of CB8. When the amount of CB8 is less than 1.0 equiv, the cobaltocenium proton resonance is shifted from the initial value for the free guest and severely broadened at room temperature (Figure 2b). This broadening is probably associated with intermediate exchange kinetics between the free and bound guests on the NMR time scale. In contrast to this finding, the exchange rate between free and CB7-bound Cob^+ is fast, as a single—and relatively narrow—average peak for the free and bound guest proton resonances can be observed in the presence of less than 1.0 equiv of CB7 at the same concentration level.[6]

The formation of the CB8 complex with Cob^+ was also confirmed by MALDI-TOF mass spectrometry experiments, where a major peak at m/z 1517, corresponding to the Cob^+@CB8 complex, was clearly observed (Figure S1, Supporting Information).

Similarly to the case of CB7,[6] the UV absorption band of cobaltocenium at 261 nm is depressed upon addition of CB8 (Figure 3). The plot of absorbance versus CB8 concentrations clearly shows behavior characterized by two straight lines intersecting at the equivalence point, which was reached exactly upon addition of 1.0 equiv of CB8. This behavior indicates quantitative complex formation at the micromolar concentrations used in these experiments and clearly suggests that the binding constant between Cob^+ and CB8 is too high ($K > 10^6$ M^{-1}) to be measured directly in this UV titration experiments.

Therefore, 1-adamantylamine was selected as the reference guest to determine the K value for the Cob^+@CB8 complex, using binding competition experiments, similar to those discussed before for the CB7-Cob^+ host–guest pair.[7] By fitting the experimental data to a competitive 1:1 binding model (Figure 4) and using the K value of 1-adamantylamine (the equilibrium association constant between 1-adamantylamine and CB8 has been reported[8] as 8.2×10^8 M^{-1}), we obtained a binding constant of 1.9×10^8 M^{-1} (see details in the Supporting Information) for the association of Cob^+ with CB8

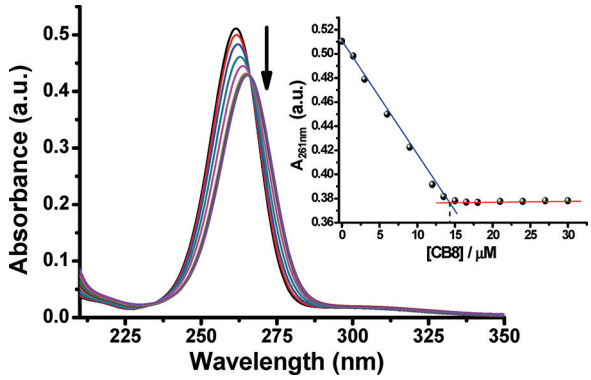

Figure 3. Electronic absorption spectra of Cob^+ (14.9 μM in pure water) in the presence of various CB8 concentrations (0–30 μM, in the direction of the arrow). The inset shows the binding isotherm recorded at 261 nm. The intersection of two straight lines appears at the concentration of 1.0 equiv of CB8.

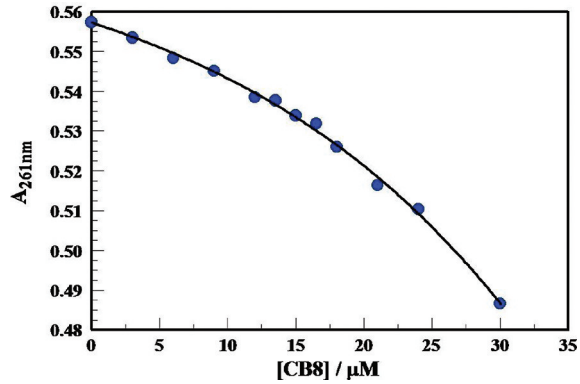

Figure 4. Plot of absorbance values of 16.3 μM Cob^+ in the presence of 30.0 μM 1-adamantylamine and increasing CB8 concentrations (0–30 μM). The curve shows the best fit of the experimental data to the competitive 1:1 binding model. The fitting affords a ratio of 0.235 between the binding constants for the CB8 complexes of Cob^+ and 1-adamantylamine.

in 50 mM sodium acetate solution. This binding constant is almost 1 order of magnitude lower than that between Cob^+ and CB7, presumably reflecting a relatively looser fit of the guest in the host cavity of the CB8 inclusion complex.

The stoichiometry of the Cob^+@CB8 inclusion complex was also verified to be 1:1 by the continuous variation method (Job plot) using UV–vis spectroscopy (Figure 5).

Since the binding affinities between Cob^+ and both hosts (CB7 and CB8) are quite high, UV–vis titrations with concentrations of guest and host in the 10–50 μM range give rise to extremely well-defined end (equivalence) points, defined by the intersection of two straight lines described by two sets of data points: (1) those obtained with titrant concentrations under 1.0 equiv and (2) those obtained with titrant concentrations above 1.0 equiv. Since Cob^+ is the UV–vis active species, we usually maintain its concentration constant through the titration so that any absorbance changes are due to the formation of the less absorbing Cob^+ inclusion complex.

The resulting UV–vis titration data can be readily used to determine the purity of a sample of CB7 or CB8. Let us assume that we prepare a solution having a molar concentration c of Cob^+. A portion of this solution (a small volume v) is used to dissolve a weighted amount m of the CBn sample. Then, the

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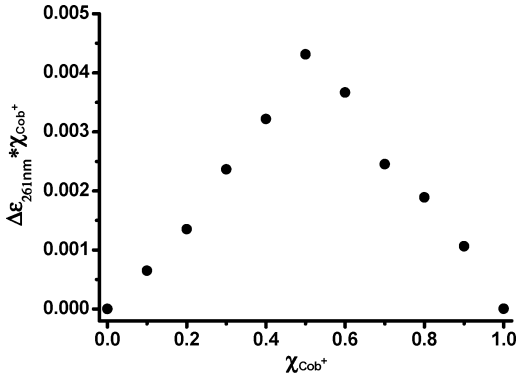

Figure 5. Job plot for the Cob^+@CB8 complex ([CB8] + [Cob^+] = 25 μM) in pure water. Molar absorptivity coefficients (ε) in μM^{-1} cm^{-1} units were used in the calculations of the values plotted in the y axis.

CBn containing solution is used to titrate an initial volume V_i of the original Cob^+ solution. The data should look like the inset of Figure 3, allowing the measurement of the volume (V_e) required to reach the equivalence point. The purity (% p) of the CBn sample can be readily calculated as

$$\%p = \frac{\mathrm{MW}\cdot c(V_i + V_e)}{m\frac{V_e}{v}} \times 100 \quad (1)$$

where MW is the nominal molecular weight of the cucurbituril host (1162 g/mol for CB7 and 1328 g/mol for CB8).

In our group, we find it more convenient to characterize the purity of the CBn sample by its "effective" or apparent molecular weight (MW_{eff}). We use the nominal formula weight (MW) of the CBn sample to calculate the concentration of the titrant solution and then use the measured concentration of CBn at the equivalence point (C_{ep}) in the titration to determine the effective molecular weight of the CBn sample, using the simple equation

$$MW_{eff} = \frac{C_{ep}}{c}\mathrm{MW} \quad (2)$$

Using this simple method to assay the purity of CBn samples, we monitored the MW_{eff} variation of a CBn sample exposed to the laboratory atmosphere as a function of time. Figure 6 clearly shows that both CB7 and CB8 had a pronounced

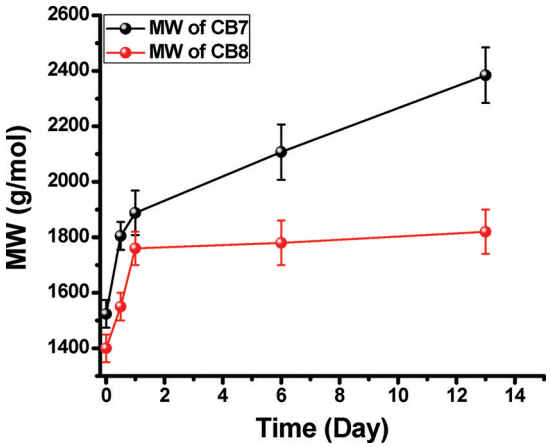

Figure 6. Variation of the effective molecular weight of CBn (n = 7, 8) as a function of time.

increase in their effective molecular weights within a few days. In contrast to CB7, whose effective MW increased monotonically, the effective MW of CB8 reached an apparent saturation level at around 1800 g/mol, and its further exposure to the atmosphere did not significantly change this value. This finding is consistent with the lower aqueous solubility of CB8 compared to CB7. Note that the effective MW of CB7 could reach values as high as 2400 g/mol, which is almost twice its formula weight! This finding suggests that, for quantitative experiments, it is necessary to calibrate the MW of CBn often.

We should point out that this method is not designed to discriminate between the various CBn hosts, particularly between CB7 and CB8, since both species bind cobaltocenium strongly. The composition of CBn mixtures can be suitably assessed by ^{1}H NMR spectroscopy.[10]

In conclusion, we describe here a simple and highly practical method to assay the purity of CB7 and CB8 samples based on their UV–vis titration with the organometallic cation cobaltocenium. The formation of the highly stable complexes Cob^+@CB7 and Cob^+@CB8 in aqueous solution leads to very well-defined end points in these titrations, which allow the straightforward calculation of the CBn sample purity.

EXPERIMENTAL SECTION

CB7 and CB8 were prepared following a reported procedure.[3] Cobaltocenium hexafluorophosphate (Cob^+PF6^-) and 1-adamantylamine were purchased from a commercial supplier and used without further purification. The concentration of Cob^+ in solution was accurately determined using its molar absorptivity coefficient (34200 M^{-1} cm^{-1} at 261 nm). The concentration of CBn (n = 7, 8) in solution was initially calculated according to its formula weight (1162 g/mol for CB7, 1328 g/mol for CB8). The UV–vis titration experiments were performed by measuring the absorbance at 261 nm of solutions containing a fixed concentration of Cob^+ and variable concentrations of CBn (in the range of 0–45 μM). Typically, in a titration experiment, two solutions were prepared. The first one (A) had a 15 μM concentration of Cob^+ in pure water and the second solution (B) contained 15 μM Cob^+ and 0.15 mM CBn (calculated from its nominal MW) in pure water. A 2.6-mL aliquot of solution A was placed in a 1.0-cm cuvette and titrated with solution B. The absorbance values for each addition were plotted versus the calculated concentrations of CBn and fitted by two straight lines intersecting with each other. From the value of the intersection (end) point, the effective MW of CBn was obtained using eq 2.

ASSOCIATED CONTENT

Supporting Information

Fitting method and equations for competition experiments between two guests and a single host. Mass spctrum of Cob^+@CB8 complex. This material is available free of charge via the Internet at http://pubs.acs.org.

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