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Original Article

Binding Study of Monohydroxy Cucurbit[7]uril with Rhodamine B

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ABSTRACT

Background: Cucurbit[7]uril (CB[7]) is the most important host among the reported cucurbit[n]urils family members, because it exhibited unique molecular recognition with exceptional applications. The binding affinity of functionalized CBs, especially mono hydroxylated CB[7] is important, because it will help to understand the difference in binding profile of functionalized CB[7] in comparison to that of the parent CB[7]. **Materials and Methods:** We have utilized Rhodamine B (RhB) to get encapsulated in monohydroxy CB[7] (CB[7]-OH) and measured the binding affinity of RhB in water. **Results:** The binding properties showed that the CB[7]-OH and RhB formed a 1:1 host-guest complex and the binding constant was found to be 3.75×10^4 M^{-1}, which is slightly lower than the binding affinity of the parent molecule CB[7], which indicates that the binding behavior of CB[7]-OH not deviated much from the parent CB[7] molecule. Electrostatic Potential studies of these two molecules also supported these results. **Conclusion:** The binding studies of monohydroxy CB[7] (CB[7]-OH) successfully studied with RhB in water.

Keywords: Cucurbit[7]uril, Encapsulation, Host-guest chemistry, Rhodamine B, Binding constant, Monohydroxy-Cucurbit[7]uril.

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INTRODUCTION

Among the various host molecules, Cucurbiturils, Cyclodextrins, Pillararenes, Dendrimers, Crown-ethers, Calixarenes and many others have gained much attention, because they have exhibited many unique applications in various scientific fields that include material science, nanotechnology, drug delivery and drug carriers, sensors, chiral chromatography and many others.[1] Therefore, the demand for these host molecules was kept increasing in order to use them in various applications. For example, cucurbiturils could able to encapsulate dyes and drug molecules effectively and these properties have been explored in dye removal from industrial effluents and drug encapsulation prevented the toxicity of the drugs.[2] An interesting feature of encapsulation of dye by CB[7] is that the encapsulation prevents the interaction of dye with the neighboring dyes and with the solvents, which provides stability to the dyes in numerous ways.[3] Thus encapsulation of dyes by CB[7] spontaneously increased the photophysical properties of the dye and that increased the absorbance or fluorescence of the dyes. This incremental fluorescence by the addition of host was used for the estimation of binding constant of CB[7] against the particular dye.[4]

Cucurbit[n]urils (CB[n], n = (5–8, 10, 14)) are the host molecules derived from the reaction of formaldehyde and glycoluril, which produced variable size of cucurbiturils and they are named based on the number of glycolurils present in the host molecule.[5] For example, CB[7] means it could have seven glycolurils units connected by the bridges of formaldehyde to get the cyclic macrocycles. Among the CB[n], CB[7] gained much attention for its superior solubility in water and that made this host molecule to become valuable in terms of applications, especially drug delivery applications.[6] Although CB[7] binds strongly with some of the mono amine or diamino molecules that are having the core of Ferrocene (Fc), Adamantane (Ad), and Diamantane (DA) derivatives with exceptionally high binding affinities (10^{12} to 10^{16} M^{-1}), which surpassed the strongest binding pair of avidin-biotin (10^{15} M^{-1}) and that led the cucurbituril chemistry to explore in areas such as protein chemistry.[7] The binding affinity of CB[7] towards many of the biologically important molecules also gained much attention for many useful applications. Thus binding studies of CB[7] have encouraged to work on various molecules, that eventually attracted many useful applications.[8] In a similar way, binding of xanthene dyes, especially RhB with CB[7] gained much attention, because RhB displayed exceptional fluorescence and exhibited stability even at low temperature.[9]

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For that reason RhB has been explored in various applications that include lasers dyes,[10] as a material of photosensitizers,[11] and also explored in high-throughput screening assays.[12] Until now, Halterman *et al.* have studied the binding affinity of CB[7] with RhB and found to be 1.1×10^5 M^{-1} by NMR and the fluorescence titrations indicated that, the RhB has the potential to bind with the CB[7] with moderate affinity.[13] Recently, many of the research groups have published the functionalization of CB[7], especially monohydroxylated CB[7] derivatives using potassium persulfate or with hydrogen peroxide and UV light combination to achieve the mono-functionalized CB[n] products.[14] However, until now none has explored the binding affinity of monohydroxylated derivative of CB[7] such as CB[7]-OH with RhB, although Kaifer *et al.* have explored with high binding substrates, but not with RhB.[14] Thus, here we have explored the binding affinity of RhB towards CB[7]-OH using UV-vis titration method. We found that the binding affinity of RhB with CB[7]-OH is much comparable to that of the parent CB[7] molecule and the binding constant was found to be 3.75×10^4 M^{-1} which is comparable and reasonable, because the deviation is within the acceptable range (Scheme 1).

MATERIALS AND METHODS

Materials

All the chemicals were purchased from the Avra chemicals India in analytical grade and the host molecule CB[7]-OH was synthesized based on the reported procedure.[15] All computational studies have been performed by free service such as WebMO server based ESP calculations (WebMO basic free license number: 2101-1001-2049) and from the Molview (https://molview.org/).

Methods

Binding constant studies

The binding constant of RhB towards CB[7]-OH was determined using USA-USB 2000 model spectrophotometer. In five different vials, 1 mL of the guest-RhB having the concentration of 10^{-4} M were added. Phosphate buffer were added sequentially in the range of 8.8, 8.6, 8.4, 8.2 and 8 mL and subsequently 0.2, 0.4, 0.6, 0.8 and 1 mL of 10^{-4} M CB[7]-OH were added, which were further diluted with water to get the concentrations of CB[7]-OH ranging from 2×10^{-6} M, 4×10^{-6} M, 6×10^{-6} M, 8×10^{-6} M and 10×10^{-6} M respectively. All the UV-vis measurements were taken into the account by taking the λ_{max} value at 554 nm. The binding constant was calculated using Benesi-Hildebrand equation as shown below.

$$\frac{1}{[A_s - A_o]} = \frac{1}{[A_s - A_o]} + \frac{1}{[A_s - A_o]} K\,[\text{Host}]$$

Where,

A_S = Absorption intensity of host (CB[7]-OH) with guest (RhB)

A_O = Absorption intensity of host (CB[7]-OH) without guest (RhB)

K = Binding constant

Based on the above equation the binding constants (K_a = association constant) can be calculated from the slope of the graph and that was found to be: CB[7]-OH for RhB is 3.75×10^4 M^{-1}

RESULTS

Binding affinity of RhB with CB[7]-OH

We have explored the binding studies of RhB with the host molecule CB[7]-OH in the phosphate buffer solution using UV-Vis spectrophotometer. We have prepared 10^{-4} solution of RhB and taken the constant volume of this solution in five different vials. To those vials, 0.2, 0.4, 0.6, 0.8 and 1 mL of 10^{-4} M CB[7]-OH were added followed by the addition 8.8, 8.6, 8.4, 8.2 and 8 mL of buffer solution to make up the total volume of 10 mL solution, which were further diluted with buffer until to get the measurable absorption range of the test solutions. In our case, the dilution have done by buffer solution to reach the concentrations of CB[7]-OH in the ranges of 2×10^{-6} M, 4×10^{-6} M, 6×10^{-6} M, 8×10^{-6} M and 10×10^{-6} M which were tested against the RhB to get the binding constant of the RhB against the host molecule CB[7]-OH.

As shown in the Figures 1 and 2, the addition of CB[7]-OH to the solution of RhB steadily increased the absorption and reached the saturated limit. From the data sets, the binding constant was calculated using Benesi-Hildebrand equation. The binding affinity (K_a) was found to be having the value of 3.75×10^4 M^{-1} (Figure 3), which is comparable to the value reported by Halterman *et al.*, which is 1.1×10^5 M^{-1} for CB[7], which is one order magnitude higher than CB[7]-OH.

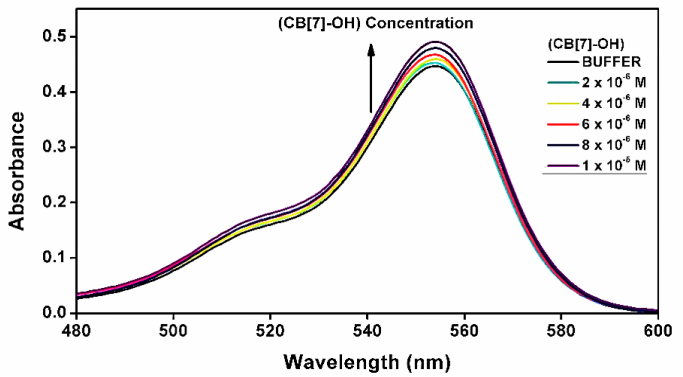
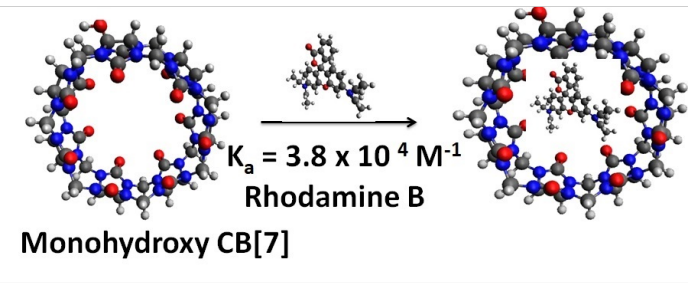


Figure 1: Change in UV-vis spectra of RhB upon addition of CB[7]-OH at various concentrations (2×10^{-6} to 10×10^{-6} M).



Scheme 1: Binding constant studies of CB[7]-OH for Rhodamine B; K_a = 3.75 × 10^4 M^{-1}.

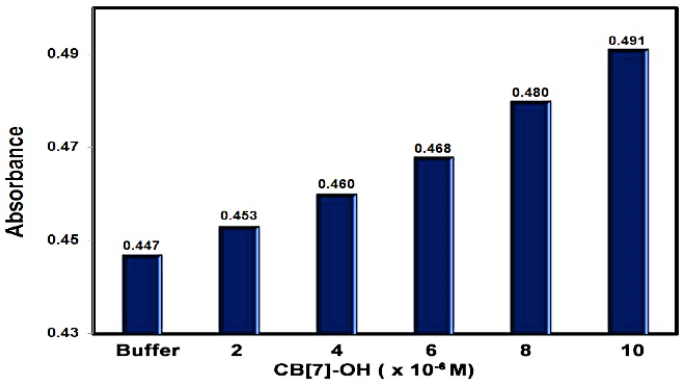


Figure 2: Quantitative increase of absorbance of RhB upon addition of increasing concentration of CB[7]-OH.

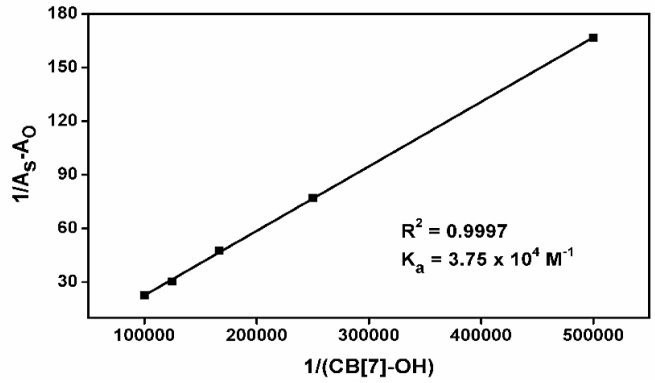


Figure 3: Plot of (1/A_S-A_O) for RhB versus 1/CB[7]-OH from the direct titration of CB[7]-OH into RhB; K_a = binding constant of CB[7]-OH for RhB.

From the Figure 4, we learned that the electrostatic potential plot of CB[7] is favorable than CB[7]-OH for the binding of RhB. In CB[7], the positive charges (blue color–outside the rim) are uniformly distributed, which is favorable for the hydrophobic attraction of N,N-diethyl part of the RhB. We have also examined the electrostatic potential plot for the guest, which has been displayed in Figure 5, from which we could understand that the charge density (negative charge, red colored) of the molecule RhB is located on one side of the N,N-diethyl portion while the charge density is less on the other side of the N,N-diethyl portion due to the dynamic conjugation shift of positive charge from one

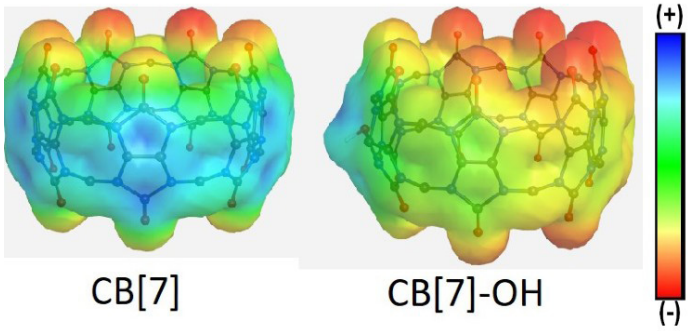


Figure 4: Comparable Electrostatic Potential plot of CB[7] and CB[7]-OH.

nitrogen to another which is carrying the ethyl groups.

DISCUSSION

RhB binding studies with CB[7]-OH

Halterman *et al.* have studied the binding affinity of ethyl ester of rhodamine B with CB[7] and found to be 2.8 × 10^6 M^{-1}. The binding mode of RhB inside the hydrophobic cavity of CB[7] was found to be through the N, N–diethyl functional group attached to the core of RhB. On the other hand, a theoretical calculation has performed with RhB species against CB[7] by Halter *et al.* and found to be having similar host-guest interactions and the binding constant was found to be 1.1 × 10^5 M^{-1}.[15] However no binding studies have been reported for CB[7]-OH using RhB as a guest, even though CB[7]/CB[7]-OH has been extensively studied with the numerous guest molecules. Here, we have established the binding affinity of RhB toward the host molecule CB[7]-OH to find out the correlation between the binding affinities of CB[7]-OH and the CB[7] to understand the influence of substitution (hydroxy) at the periphery of CB[7]. From the Figure 3, we established the binding affinity of RhB for CB[7]-OH, which was found to be 3.75 × 10^4 M^{-1}. However, the binding affinity of RhB towards CB[7] was found to be 1.1 × 10^5 M^{-1} for CB[7]. Thus, RhB binds strongly with CB[7] compare to that of CB[7]-OH. Similarly, in an another study rhodamine ethyl ester derivative was found to be having the binding affinity of 2.8 × 10^6 M^{-1}, which is two order magnitude higher than CB[7]-OH. The reason behind this slight difference might be due to the structural changes of CB[7]-OH, compared to that of the parent molecule, CB[7].

ESP Structural comparison of CB[7]-OH and CB[7]

We have taken into the consideration of structural changes and measured the electrostatic potential of these molecules by computational software offered by Webmo (a free server based software). Halterman *et al.* have clearly studied the binding mode of RhB with CB[7] and indicated that CB[7] may be exchanging the N-diethyl ends of the RhB core by the associative mechanism to create a unstable 2:1 of CB[7]: RhB. Nevertheless, it is evident

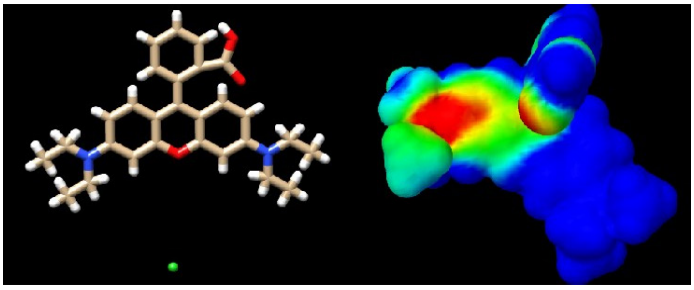


Figure 5: Rhodamine B structures: 3D image (left) and electrostatic potential of RhB (right, Molview).

from the electrostatic potential; which displayed a similar charge dispersion (Figure 5) and indicating that one of the portion of the N,N-diethyl portion alone can preferably move into the CB[7] cavity. Since they are identical groups, it would be difficult to pin out which nitrogen gets involved into the CB[7] due to the symmetrical nature of the molecule. As shown Figure 4, there is considerable changes in the electrostatic potential between the CB[7] and CB[7]-OH. As predicted by Kim *et al*. the hydrophobic interior of CB[7] is highly hydrophobic (blue colored), while CB[7]-OH is not that much hydrophobic, but comparable (not red colored); which indicates that there is a moderate changes occurred in the hydrophobic nature of the CB[7] by the presence of one hydroxyl group, which ultimately reflected in the guest binding potential of the guest. However, the electrostatic potential of rhodamine B (Figure 5) has both hydrophobic (blue) and hydrophilic regions (red). As demonstrated here, the binding constant difference between CB[7] and CB[7]-OH towards RhB is more closer to each other, indicating that CB[7]-OH binding property may not change by attaching one OH group on the periphery of the cucurbiturils. Comparison study of electrostatic potential of fully substituted hydroxyl derivatives such as CB[7]-$(OH)_{14}$ and CB[7] is in progress.

CONCLUSION

In conclusion, we have measured the binding affinity of CB[7]-OH against RhB using UV-vis spectrophotometer and the binding constant was found to be 3.75×10^4 M^{-1}, which is comparable binding constant with the parent CB[7] (1.6×10^5 M^{-1}) host molecule.[16] We have also compared the electrostatic potential of CB[7] with CB[7]-OH. From the electrostatic potential studies, we found not much difference in their hydrophobic cavity of these host molecules, which is supported by the binding constant differences between CB[7]-OH and CB[7].

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CB[7]: Cucurbit[7]uril; **CB[7]-OH:** Monohydroxy Cucurbit[7]uril; **CB[7]-$(OH)_{14}$:** Perhydroxy Cucurbit[7]uril; **RhB:** Rhodamine B; **Ad:** Adamantane; **DA:** diamantine; **Fc:** Ferrocene; **NMR:** Nuclear Magnetic Resonance spectroscopy; **UV-vis:** UV-visible spectroscopy; **ESP:** Electrostatic Potential.

SUMMARY

- Recent years, cucurbit[7]uril and monohydroxy CB[7] has gained much attention due their excellent binding affinity towards dyes, drugs and biologically important molecules.
- The solubility of hydroxylated cucurbit[n]uril in water is greater than the parent cucurbit[n]urils.
- Therefore, binding studies of rhodamine B towards CB[7[-OH is important to understand the binding profile of recently developed monohydroxylated derivative, CB[7]-OH.
- The binding properties showed that the CB[7]-OH and RhB formed a 1:1 host-guest complex and the binding constant was found to be 3.75×10^4 M^{-1}, which is slightly lower than the binding affinity of the parent molecule CB[7].
- Electrostatic potential studies of CB[7]-OH and RhB helped to understand the binding difference of RhB towards CB[7]-OH and CB[7].

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