

# Association constant of $\beta$ -cyclodextrin with carboranes, adamantane, and their derivatives using displacement binding technique

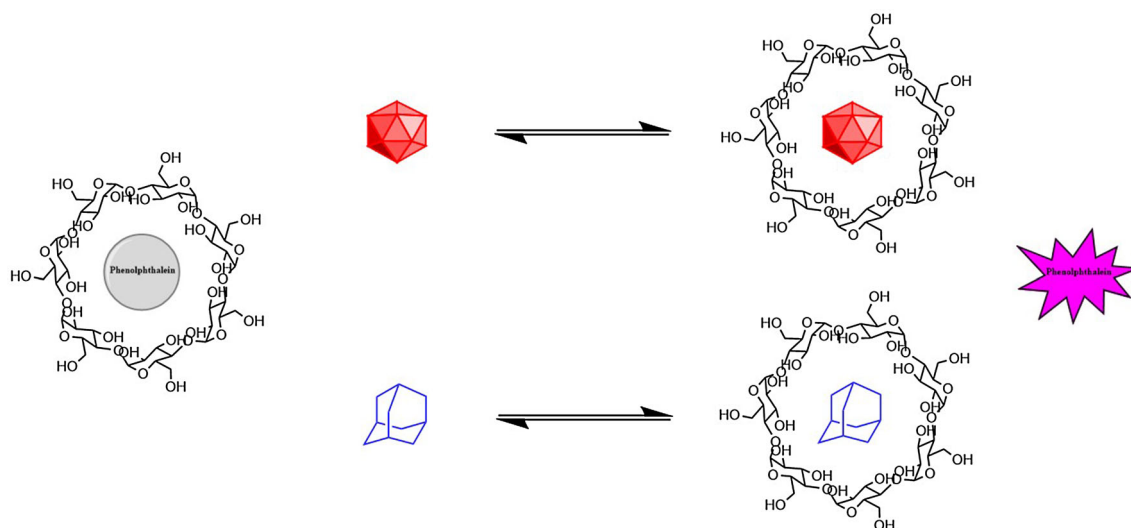
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**Abstract** The association constants ( $K'_a$ ) of unsubstituted *o*-, *m*-, and *p*-carborane, as well as that of adamantane, with  $\beta$ -Cyclodextrin are reported for the first time using displacement binding in an aqueous solution. The  $K'_a$  of several derivatives of these species are reported as well. The limitations of the displacement binding technique are also explored. Although hydrophobicity plays a major role in the association with  $\beta$ -CD, unsubstituted *o*-carborane,

which is the least hydrophobic of the carborane derivatives, exhibits the highest  $K'_a$  of  $2690\text{ M}^{-1}$ . The  $K'_a$  values for the *m*- and *p*-carborane isomers decrease with decreasing dipole moment ( $1830\text{ M}^{-1}$  and  $1560\text{ M}^{-1}$  respectively). Unsubstituted adamantane exhibits a  $K'_a$  value lower than each of the three carborane isomers at  $1410\text{ M}^{-1}$ .

*Graphical Abstract*



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**Keywords** Adamantane · Association constant · Carborane ·  $\beta$ -Cyclodextrin · Displacement binding · Inclusion

## Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of six, seven, or eight units of  $\alpha$ -D-glucopyranoside forming  $\alpha$ -cyclodextrin ( $\alpha$ -CD),  $\beta$ -cyclodextrin ( $\beta$ -CD),

and  $\gamma$ -cyclodextrin ( $\gamma$ -CD), respectively. In solution, CDs exhibit a truncated cone shape and possess a hydrophobic cavity that varies in size, depending on the number of glucopyranoside units, as well as a hydrophilic outer surface owing to the large number of hydroxides presented outward [1]. CDs have been extensively studied for their ability to form host–guest complexes with lipophilic molecules. The formation of such complexes have been used to increase the solubility and bioavailability of lipophilic drugs and their dissociation may be used to optimize drug delivery [1, 2]. Depending on the specific CD and the size of the guest lipophilic molecule, these can form either a 1:1 or a 1:2 complex, where two CDs encapsulate a single guest molecule.

Dicarba-*closo*-dodecaboranes (CBs) have been investigated as potential building blocks for pharmaceuticals [3, 4]. CBs are extraordinarily hydrophobic. Owing to the hydridic nature of their boron-hydrogen bonds, they may interact with biomolecules through unique proton-hydride hydrogen bonds (dihydrogen bonds) [5]. In recent years, there has been an increasing interest in the use of carboranes for biomedical applications. Such interests include their use as pharmacophores in the development of new drugs for a variety of illnesses [3, 6]. They have become attractive scaffolds for development of therapeutic agents and for boron neutron capture therapy (BNCT) due to their high abundance of boron [7–11]. The high affinity of carboranes to  $\beta$ -CD has been utilized to develop host–guest systems, such as a dynamic interface for bioactive surfaces [12]. The inclusion of *o*-CB, and adamantane with naphthalene- $d_8$ - $\gamma$ -CD complex to form a ternary complex has shown to effect the luminescence type of naphthalene- $d_8$  [13].

One potential drawback to utilizing CBs in drug design is a decrease in the bioavailability of such molecules due to their hydrophobicity. Both CBs and adamantane have been reported to bind to  $\beta$ -CD with high  $K'_a$  (reports as high as  $1 \times 10^6 \text{ M}^{-1}$  for carborane derivatives, and  $4.2 \pm 0.1 \times 10^4$  for adamantane derivatives) [14–17].  $\beta$ -CD has been shown to form a 1:1 complex with *o*-carborane [18]. Depending on the conformation of  $\beta$ -CD, its interior cavity has a height of 8.81–5.03 Å, top diameter of 7.92–5.28 Å, and bottom diameter of 8.53–7.60 Å [19]. However, most literature report the interior cavity's bottom diameter as 7.6–7.8 Å [1, 20]. CBs have a diameter of 7.2 Å, while adamantane's diameter is  $6.34 \pm 0.04 \text{ Å}$  [21, 22]. The structures of the CB isomers and adamantane are depicted in Fig. 1.

It has been proposed that 1,2-dicarba-*closo*-dodecaborane (*o*-CB) forms a 1:1 complex with  $\beta$ -CD due to its nearly perfect fit inside the hydrophobic cavity [18]. In a separate study, phenyl-substituted CB was shown to form a robust 2:1 complex with  $\beta$ -CD [23]. In order to avoid 2:1

complex formation, the species tested herein contain only one possible site for inclusion with  $\beta$ -CD and, in the examples of substituted species, only hydrophilic substituents were selected.

Before selecting the spectral displacement technique to measure the  $K'_a$  of the present species, other methods to measure the association constants and their limitations were studied. Some of the common methods to measure  $K'_a$  include: NMR titration technique, spectral displacement technique, and isothermal titration calorimetry (ITC). The ITC technique was initially attempted by the authors for the present study. However, it was determined to be unsuitable, as the preparation of solutions of the host/guest molecules required various organic solvents; their mixture resulted in aberrant measurements due to the heat of solvation.

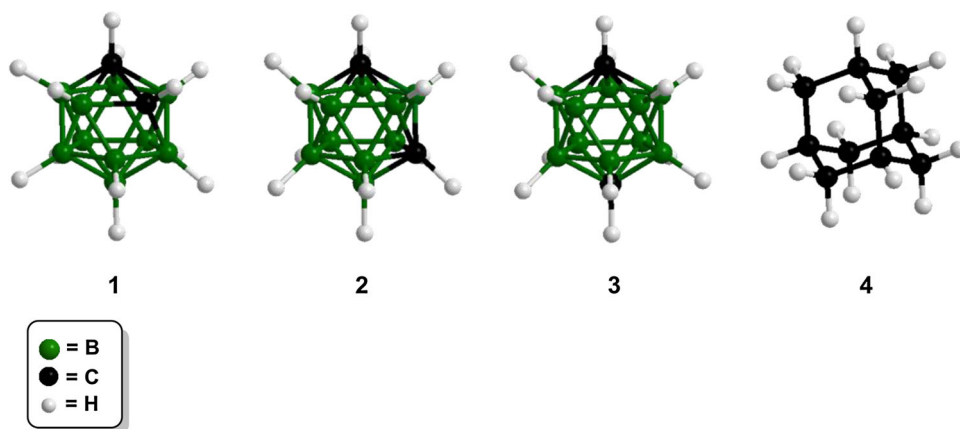
The NMR titration technique was rejected, as it necessitates the use of  $D_2O$  as the solvent, in which both  $\beta$ -CD and the guest molecules have poor solubility under neutral conditions. This technique also requires the use of an internal standard. Such a technique may be inappropriate for measuring association constants for carborane and adamantane derivatives, as the magnitude of some of these species lie above the range appropriate for this technique:  $10 \times 10^4 \text{ M}^{-1}$  [24].

For non chromophoric guest molecules, spectral displacement technique can be utilized in presence of a competing chromophore. The absorptivity of the chromophore must change significantly between its bound and unbound states; ideally, it will be strongly absorbant in one state and non-absorbant in the other. The addition of analyte displaces some of the bound chromophore and the resulting change in absorptivity of the chromophore is measured. The association constant of the analyte may be calculated from the change in absorptivity of the chromophore and its known  $K_a$  value. The specific equations used are introduced in the experimental section.

To obtain accurate measurements using the spectral displacement technique, one must select a chromophore that possesses an equal, or higher, association constant than that of competing guest [14]. For the present study, the displaceable chromophore phenolphthalein (PP) was selected since its association constant with  $\beta$ -CD ( $3.46 \times 10^4 \text{ M}^{-1}$ ) is higher than those of any guest molecules that tested herein. The use of methyl orange was considered, however, its  $K_a$  value ( $3.24 \times 10^3 \text{ M}^{-1}$ ) is 10-fold smaller than that of PP and its use would have resulted in erroneously high  $K_a$  values for other guest molecules.

In order to ensure accurate results, the concentration of PP and the guest molecules were kept lower than that of  $\beta$ -

**Fig. 1** *Ortho*- (1), *meta*- (2), and *para*-carborane (3), Adamantine (4)

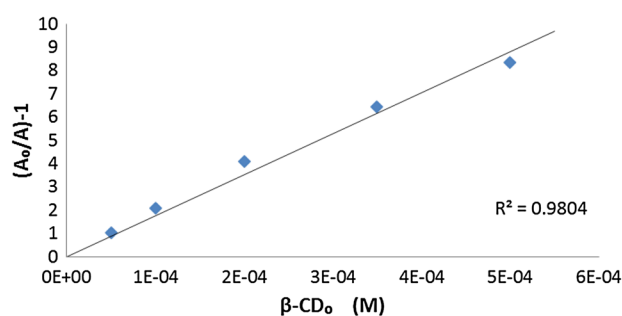


CD to minimize the potential of 2:1 complex formation. This was also necessary, since most of the analytes tested were poorly soluble in aqueous solutions and their solubilization was only possible due to their inclusion within the cavity of  $\beta$ -CD. Higher concentrations of lipophilic analytes would precipitate or crystallize over time. The carborane derivatives utilized and the methods employed for their syntheses are depicted in Scheme 1.

## Results and discussion

### Association constant ( $K_a$ ) of PP with $\beta$ -CD

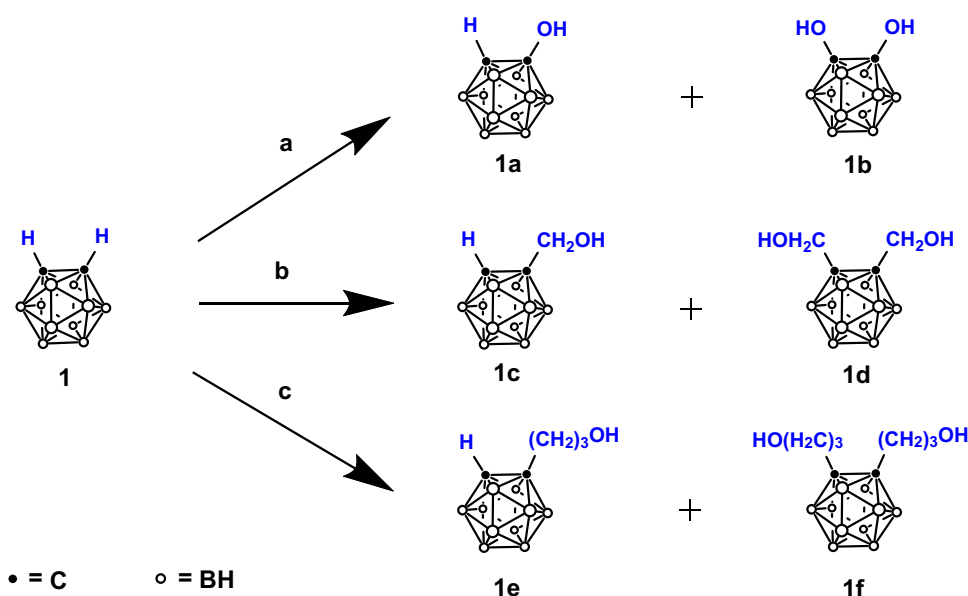
To establish that  $\beta$ -CD forms a 1:1 complex with PP,  $(A_0/A) - 1$  was plotted against  $[\beta\text{-CD}]_0$  (Fig. 2).  $A_0$  and  $A$  are the peak absorbances of PP in absence and presence of  $\beta$ -CD, respectively at 552 nm, and  $[\beta\text{-CD}]_0$  is the total



**Fig. 2** Plot of  $(A_0/A) - 1$  against  $\beta\text{-CD}_0$  for PP:  $\beta\text{-CD}$  complex at 22 °C.  $[\text{PP}]_0 = 3 \times 10^{-5}$  M and  $\beta\text{-CD} = 5 \times 10^{-5}$  to  $5 \times 10^{-4}$  M, in  $\text{Na}_2\text{CO}_3$  buffer ( $4 \times 10^{-3}$  M,  $\text{pH} \approx 10.65$ ). The concentration of PP was determined by absorptivity at 552 nm

concentration of  $\beta$ -CD. The linearity of the plot over the concentration range employed indicates that a 1:1 complex is formed [25].

**Scheme 1** Synthesis of carborane derivatives (1a–1f). The *meta* isomers (2a–2d) were synthesized in a similar fashion. *a* (i) *n*-BuLi,  $\text{B}(\text{OMe})_3$ ,  $\text{Et}_2\text{O}$ , 0 °C; (ii) AcOH,  $\text{H}_2\text{O}_2$ , rt [31]. *b* (i) *n*-BuLi, Benzene: $\text{Et}_2\text{O}$ , 0 °C—rt; (ii) PFA, 0 °C—rt, 2 N HCl [32]. *c* (i) *n*-BuLi, oxetane; (ii) TBAF [33]

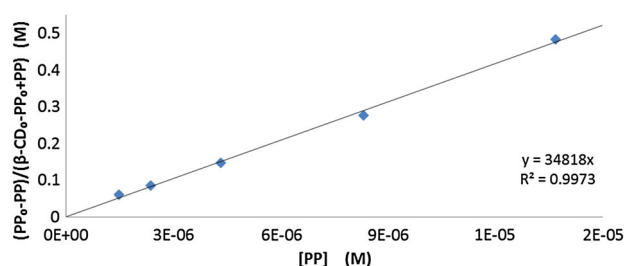


The association constant ( $K_a$ ) of PP with  $\beta$ -CD was then determined by plotting  $([PP]_0 - [PP])/([\beta\text{-CD}]_0 - [PP]_0 + [PP])$  against  $[PP]$ , and fitting the data to a linear equation, the slope of which is equal to  $K_a$  ( $3.46 \times 10^4 \text{ M}^{-1}$ ) (Fig. 3). Here  $[PP]_0$  is the total concentrations of PP, and  $[PP]$  is the concentration of non-complexed PP, which was calculated using the calibration curve and its measured absorbance at 552 nm. Our measured value of  $K_a$  for PP is comparable to those found in literature which range from  $3.4 \times 10^4 \text{ M}^{-1}$  to  $3.1 \times 10^4 \text{ M}^{-1}$  [15, 25]. Others have reported values of  $K_a$  for PP that were slightly lower; this may be due to the fact that those authors prepared their PP solution using ethanol and their mixed-solvent system contained 5 % ethanol [14, 26]. The inclusion of ethanol in those studies would also act as a competing guest, since ethanol is known to form an inclusion complex with  $\beta$ -CD and its association constant was reported to be  $37.55 \text{ M}^{-1}$  [27].

### Association constant ( $K'_a$ ) of CBs, adamantane, and their derivatives with $\beta$ -CD

For the first time, we report the association constants for the unsubstituted carborane isomers, as well as for adamantane with  $\beta$ -cyclodextrin in an aqueous solution using the spectral displacement technique. Several derivatives of these molecules have also been investigated and their  $K_a$  values determined (Fig. 4). The results from this study are summarized in Table 1. Between the unsubstituted carborane isomers and adamantane, a trend is observed where *o*-CB has the highest  $K'_a$  value followed by *m*-CB, *p*-CB, and adamantane respectively. Higher  $K'_a$  values might be expected for carboranes as their nearly spherical shape may be complimentary to the inside cavity of  $\beta$ -CD. They are also more hydrophobic than adamantane and would therefore have a higher affinity toward the comparably sized hydrophobic cavity of  $\beta$ -CD.

Carboranes might also be expected to have higher  $K'_a$  values than adamantane for entropic reasons. Carboranes

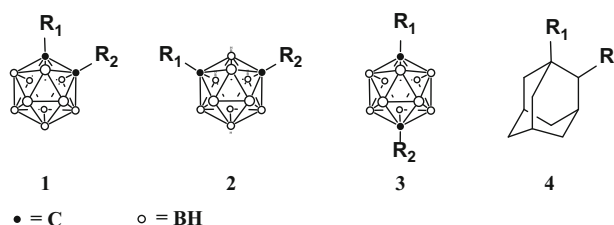


**Fig. 3** Plot of  $([PP]_0 - [PP])/([\beta\text{-CD}]_0 - [PP]_0 + [PP])$  against  $[PP]$  for  $\beta$ -CD:PP complex at 22 °C.  $[PP]_0 = 3 \times 10^{-5} \text{ M}$  and  $\beta$ -CD =  $5 \times 10^{-5}$  to  $6 \times 10^{-4} \text{ M}$ , in  $\text{Na}_2\text{CO}_3$  buffer ( $4 \times 10^{-3} \text{ M}$ ,  $\text{pH} \approx 10.65$ ). The concentration of PP was determined by absorbance at 552 nm

have a rigid structure owing to their 26 extensively delocalized framework bonding electrons [6]. In contrast, the structure of adamantane allows for increased bending and stretching of the C–C bonds and the molecule has higher degrees of freedom in solution than carborane. The formation of an adamantane  $\beta$ -CD complex results in a larger decrease in entropy and is therefore entropically less favorable than the formation of a carborane  $\beta$ -CD complex.

Although the difference in  $K'_a$  was not very large, the fact that *o*-CB exhibits the highest  $K'_a$  value was unexpected as *p*-CB is the most hydrophobic of the three carborane isomers; followed by *m*-CB, with *o*-CB the least hydrophobic of the three. The trend of  $K'_a$  values observed is opposite that of hydrophobicity. Given that the three carborane isomers are essentially identical in size and shape, their differences in dipole moment must account for the significantly different  $K'_a$  values. *ortho*-CB has the highest dipole moment, followed by *m*-CB and then *p*-CB [6]. Although these are the first accurately reported association constants for the three unsubstituted carborane isomers, this trend has been previously observed for their derivatives [16, 17]. The dipole moment unique to each CB isomer is owing to the differing locations of the two carbon atoms in each cage (Fig. 1). Differences in the electronegativities of carbon and boron lead to different partial charges on these atoms and also on their respective bound hydrogen atoms. It is likely that these differences lead to more favorable interactions for some of the cage vertices with  $\beta$ -CD, resulting in a specific orientation for each cage inside the cavity of  $\beta$ -CD, ultimately effecting their  $K'_a$  values [16].

The derivatives used in this study were mono- and di-substituted carborane and adamantane alcohols. Compound **2b** was readily soluble in water and in comparison with most other carborane derivatives used in this study, was the most hydrophilic and thus exhibited the lowest  $K'_a$  value. Other compounds that were readily soluble in the aqueous buffer also exhibited low  $K'_a$  values. These include **1a**, **1b**, **2a**, and **4a**, which were mono- and di-substituted hydroxyl derivatives. By distancing the hydrophilic functional group from the bulky lipophilic moiety by one carbon, all mono-



**Fig. 4** Carborane derivatives: *ortho*- (1), *meta*- (2), and *para*-carborane (3). Adamantane derivatives (4)

**Table 1** Association constant  $K'_a$  ( $M^{-1}$ ) and the standard error of the estimate of carborane and adamantane derivatives with  $\beta$ -CD at 22 °C, in 4 mM  $Na_2CO_3$  buffer (pH 10.65)

	R1	R2	<i>o</i> -CB (1)	<i>m</i> -CB (2)	<i>p</i> -CB (3)	Adamantane (4)
	H	H	$2.69E+03 \pm 7.41E-03$	$1.83E+03 \pm 5.16E-03$	$1.56E+03 \pm 7.15E-03$	$1.41E+03 \pm 3.93E-03$
<b>a</b>	H	OH	$1.53E+03 \pm 1.38E-02$	$8.59E+02 \pm 5.96E-03$	n.a.	$1.52E+03 \pm 9.24E-03$
<b>b</b>	OH	OH	$1.66E+03 \pm 3.81E-03$	$1.52E+02 \pm 1.97E-03$	n.a.	n.a.
<b>c</b>	H	CH <sub>2</sub> OH	$1.80E+03 \pm 4.49E-03$	$2.34E+03 \pm 2.21E-03$	n.a.	$1.91E+03 \pm 7.17E-03$
<b>d</b>	CH <sub>2</sub> OH	CH <sub>2</sub> OH	$1.59E+03 \pm 2.85E-03$	$1.47E+03 \pm 1.69E-03$	n.a.	n.a.
<b>e</b>	H	(CH <sub>2</sub> ) <sub>3</sub> OH	$2.28E+03 \pm 5.08E-03$	n.a.	n.a.	n.a.
<b>f</b>	(CH <sub>2</sub> ) <sub>3</sub> OH	(CH <sub>2</sub> ) <sub>3</sub> OH	$2.40E+03 \pm 6.52E-03$	n.a.	n.a.	n.a.

n.a. Not available

substituted guest molecules experienced a slight increase in  $K'_a$  (**1c**, **2c**, **4c**). With the bulky hydrophobic CB or adamantane moiety occupying the inside the  $\beta$ -CD cavity, the hydroxy groups on **1c**, **2c**, and **4c** may be able to form hydrogen bonds with the hydroxy groups on the rim of  $\beta$ -CD, slightly increasing the  $K'_a$  values. Along with the orientation of the substituents, this may explain the increase in  $K'_a$  between **2** and **2c**, as well as **4** and **4c**.  $K'_a$  values decreased for di-substituted derivatives **1d**, and **2d**. Having two hydroxyl groups increased the hydrophilicity and, as in the case of compound **2b**, this increased the solubility, resulting in a decreased value for  $K'_a$ .

The  $K'_a$  for compounds **1a-c** and **2a-c** were previously reported [16, 17]. However, in those studies, the NMR titration technique utilizing neutral D<sub>2</sub>O at 30 °C was utilized. The reported value for compound **1c** was  $2.40 \times 10^3 M^{-1}$  which is very similar to our value ( $2.34 \times 10^3 M^{-1}$ ). However, the values reported for **1a**, **1b**, **2a**, and **2b** were 20–30 fold larger, while the value reported for **1c** was 4.5 times larger. In the previous studies, the authors claimed that it was not possible to determine  $K'_a$  for unsubstituted CB derivatives due to poor solubility. The range of guest concentrations used in those studies was not apparent, but the authors presented a graph of NMR titrations of carborane alcohols, which indicated that the changes in chemical shifts plateaued after one equivalent of guest molecule to  $\beta$ -CD had been added. We suggest that the hydrogen bonding between the hydroxyl groups of  $\beta$ -CD and on the guest molecules, among other interactions, had an impact on the inclusion process. The authors of the previous study also stated that the value they measured for compound **1b** ( $K'_a > 1 \times 10^6 M^{-1}$ ) was close to the limit of NMR titration method. However, previous studies have suggested that the NMR titration method is useful for the measurement of association constants that range between 10 and  $10^4 M^{-1}$  [24]. The values reported for **1b** ( $K'_a = 6.0 \times 10^5 M^{-1}$ ), **2a** ( $K'_a = 4.15 \times 10^4 M^{-1}$ ), and **2b** ( $K'_a = 2.53 \times 10^4 M^{-1}$ ) were above this range [16, 17].

The effect of substituent carbon chain length was tested with compound **1e**. Adding a lipophilic three-carbon long chain slightly increased  $K'_a$  over that of **2e**. The addition of a second such lipophilic substituent in **1f** caused an additional increase in  $K'_a$ . This can be explained by considering the ratio of CH<sub>2</sub> to OH for each substituent, which is 3:1 for **1f**, versus 1:1 for **1d**. The increased lipophilicity imparted by the hydrocarbon chains results in somewhat higher  $K'_a$  values. It is also important to consider the effect of orientation of the ligands on CBs and the possible effect of hydrogen bonding between the hydroxy groups on the guest molecules with the hydroxy groups on  $\beta$ -CD.

The  $K'_a$  values for compounds **1e** and **1f** had previously been reported ( $K'_a$  of **1e** reported to be  $1.14 \times 10^5 M^{-1}$  and **1f** to be  $1.07 \times 10^5 M^{-1}$ ) [15]. These values are approximately 20-fold larger than those reported here. In those studies, the authors reportedly used similar displacement technique using PP in 0.05 M borate buffer (pH 10.5). However, no experimental data was provided, nor were the ranges of concentrations used for the guest molecules. Previous reports of high  $K'_a$  values utilized concentrations of guest molecules higher than that of  $\beta$ -CD, resulting in erroneously high  $K'_a$  values [15].

## Conclusion

The association constants for unsubstituted *o*-, *m*-, and *p*-carborane, as well as that of adamantane with  $\beta$ -CD are reported for the first time using the displacement binding technique in an aqueous solution under carefully controlled conditions. The  $K'_a$  values for these species are  $2.69 \times 10^3$ ,  $1.83 \times 10^3$ ,  $1.56 \times 10^3$ , and  $1.41 \times 10^3 M^{-1}$ , respectively, with *o*-CB exhibiting the highest  $K'_a$  value. Previously reported  $K'_a$  values for **1b**, **2a**, and **2b** ( $6.0 \times 10^5$ ,  $4.15 \times 10^4$ , and  $2.53 \times 10^4 M^{-1}$ , respectively) were found to be erroneously high and instead equal  $1.66 \times 10^3$ ,  $8.59 \times 10^2$ , and  $1.52 \times 10^2 M^{-1}$ , respectively. These new



values are approximately 20-fold smaller. The  $K'_a$  for compound **1e** and **1f** were previously reported to be  $1.14 \times 10^5$ , and  $1.07 \times 10^5 \text{ M}^{-1}$ , respectively, but instead found to be  $2.28 \times 10^3$ , and  $2.40 \times 10^3 \text{ M}^{-1}$  (also approximately 20-fold smaller).

## Experimental

### Materials

$\beta$ -CD was purchased from Sigma-Aldrich and was further purified by recrystallizing three times [28]. A water content of 13.9 % was measured using thermogravimetric analysis (TGA). The  $\beta$ -CD was dried in a vacuum oven at 90 °C for 1 day, then stored in a vacuum desiccator in the presence of phosphorus pentoxide ( $\text{P}_2\text{O}_5$ ). *o*-, *m*-, and *p*-carborane (*o*-CB, *m*-CB, *p*-CB) were purchased from Katchem Ltd., 1-adamantanemethanol (1Ad- $\text{CH}_2\text{OH}$ ), phenolphthalein (PP), *n*-butyllithium (*n*-BuLi), trimethyl borate ( $\text{B}(\text{OMe})_3$ ), paraformaldehyde (PFA), oxetane, tetra-*n*-butylammonium fluoride (TBAF), sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), glacial acetic acid (AcOH), 37 % hydrochloric acid (HCl), 30 % hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), and all solvents were purchased from Sigma-Aldrich. Adamantane (Ad), 1-adamantanol (1Ad-OH) were purchased from Acros Organics. All organic solvents were dried over 3 Å molecular sieves (20 % m/v) for at least 3 days [29]. All chemicals were used without further modifications, unless stated otherwise. Triply distilled water was used throughout these studies.

### General

Using  $\text{Na}_2\text{CO}_3$  buffer ( $4 \times 10^{-3} \text{ M}$ ,  $\text{pH} \approx 10.65$ ), a stock solution of PP ( $1.5 \times 10^{-3} \text{ M}$ ) was prepared. This stock solution was then used to make a series of dilutions ranging from  $3 \times 10^{-6} \text{ M}$  to  $3 \times 10^{-5} \text{ M}$  PP. A buffer system with pH of 9.5–10.5 is typically utilized since PP in basic aqueous solutions has a fuchsia color, the absorption of which is measurable in the visible region using a spectrophotometer [15, 30]. Since PP has low solubility in water, a slightly higher pH 10.65 was used so that the use of ethanol or other organic solvents, which interfere with the results, may be avoided. Solution absorptivity was measured at 552 nm using a Varian CARY 300 Conc UV–Visible spectrophotometer at ambient temperature (22 °C). A calibration curve was prepared to measure the concentration of free PP in solution (Fig. 5). Each prepared sample was sonicated for 20 s using SONICS vibra-cell probe at  $20 \text{ kHz} \pm 50 \text{ Hz}$  at 40 % amplitude which mildly heated the solutions. These solutions were allowed to reach ambient temperature before spectroscopic measurements

were made. The procedure and the calculations described below are very similar to those described elsewhere [26].

### Association constant ( $K_a$ ) of PP with $\beta$ -CD

To measure the  $K_a$  of PP with  $\beta$ -CD, a series of solutions in  $\text{Na}_2\text{CO}_3$  buffer were made, keeping the concentration of PP constant at ( $3 \times 10^{-5} \text{ M}$ ), while varying the concentration of  $\beta$ -CD between  $5 \times 10^{-5} \text{ M}$  to  $6 \times 10^{-4} \text{ M}$ . The  $K_a$  was calculated using the following equation:

$$K_a = \frac{[\text{PP}]_0 - [\text{PP}]}{[\text{PP}]([\beta\text{-CD}]_0 - [\text{PP}]_0 + [\text{PP}])} \quad (1)$$

Using least squares analysis, the slope of the graph obtained provided a value for  $K_a$ .

### Association constant ( $K'_a$ ) of guest molecules with $\beta$ -CD

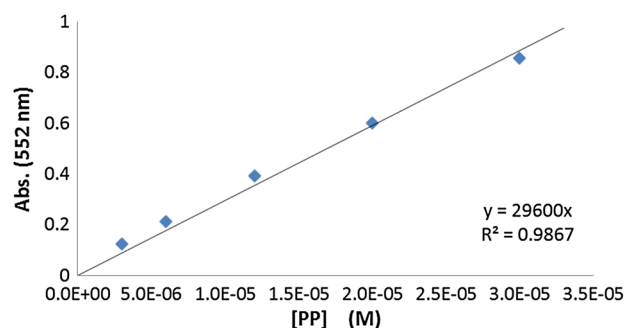
Assuming 1:1 complexations of both PP and guest molecule (G) with  $\beta$ -CD and no interaction between the PP and G, the total concentration of the host ( $[\beta\text{-CD}]_0$ ), chromophore ( $[\text{PP}]_0$ ), and guest ( $[\text{G}]_0$ ) molecule may be calculated using the following equations:

$$[\beta\text{-CD}]^0 = [\beta\text{-CD}] + K_a[\beta\text{-CD}]_0[\text{PP}] + K'_a[\beta\text{-CD}][\text{G}] \quad (2)$$

$$[\text{PP}]_0 = [\text{PP}] + K_a[\beta\text{-CD}][\text{PP}] \quad (3)$$

$$[\text{G}]_0 = [\text{G}] + K'_a[\beta\text{-CD}][\text{G}] \quad (4)$$

where  $[\text{G}]$  and  $[\beta\text{-CD}]$  are the non-complexed concentrations of guest molecule and  $\beta$ -CD, respectively.  $K'_a$ , and  $K_a$  are the association constants of the guest molecule and PP, respectively. Since  $[\text{PP}]$  is the only value that can be measured spectroscopically, these equations may be rearranged so that  $K'_a$  may be calculated based on the known initial concentrations,  $[\text{PP}]$ , and  $K_a$  using the following equation:



**Fig. 5** Calibration curve for PP solution measured at 552 nm.  $[\text{PP}] = 3 \times 10^{-6}$  to  $3 \times 10^{-5} \text{ M}$ , in  $\text{Na}_2\text{CO}_3$  buffer ( $4 \times 10^{-3} \text{ M}$ ,  $\text{pH} \approx 10.65$ ) at 22 °C

$$K_a = \frac{\left\{ \frac{K_a[PP]([\beta-CD]^0 - [PP]^0 + [PP])}{[PP]^0 - [PP]} \right\}}{\frac{[G]_0 - [\beta-CD]_0 + ([PP]_0 - [PP])(K_a[PP] + 1)}{K_a[PP]}} \quad (5)$$

Using least squares analysis, the slope of the linear fragment of a plot of Eq. 5 will equal  $K_a'$ . Precipitation was observed when the concentration of lipophilic analytes were higher than that of  $\beta$ -CD, resulting in nonlinear plots. This behavior was not observed for analytes that were readily soluble in the buffer. In order to avoid precipitation, the concentrations of all guest molecules were maintained below that of  $\beta$ -CD and ranged between  $(1-9) \times 10^{-5}$  M. Only concentrations where all components remained in solution were considered. All guest molecules were stable in alkaline buffer (pH 10.65).

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