Journal of Inclusion Phenomena and Macrocyclic Chemistry (2024) 104:129–136
https://doi.org/10.1007/s10847-024-01222-8

ORIGINAL ARTICLE

NMR studies of complex formation between natural cyclodextrins and benzene

Gabriela Szczupaj[1] · Jacek Wójcik[1] · Andrzej Ejchart[2] · Michał Nowakowski[1]

Received: 19 December 2023 / Accepted: 30 January 2024 / Published online: 10 February 2024

Abstract
Inclusion complexes of benzene (Bz) with cyclodextrins (CD) have been investigated so far using non-NMR techniques in various solvents resulting in conflicting data. Here, the first application of NMR spectroscopy in combination with rigorous statistical analysis of the results has allowed us to determine accurately the stoichiometry of complexes and their association constants. Titration measurements have been performed by ^{1}H NMR spectroscopy in D_2O at a magnetic field B_0 of 14.1 T. αCD and γCD host molecules form weak 1 : 1 complexes with Bz. In contrast, Bz and βCD build 1 : 1 and 2 : 1 complexes coexisting in solution with large binding constants. Binding of second benzene molecule is strongly cooperative.

Keywords Benzene · Cyclodextrins · Inclusion complexes · NMR spectroscopy · NMR titration

Introduction

Components of supramolecular complexes studied in this research have been known in chemistry for a very long time. Benzene (Bz) is a hydrocarbon that was first isolated in its pure form in 1825 by Michael Faraday [1]. Its six-membered cyclic structure was proposed in 1865 by August Kekulé and confirmed in 1929 using X-ray diffraction methods by Kathleen Lonsdale [2]. Cyclodextrins (CD), carbohydrates obtained from starch, were first mentioned in scientific reports in 1891 by Antoine Villiers. In the late 1950s, Dexter French and co-workers determined the molecular weight, exact chemical structure, dimensions and bond types of the three naturally occurring basic cyclodextrins: αCD, βCD and γCD. At this time, the first studies on the formation of inclusion complexes by cyclodextrins accepting small molecules into their cavity were also described [3].

Cyclodextrins (CDs) are macrocyclic oligosaccharides composed of a number of glucopyranoside units bound together by α-1,4 bonds. The naturally occurring α, β and γ cyclodextrins (αCD, βCD, and γCD) consist of six, seven, and eight glucopyranose units, respectively [4]. They are obtained by enzymatic starch degradation [4–6]. CDs, whose shape remains a truncated cone, contain a lipophilic central cavity paved with apolar glucopyranose H3 and H5 hydrogens and endocyclic oxygens suitable for entrapping nonpolar molecules. Outer CD surface is hydrophilic owing to hydroxyl groups orientated outwards [6, 7]. The size of CD cavity allows for accommodating many low and medium molecular weight compounds (approximately ≤ 1000 daltons). α- and γ-cyclodextrins dissolve well in water in contrary to βCD exhibiting an order of magnitude smaller solubility. However, at high pH, ionization of hydroxyl groups increases solubility of βCD in water [8]. It is also noteworthy that solubilities of all three CDs in D_2O are considerably diminished in comparison with those in H_2O [9]. CDs are also soluble in other polar solvents, dimethylsulfoxide or dimethylformamide, but insoluble in nonpolar solvents. In aqueous solutions, CDs can form host–guest inclusion complexes with many partially or fully lipophilic molecules often increasing a guest solubility. Hence their wide application in chemistry, pharmacy, or food industry as drug or fragrance carriers [4, 6, 10, 11]. Their complexes with harmful chemicals are regarded as one of the methods

Jacek Wójcik guest scientist.

✉ Michał Nowakowski
lyam@chem.uw.edu.pl

[1] Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Żwirki i Wigury 101, Warszawa 02-089, Poland

[2] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5A, Warszawa 02- 106, Poland

allowing to remove pollutants from the environment [12–15]. Besides αCD, βCD, and γCD larger cyclodextrins built up of dozens of glucopyranose units exist [16, 17]. One has to be aware, however, that large cyclodextrins display strong internal mobility of macrocycle [18] resulting in their irregular shape [19] and are prone to the macrocycle ring opening owing to hydrolysis of glycosidic bond not only under acidic conditions [20] but also at room temperature and neutral pH. This makes questionable the use of large CDs as host molecules.

Benzene is currently produced from fossil resources. It has long been classified as a highly carcinogenic compound and therefore its toxicity has been the subject of much research [21]. Despite this, its production is increasing rapidly worldwide [22] because the compound is used to obtain numerous precursors of commonly used polymers and resins. Benzene at room temperature under atmospheric pressure is a colorless liquid with high volatility (94.8 mm Hg − 12.64 kPa at 25 °C) [23] slightly soluble in water (1.79 g/dm^3) [24].

Besides NMR spectroscopy a number of physicochemical methods are used for investigating the formation of inclusion complexes: microcalorimetry (ITC, DSC), UV-VIS absorption spectroscopy, fluorescence, circular dichroism (CD), Fourier transform infrared spectroscopy (FTIR), high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), ionization mass spectrometry (ESI-MS), or phase solubility diagrams [28, 29]. Obviously some methods are better suited to study such complexes than others, and among the non-separation methods NMR spectroscopy is one of the most widely used because it delivers a wealth of highly reliable quantitative information at the atomic resolution level providing several independent data sets for the evaluation of association constants K as well as an insight into the conformation of the formed supramolecular complexes [30, 31].

The aim of the present study is to obtain reliable stoichiometries and association constants for inclusion complexes of natural cyclodextrins with benzene in the most natural environment, water. Previous data published for these systems are inconsistent. The association constants of these complexes have been determined by several methods, such as volatilization or transport methods, UV absorption, fluorescence or isothermal titration calorimetry. However, the reported results concerning stoichiometry and association constants are contradictory. They have been collected in the Table 1. It has to be pointed out that the method best suited for studying molecular complexes, the nuclear magnetic resonance (NMR), has not yet been exploited due to poor benzene solubility in water and inherently low NMR sensitivity. Recently, NMR sensitivity has significantly increased due to technical progress in NMR equipment making feasible the NMR titration experiments of Bz/CD complexes. Thus, we reinvestigated inclusion complexes formation of benzene and natural cyclodextrins examining a range of possible stoichiometries.

Experimental

Materials and sample preparation

Benzene (Bz) p.a.,from OBRPCh (Płock, Poland) was distilled from P_2O_5 before use. α-, β- and γ-cyclodextrins were from Sigma-Aldrich (St. Louis, MO) and were used without further purification. Dimethyl sulfone was manufactured by Alfa Aesar. D_2O from Armar Chemicals (Dottingen, Switzerland) contained 99.8% D. A solution of Bz in D_2O was prepared by stirring for 5 min. A part of the Bz solution was separated from the rest and cyclodextrin was added to it in

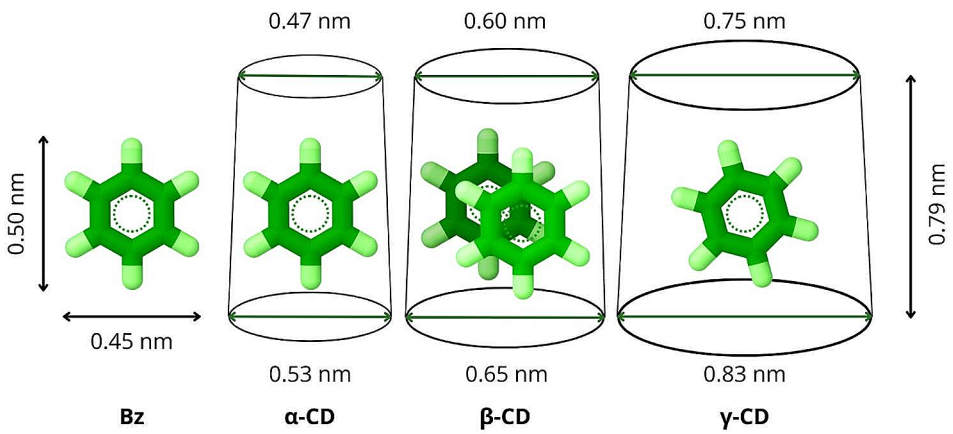

Fig. 1 Molecular dimensions of benzene molecule and inner cavities in cyclodextrins. The diagrams are drawn to scale. Benzene molecule was built using accepted bond lengths [25]. For cyclodextrins, data the most frequently quoted selected from inconsistent literature data are used [4, 6, 19, 26, 27]

Table 1 Stoichiometries, association constants and experimental conditions reported in the literature for Bz/CD inclusion complexes in aqueous solutions

CD	stoich	K [dm^3/mol]	method	t [°C]	c(Bz) [$mmol/dm^3$]	Ref.
α	1:1	28.6 (1.4)	UV absorption & fluorescence	25	0.02–0.2 ??	[32]
α	1:1	31.6 (0.1)	vapor pressure/solubility	25	0–16	[33]
	2:1	[a] 97 (4)				
	1:2	[a] 325 (11)				
α	1:1	17 (2)	volatilization	25		[34]
	1:2	17 (3)				
α	1:1	19 (1)	static HSGC	25	var.	[35]
	1:2	16 (13)				
β	1:1	194 (9)	UV absorption & fluorescence	25	20–200 ??	[32]
β	1:1	169 (1)	vapor pressure/solubility	25	0–16	[33]
	2:1	[a] 2270 (30)				
β	1:1	120 (10)	volatilization	25		[34]
β	1:1	196 (10)	UV absorption & fluorescence	28		[36]
β	1:1	86	UV absorption	25	0.3	[37]
β	1:1	107 (31)	ITC	25	22.3 (sat)	[38]
	2:1	1000 (3000)				
γ	1:1	9.1 (0.1)	vapor pressure/solubility	25	0–16	[33]
γ	1:1	12 (2)	volatilization	25		[34]

[a] K $[dm^3/mol]^2$

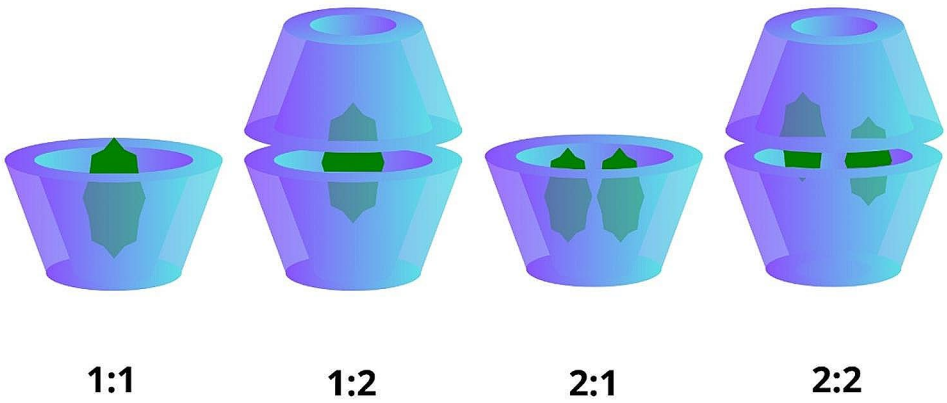

Fig. 2 Visualization of stechiometries used in the present study. G:H from left to right: 1:1, 1:2, 2:1, 2:2

excess over Bz. In the case of βCD the excess was limited since its solubility in D_2O is lower than those of αCD and γCD [9].

Cyclodextrins contain an imprecisely known amount of water because they crystallize from water as hydrates of variable composition [19, 27, 39, 40]. Therefore, the concentration of cyclodextrin was determined by integrating the ^{1}H NMR signal of its anomeric protons relative to the six protons of dimethylsulfone of known concentration. Dimethylsulfone is considered the standard for quantitative NMR studies [41, 42]. Since the longitudinal T_1 relaxation time of dimethylsulfone is long ($T_1 = 6.17 \pm 0.01$ s at $T = 298$ K, $B_0 = 14.1$ T, concentration 10 $mmol/dm^3$), an extended relaxation time between scans was used.

Basic solutions, containing either Bz or Bz and cyclodextrin, were mixed afterwards together in order to prepare eight to eleven NMR samples (0.65 cm^3 total volume) of various CD/Bz molar ratios, R, so that the concentrations of Bz remained constant during the titrations; 1.0, 0.3, and 0.4 $mmol/dm^3$ for αCD, βCD, and γCD experiments, respectively. Four groups of CD protons which do not superpose one another (H1, H3, H2 + H4, H5 + H6,6′) can be integrated accurately. Therefore, integration of Bz/CD ^{1}H NMR spectra delivers four values of molar ratios R. R values from six independent measurements have been averaged allowing to estimate their accuracies which are better than 4%. Precaution has to be undertaken concerning the Bz concentration. Benzene is very volatile and easily evaporates from a solution. Therefore, NMR tubes with Bz/CD solutions

were flame sealed immediately after filling to avoid the slow evaporation of benzene.

NMR measurements

All measurements have been performed at a magnetic field of 14.1 T, using a Varian DDR2 600 MHz spectrometer equipped with a triax probe. NMR spectra of each sample have been measured six times at a temperature carefully adjusted to 298.1 K with an accuracy of 0.1 K and checked by an ethylene glycol reference sample [43]. All chemical shifts in ^{1}H NMR spectra are reported with respect to external TSP-d4. ^{1}H chemical shifts have been determined from 1D ^{1}H NMR spectra measured with a 60 s relaxation delay and a 1.5 s acquisition time. A long repetition time allows avoiding the partial saturation of benzene protons displaying extensive longitudinal relaxation time 14.6 ± 0.4 s. Acquisition parameters are as follows: sweep width, 9615.38 Hz, number of acquired data points, 28 846, and 32 scans. FIDs have been processed with 1.5 Hz line broadening and zero-filled to 128 k points, resulting in a 0.07 Hz spectrum digital resolution. The accuracy of the chemical shift readout is not better than 0.0001 ppm. Stability of benzene resonance frequency in the series of spectra is not worse than 0.22 Hz (0.00037 ppm). Hence error introduced by the accuracy of benzene resonance frequencies is negligible in comparison with the determination of host/guest molar ratios.

Analysis of titration data

In the case of several competing reactions of complex formation the best approach is to fit the titration data to different stoichiometry models and choose the best among them on the basis of statistical tests [30, 44–46]. This approach has been used in the present analysis of Bz/CD complexes. Changes in the ^{1}H chemical shifts of protons in benzene, as a function of CD/Bz molar ratios, R = $[H]_0/[G]_0$, have been analyzed assuming relevant stoichiometry. $[H]_0$ and $[G]_0$ denote the initial molar concentrations of the host (in this work cyclodextrin) and guest (in this work benzene), respectively. For all three complexes (Bz/αCD, Bz/βCD, Bz/γCD) four stoichiometry models have been compared: 1 : 1, 2 : 1, 1 : 2, and 2 : 2. All the pertinent equations defining macroscopic association constants for each model are given explicitly in Supplementary Materials.

Since one signal of Bz protons is observed, the exchange between the free and complexed Bz molecules is fast on the chemical shift timescale. Therefore, the observed chemical shifts are the mole fraction weighted averages of the chemical shifts specific for the free and complexed Bz molecules [30, 31]. The explicit equations for the discussed stoichiometry models are given in Supplementary Materials.

The model parameters, association constants K_i and complexation shifts CS_i are determined by fitting the chemical shifts calculated for the specific model to the experimental ones. The least-squares procedure uses a Fortran routine written in-house optimizing the model parameters that consists of minimization through a grid search of the target function RSS (the sum of squares of residuals), where N is the number of different molar ratios R.

$$RSS = \sum_{i=1}^{N} (\delta_{i,obs} - \delta_{i,calc})^2 \quad (1)$$

The confidence limits of fitted parameters are estimated by the use of F−statistics [47, 48].

Results and discussion

As mentioned above, four stoichiometry models (1 : 1, 2 : 1, 1 : 2, and 2 : 2) were fitted to all three Bz/CD complexes. Selection of the best stoichiometry based on the comparison of the target function values, *RSS*, and/or determination of Fisher-Snedecor F−statistics, if applicable.

Bz/αCD and Bz/γCD inclusion complexes

The stoichiometry 1 : 1 describes two complexes, Bz/αCD and Bz/γCD, in the best manner as can be concluded from the data given in Table 2. Appropriate plots of experimental data titration curves and calculated for 1 : 1 stoichiometry are shown in Figs. S1 and S2.

Results of numerical analyses performed with initial assumption of 1 : 2 or 2 : 2 stoichiometry for Bz/αCD complex always resulted in $K_2 = 0$ thus converging back to the 1 : 1 stoichiometry. The same was true for Bz/γCD complex

Table 2 Values of target function *RSS*, in 10^{-5}ppm^2, and parameters characterizing 1 : 1 complexes: association constant K_1 [dm^3/mol] and complexation shift CS_1 [ppm]

G : H	Bz/αCD	Bz/γCD
1 : 1	3.8982	0.7317
1 : 2	3.8981 [a]	0.7336 [a]
2 : 2	3.8971 [a]	0.7277 [c]
2 : 1	3.8563 [b]	0.7323 [a]
K_1 (1 : 1)	25.1 ± 0.5	2.1 ± 0.2
CS_1 (1 : 1)	0.164 ± 0.002	0.093 ± 0.007

[a] $K_2 = 0$. Calculations converge back to the 1 : 1 stoichiometry

[b] comparison of *RSS* values for 2 : 1 and 1 : 1 stoichiometries gives statistics $F = 0.24$ corresponding to the probability $p = 0.79$ which is much larger than commonly accepted rejection probability $p \le 0.05$

[c] comparison of *RSS* values for 2 : 2 and 1 : 1 stoichiometries gives statistics $F = 0.17$ corresponding to the probability $p = 0.84$ which is much larger than commonly accepted rejection probability $p \le 0.05$

and stoichiometries 1 : 2 or 2 : 1. Experimental data fit slightly better to 2 : 1 than to 1 : 1 Bz/αCD complex. The *RSS* improvement, however, is statistically insignificant. Determination of confidence limits of K_1 and K_2 association constants (2 : 1) are shown in Figs. S3 and S4. Moreover, association constant K_2 displays absurd confidence limits (cf. Fig. S4). Similar situation took place during comparison of 2 : 2 and 1 : 1 Bz/γCD complexes. Therefore, one can safely conclude that Bz/αCD and Bz/γCD form 1 : 1 complexes.

Both, association constant and complexation shift for Bz/γCD 1 : 1 complex are smaller than the corresponding parameters of Bz/αCD complex. In much more spacious cavity of γCD the benzene molecule can probably freely rotate inside the cyclodextrin. In consequence association constant is an order of magnitude smaller. Smaller complexation shift ensues from weaker interactions between benzene molecule and γCD cavity.

Bz/βCD inclusion complex

In contrast, the parameters obtained for the Bz/βCD complex clearly differ from those discussed above. Among four stechiometries used to describe titration experiment for Bz/βCD complex the best fit and the smallest *RSS* value were obtained when two molecules of benzene were located in the βCD inner space; 2 : 1 stoichiometry (Table 3) resulting in the association constants $K_1 = 405 \pm 18$ dm^3/mol, $K_2 = 6000 \pm 1100$ dm^3/mol, and complexation shifts $CS_1 = 0.080 \pm 0.002$ ppm, $CS_2 = -0.038 \pm 0.004$ ppm.

Table 3 Values of target function *RSS*, in $10^{-5}ppm^2$ for Bz/βCD inclusion complex

G : H	RSS
1 : 1	1.1865
1 : 2	0.7201
2 : 2	0.8201
2 : 1	0.2439

Titration curve calculated applying the best fit parameters and experimental data are shown in Fig. 3. Determination of parameter confidence limits are presented in Figs. S5 - S8.

Binding of a second benzene molecule to the 1 : 1 complex is strongly cooperative. The cooperativity factor, *c*, has been delimited from the macroscopic association constants K_1 and K_2applying formula $c \geq 4 \cdot K_2/K_1$ [49]. The obtained value, $c \geq 59 \pm 11$, is markedly larger than the cooperativity factors for parent complex naphthalene/βCD, 5.2 ± 0.3 and 25.4 ± 1.7 for 2 : 1 and 2 : 2 stechiometries, respectively [46]. One can safely conclude that two benzene molecules

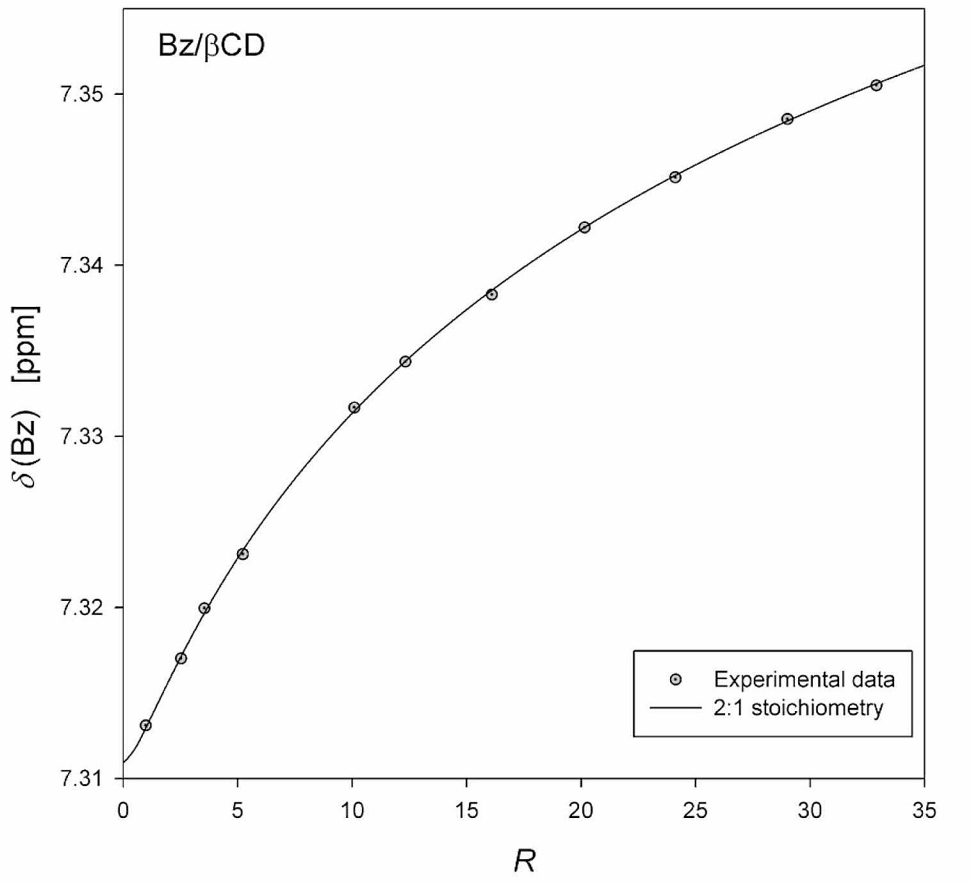

Fig. 3 Experimental data measured for Bz/βCD complex and the titration curve calculated using the best fit parameters: 2 : 1 stoichiometry, $K_1 = 405 \pm 18$ dm^3/mol, $K_2 = 6000 \pm 1100$ dm^3/mol, $CS_1 = 0.080 \pm 0.002$ ppm, $CS_2 = -0.038 \pm 0.004$ ppm

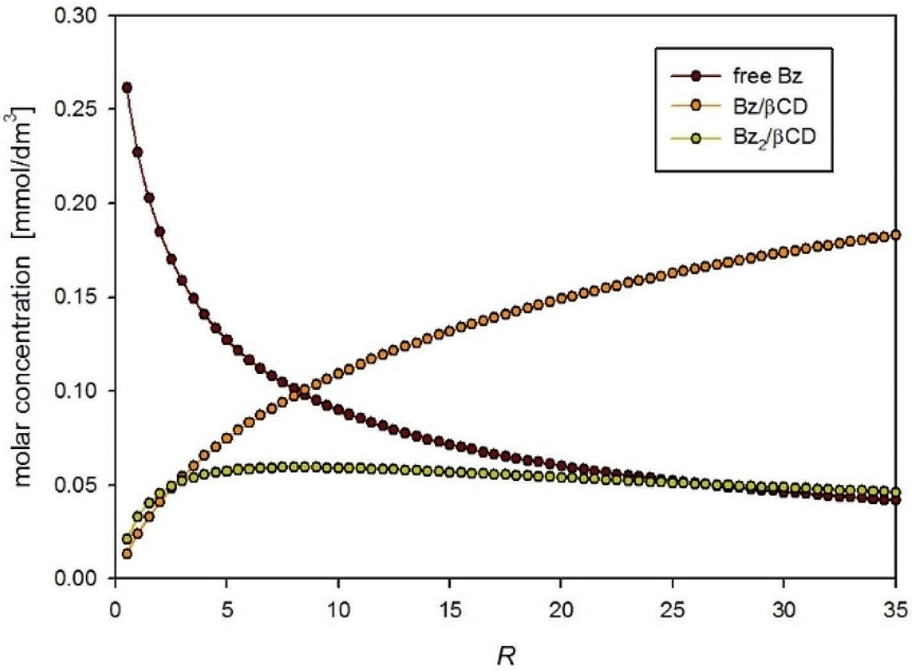

Fig. 4 Molar concentrations of species containing benzene molecules in Bz/βCD solution. Calculation have been performed using the best fit parameters for 2 : 1 stoichiometry. Experimental range of *R* values: (0.0, 32.9)

fit better to the βCD cavity than two larger naphthalene molecules. For comparison purposes it should be also recalled that the cooperativity factors for 1 : 2 inclusion complexes of (+)-fenchon/αCD and (−)-fenchone/αCD are 42.6 ± 3.0 and 9.9 ± 0.7, respectively [45].

While K_1 association constant and corresponding complexation shifts CS_1 are determined with good accuracy, parameters of the step binding, K_2 and CS_2, are calculated much less precisely. Several studies have reported 1 : 2 stoichiometry and large overall association constant for camphor/αCD complexes [50–52]. Dodziuk et al. [53] reported that analyzing titration data of (+)-camphor/αCD system were not able to partition overall association constant into stepwise constants because such partitioning becomes equivocal for $K_1 << K_2$. In the case of Bz/βCD complex the separation of stepwise association constants was possible, but accuracy of determined parameters suffered seriously. It ensues from the fact that concentration of G_2H complex is virtually constant from βCD/Bz molar ratio $R = [H]_0/[G]_0 \approx 4$ (Fig. 4). Therefore, the observed chemical shift of benzene becomes effectively decoupled from further *R* changes.

Generally, the protection of Bz molecules from solvent in the CD cavity results in the deshielding effect of guest protons. Therefore, CS_1 values in all studied complexes are positive. On the other hand, the interaction of two π electron systems in 2 : 1 Bz/βCD complex causes a shielding effect of the involved protons [54]. These two effects interplay and their combined effect depends on the mutual orientation of complex components. Hence, it is not surprising that CS_2 in Bz/βCD complex is negative.

Conclusion

^{1}H NMR titration experiments carried out at a magnetic field of 14.1 T, coupled with rigorous statistical analysis of the results, allowed the exact determination of the stoichiometry and corresponding association constants of the benzene complexes with the three natural cyclodextrins in aqueous solutions. Furthermore, the parameter values obtained provide insights into the structures of the complexes studied based on chemical intuition. The supramolecular complex of benzene with βCD differs from the association schemes with αCD and γCD. Bz/βCD displays well-defined 2 : 1 stoichiometry and a considerable cooperativity coefficient. Complexes Bz/αCD and Bz/γCD are described by 1 : 1 stoichiometry. Both complexes are weak but the latter is extremely weak ($K_1 = 2.1$ dm^3/mol) owing to large γCD cavity which allows for unrestricted motion of benzene inside the host molecule.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10847-024-01222-8.

Author contributions AE and MN: conceptualization; GS, MN and JW: samples preparation; GS and MN: measurements; GS and AE: data analysis; GS, JW, AE and MN: manuscript preparations.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

References

1. Faraday, M.: On new compounds of carbon and, and on certain other products obtained during the decomposition of oil by heat. Philos. Trans. R Soc. **115**, 440–466 (1825)
2. Lonsdale, K.: The structure of the Benzene Ring in Hexamethylbenzene. Proc. R Soc. **123A**, 494–515 (1929)
3. Crini, G.: Review: A history of Cyclodextrins. Chem. Rev. **114**, 10940–10975 (2014)
4. Del Valle, E.M.M.: Cyclodextrins and their uses: A review. Process. Biochem. **39**, 1033–1046 (2004)
5. Biwer, A., Antranikian, G., Heinzle, E.: Enzymatic production of cyclodextrins. Appl. Microbiol. Biotechnol. **59**, 609–617 (2002)
6. Szejtli, J.: Introduction and general overview of cyclodextrin chemistry. Chem. Rev. **98**, 1743–1753 (1998)
7. Rekharsky, M.V., Inoue, Y.: Complexation Thermodynamics of Cyclodextrins. Chem. Rev. **98**, 1875–1917 (1998)
8. Pharr, D.Y., Fu, Z.S., Smith, T.K., Hinze, W.L.: Solubilization of Cyclodextrins for Analytical Applications. Anal. Chem. **61**, 275–279 (1989)
9. Sabadini, E., Cosgrovea, T., do, Carmo Egídio, F.: Solubility of cyclomaltooligosaccharides (cyclodextrins) in H_2O and D_2O: a comparative study. Carbohydr. Res. 341, 270–274 (2006)
10. Loftsson, T., Duchene, D.: Cyclodextrins and their pharmaceutical applications. Int. J. Pharm. **329**, 1–11 (2007)
11. di Cagno, M.P.: The potential of cyclodextrins as novel active Pharmaceutical ingredients: A short overview. Molecules. **22**, 1–14 (2017)
12. Sikder, M.T., Rahman, M.M., Jakariya, M., Hosokawa, T., Kurasaki, M., Saito, T.: Remediation of water pollution with native cyclodextrins and modified cyclodextrins: A comparative overview and perspectives. Chem. Eng. J. **355**, 920–941 (2019)
13. Tian, B., Hua, S., Tian, Y., Liu, J.: Cyclodextrin-based adsorbents for the removal of pollutants from wastewater: A review. Environ. Sci. Pollut Res. **28**, 1317–1340 (2021)
14. Blach, P., Fourmentin, S., Landy, D., Cazier, F., Surpateanu, G.: Cyclodextrins: A new efficient absorbent to treat waste gas streams. Chemosphere. **70**, 374–380 (2008)
15. Park, K.H., Choi, J.M., Cho, E., Jung, S.: Enhanced solubilization of Fluoranthene by Hydroxypropyl β-Cyclodextrin Oligomer for Bioremediation. Polymers. **10**, 111 (2018)
16. Terada, Y., Yanase, M., Takata, H., Takaha, T., Okada, S.: Cyclodextrins are not the major cyclic α-1,4-glucans produced by the initial action of cyclodextrin glucanotransferase on amylose. J. Biol. Chem. **272**, 15729–15733 (1997)
17. Ueda, H., Endo, T.: Large-ring cyclodextrins. In: Dodziuk, H. (ed.) Cyclodextrins and Their Complexes, pp. 370–380. Wiley-VCH, Weinheim (2006)
18. Bernatowicz, P., Ruszczynska-Bartnik, K., Ejchart, A., Dodziuk, H., Kaczorowska, E., Ueda, H.: Carbon-13 NMR relaxation study of the internal dynamics in cyclodextrins in isotropic solution. J. Phys. Chem. B. **114**, 59–65 (2010)
19. Lichtenthaler, F.W., Immel, S.: On the hydrophobic characteristics of cyclodextrins: Computer-aided visualization of molecular lipophilicity patterns. Liebig's Ann. 27–37 (1996)
20. Ueda, H., Wakisawa, M., Nagase, H., Takaha, T., Okada, S.: Physicochemical properties of large-ring cyclodextrins (CD_{18}~CD_{21}). J. Incl. Phenom. Macrocycl. Chem. **44**, 403–405 (2002)
21. Snyder, R.: Recent developments in the understanding of Benzene Toxicity and Leukemogenesis. Drug Chem. Toxicol. **23**, 13–25 (2000)
22. https://www.statista.com/statistics/1245172/benzene-market-volume-worldwide/ (accessed November 7, 2023)
23. Daubert, T.E., Danner, R.P., Washington: D.C. Taylor and Francis, 361 (1989)
24. May, W.E., et al.: J. Chem. Ref. Data. **28**, 197–0200 (1983)
25. Kınaytürk, N.K., Kalaycı, T., Tunalı, B., Altuğ, D.T.: A spectroscopic approach to compare the quantum chemical calculations and experimental characteristics of some organic molecules; Benzene, Toluene, P-Xylene, P-Toluidine. Chem. Phys. **570**, 111905 (2023)
26. Li, S., Purdy, W.C.: Cyclodextrins and their applications in analytical chemistry. Chem. Rev. **92**, 1457–1470 (1992)
27. Saenger, W., Jacob, J., Gessler, K., Steiner, T., Hoffmann, D., Sanbe, H., Koizumi, K., Smith, S.M., Takaha, T.: Structures of the common cyclodextrins and their larger Analoguess beyond the Doughnut. Chem. Rev. **98**, 1787–1802 (1998)
28. Schneider, H.J., Yatsimirsky, A.K.: Principles and methods in supramolecular chemistry. Wiley (2000)
29. Dodziuk, H. (ed.): Cyclodextrins and Their Complexes, Ch. 10. Wiley-VCH, Weinheim (2006)
30. Thordarson, P.: Determining association constants from titration experiments in supramolecular chemistry. Chem. Soc. Rev. **40**, 1305–1323 (2011)
31. Fielding, L.: Determination of Association Constants (K_a) from solution NMR data. Tetrahedron. **56**, 6151–6170 (2000)
32. Guo, Q.X., Luo, S.H., Liu, Y.C.: Substituent effects on the driving force for inclusion complexation of α- and β-Cyclodextrin with Monosubstituted benzene derivatives. J. Inclusion Phenom. Mol. Recognit. Chem. **30**, 173–182 (1998)
33. Tucker, E.E., Christian, S.D.: Vapor pressure studies of benzene-cyclodextrin inclusion complexes in aqueous solution. J. Am. Chem. Soc. **106**, 1942–1945 (1984)
34. Sanemasa, I., Akamine, Y.: Association of benzene and alkylbenzenes with cyclodextrins in aqueous medium. Bull. Chem. Soc. Jpn. **60**, 2059–2066 (1987)
35. Saito, Y., Yoshihara, K., Tanemura, I., Ueda, H., Sato, T.: Determination of the stability constants of benzene and alkylbenzenes with α-cyclodextrin by static head-space gas chromatography. Chem. Pharm. Bull. **45**, 1711–1713 (1997)
36. Hoshino, M., Imamura, M., Ikehara, K., Hama, Y.: Fluorescence enhancement of benzene derivatives by forming inclusion complexes with β-Cyclodextrin in Aqueous solutions. J. Phys. Chem. **65**, 1820–1823 (1981)
37. Trofymchuk, I.M., Belyakova, L.A., Grebenyuk, A.G.: Study of complex formation between β-cyclodextrin and benzene. J. Incl. Phenom. Macrocycl. Chem. **69**, 371–375 (2011)
38. Gómez-Orellana, I., Hallén, D.: The thermodynamics of the binding of benzene to β-cyclodextrin in aqueous solution. Thermochim. Acta. **221**, 183–193 (1993)
39. Connors, K.A.: The stability of cyclodextrin complexes in solution. Chem. Rev. **97**, 1325–1357 (1997)
40. Dang, Z., Song, L.X., Guo, X.Q., Du, F.Y., Yang, J., Yang, J.: Applications of powder X-Ray diffraction to inclusion complexes of Cyclodextrins. Curr. Org. Chem. **15**, 848–861 (2011)
41. Wells, R.J., Cheung, J., Hook, M.J.: Dimethylsulfone as a universal standard for analysis of organics by QNMR. Accrd Qual. Assur. **9**, 450–456 (2004). https://doi.org/10.1007/s00769-004-0779-0
42. qNMR Internal Standard Reference Data (ISRD): Dimethyl sulfone ($DMSO_2$) [ISRD-02]. Rapport BIMP – 2018/04

43. Rainford, D.S., Fisk, C.L., Becker, E.D.: Calibration of methanol and Ethylene Glycol Nuclear magnetic resonance thermometers. Anal. Chem. **51**, 2050–2051 (1979)
44. Dodziuk, H., Kozminski, W., Ejchart, A.: NMR studies of Chiral Recognition by Cyclodextrins. Chirality. **16**, 90–105 (2004)
45. Nowakowski, M., Ejchart, A.: Complex formation of fenchone with α-cyclodextrin: NMR titrations. J. Inclusion Phenom. Macrocycl. Chem. **79**, 337–342 (2014)
46. Jopa, S., Ejchart, A., Wójcik, J., Nowakowski, M.: NMR studies of inclusion complexes: Naphthalene and natural cyclodextrins. Phys. Chem. Chem. Phys. **24**, 13690–13697 (2022). https://doi.org/10.1039/D2CP01152B
47. Johnson, M.L., Faunt, L.M.: Parameter estimation by least-squares methods. Methods Enzymol. **210**, 1–37 (1992)
48. Al-Soufi, W., Cabrer, P.R., Jover, A., Budal, R.M., Tato, J.V.: Determination of second-order association constants by global analysis of ^{1}H and ^{13}C NMR chemical shifts. Application to the complexation of sodium fusidate and potassium helvolate by β- and γ-cyclodextrin. Steroids. **68**, 43–53 (2003)
49. Freire, E., Schön, A., Velazquez-Campoy, A.: Isothermal titration calorimetry: General formalism using binding polynomials. Methods Enzymol. **455**, 127–155 (2009)
50. Dodziuk, H., Ejchart, A., Lukin, O., Vysotsky, M.O.: ^{1}H and ^{13}C NMR and molecular dynamics study of chiral recognition of camphor enantiomers by α-cyclodextrin. J. Org. Chem. **64**, 1503–1507 (1999)
51. Dodziuk, H., Kozminski, W., Dolgonos, G.: The differences between the ΔH and ΔS values of the 1:2 complex of camphor enantiomers with α-cyclodextrin determined by NMR titration and the results obtained by other techniques. Pol. J. Chem. **77**, 251–255 (2003)
52. Bernatowicz, P., Nowakowski, M., Dodziuk, H., Ejchart, A.: Determination of association constants at moderately fast chemical exchange: Complexation of camphor enantiomers by αcyclodextrin. J. Magn. Reson. **181**, 304–309 (2006)
53. Dodziuk, H., Nowinski, K.S., Kozminski, W., Dolgonos, G.: On the impossibility of determination of stepwise binding constants for the 1: 2 complex of (+)–camphor with α–cyclodextrin. Org. Biomol. Chem. **1**, 581–584 (2003)
54. Majumder, M., Sathyamurthy, N.: A theoretical investigation on the effect of π–π stacking interaction on. Chem. Acc. **131**, 1092 (2012)