

Host–guest interactions between niobocene dichloride and α -, β -, and γ -cyclodextrins: preparation and characterization

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Abstract The possible inclusion complexes of Cp_2NbCl_2 into α -, β -, and γ -CD hosts have been investigated. The existence of a true inclusion complex in the solid state was confirmed by a combination of thermogravimetric analysis, FTIR, PXRD, and ^{13}C CP-MAS NMR spectroscopies. The solid-state results demonstrated that α -cyclodextrin does not form inclusion complexes with Cp_2NbCl_2 whereas β - and γ -cyclodextrins do form such complexes. PXRD, NMR, and thermal analysis showed that the organometallic molecules of $\text{Cp}_2\text{NbCl}_2\text{OH}$ are included in the cavities of β - and γ -cyclodextrins, possibly adopting a symmetrical conformation in the complex, with each glucose unit in a similar environment. In solution, ^1H NMR experiments suggest that niobocene has a shallow penetration on the β -CD leading to upfield shift on H-3 signal with a minor perturbation on the H-5 proton while for γ -CD, both H-3 and H-5 are shifted upfield substantially. This suggests that niobocene penetrates deeper into the γ -CD cavity than in the β -CD cavity, as a result of the cavity size.

Keywords Cyclodextrin · Niobocene · Inclusion complex · PXRD · ^{13}C CP-MAS

Introduction

Metallocene dihalides and pseudo halides of general formula Cp_2MX_2 ($\text{M} = \text{Ti}, \text{V}, \text{Nb}, \text{Mo}$; $\text{X} = \text{Cl}, \text{Br}, \text{CN}, \text{SCN}$) have shown activity on a wide variety of murine and human tumors [1–17]. Of the metallocenes tested, titanocene dichloride showed to be the most active species followed by vanadocene-, molybdenocene-, and niobocene dichloride [1–5, 7, 8]. Although they belong to the same class of complexes, they have different chemical and biochemical behaviors. One of the major complications of these metallocene complexes is the low hydrolytic stability at physiological pH [18, 19]. This has hindered to study these complexes mechanistically and as a result limiting their pharmacological use. One way to protect these species from extensive hydrolysis is to encapsulate them into macromolecule such as cyclodextrins, Scheme 1.

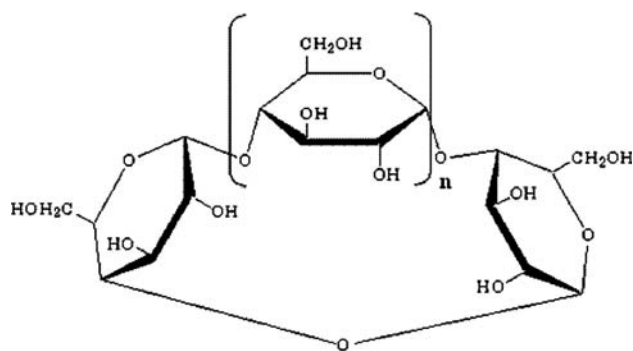
Cyclodextrins (CDs) act as a molecular host to a variety of guests: ions, metal complexes, polar, and non-polar organic molecules [20]. These inclusion complexes have found pharmaceutical applications due to the increased aqueous solubility of the drugs, better oral absorption, and their improved stability towards heat, light, oxidizing reagents, and acidic conditions. Cyclodextrins are known to form stable inclusion compounds with a variety of organometallic species including ferrocene and its derivatives [21], and sandwich complexes of molybdenum [22].

The encapsulation of Cp_2TiCl_2 in cyclodextrins was reported in 1999 by Turel et al. [23]. Precedents in this area have shown that the inclusion complexes are formed by the interaction of the metallocenes in the CD cavity and their penetration depends on the length of the cyclic oligosaccharides. According to this report, titanocene dichloride can be encapsulated in the larger cyclodextrins, β - and γ -CD, and not in the smaller α -cyclodextrin. Evidently the

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Scheme 1

size of the cavity plays an important role in the inclusion complex. More recently, the CD–Cp₂VCl₂ inclusion complexes have been studied by EPR spectroscopic methods [24]. The *g*-tensor and the hyperfine anisotropic *A* values demonstrated that vanadocene dichloride and 1,1'-dimethyl vanadocene dichloride were encapsulated in the β- and γ-CD and the rhombic symmetry was distorted as expected for encapsulated vanadium species, while the α-cyclodextrin can not encapsulate vanadocene complexes. In this regard, the anisotropic EPR spectral data demonstrated that only in the β- and γ-CD, the hyperfine interactions with the vanadium nucleus can be observed as a result of the vanadocene inclusion. On other report, the β-CD–molybdenocene dichloride inclusion complex was characterized by physical methods and ab initio calculations [25]. The predicted geometry of β-CD–molybdenocene dichloride inclusion complex is, only one of the Cp ligands inside the cavity of the cyclodextrin D-glucopyranose units.

Our research involves many aspects of the metallocene antitumor agents, from synthesis and structure modification, spectroscopic studies and development of analytical methodology to preparation and characterization of the inclusion complexes of metallocene antitumor agents, in order to afford thermally and hydrolytically stable species. Herein we report the interaction of niobocene dichloride (Cp₂NbCl₂) with α-, β-, and γ-cyclodextrins and their solution and solid-state characterization.

Experimental

Material and methods

α-CD (Sigma-Aldrich), β-CD (Aldrich), and γ-CD (Fluka) were obtained commercially. The water content was determined by TGA and the equivalents of cyclodextrins (in the reactions) were corrected by this factor. Cp₂NbCl₂ was purchased from Aldrich and used as received. The inclusion complexes were prepared using CDs (α-, β-, and γ), and metal dichloride.

The thermal analysis experiments were performed using a TAQ100 (DSC) and TAQ500 (TGA) instrument. The heating rate was 3 °C/min for DSC analysis and 10 °C/min for TGA analysis. A differential scanning calorimetry interfaced to a PC was used to measure the thermal properties of the inclusion compounds. The calorimetry operated with a nitrogen flow of 50 mL/min. The temperature of the calorimeter was calibrated from the observed melting points of indium. FTIR data were collected on a Nexus 670 spectrometer using Thunderdome ATR. Powder X-ray diffraction (PXRD) data were collected on a Siemens D5000 diffractometer using CuK_α radiation (= 1.5418 Å).

¹H NMR spectra were recorded on a Bruker Avance 500 MHz and chemical shifts are referenced to HOD/H₂O as internal reference. ¹³C CP-MAS spectra were recorded on a Bruker Avance 500 WB spectrometer at 125.75 MHz with a 4.00 μs ¹³C 90° pulse with ¹H decoupling, spinning rate of 11 kHz, a delay of 1 s between pulses using a 4 mm CPMAS probe.

Synthesis of CD–Cp₂NbCl₂ inclusion complexes

A total of 0.2 mmol of CD (corrected for water content) was dissolved in 30 mL of deionized water and 0.2 mmol of Cp₂NbCl₂ was added. After stirring for 30 min, a light yellow solution resulted that was lyophilized to obtain amorphous voluminous solid product.

Anal. Calc. for “α-CD–Cp₂NbCl₂OH · 6H₂O” [(C₃₆H₆₀O₃₀)–(C₁₀H₁₁NbCl₂O) · 6H₂O]: C, 39.70; H, 6.01; Cl, 5.09. Found: C, 40.14; H, 6.03; Cl, 4.80 IR(KBr) cm⁻¹: 3365(m), 2933(w), 1332(m), 1151(m), 1077(m), 1028(s), 950(w).

Anal. Calc. for β-CD–Cp₂NbCl₂OH · 9H₂O [(C₄₂H₇₀O₃₅)–(C₁₀H₁₁NbCl₂O) · 9H₂O]: C, 38.84; H, 6.20; Cl, 4.41. Found: C, 38.84; H, 6.11; Cl, 4.21. IR(KBr) cm⁻¹: 3332(bm), 2927(w), 1332(w), 1154(w), 1079(w), 1028(s), 938(w), 847(w).

Anal. Calc. for γ-CD–Cp₂NbCl₂OH · 9H₂O [(C₄₈H₈₀O₄₀)–(C₁₀H₁₁NbCl₂O) · 9H₂O]: C, 39.35; H, 6.21; Cl, 4.01. Found: C, 39.67; H, 6.19; Cl, 3.92. IR(KBr) cm⁻¹: 3337(bw), 1150(w), 1079(w), 1022(s), 931(w), 843(w).

Physical mixtures were prepared by mixing equimolar amounts of cyclodextrin and Cp₂NbCl₂OH in a bench top tumbler blender.

Results

It is known that niobocene dichloride (Cp₂NbCl₂), (one of the organometallic antitumor agents) dissolves in water and

undergoes an oxidation and hydrolysis processes to yield $\text{Cp}_2\text{NbCl}_2\text{OH}$ species [26]. This species has been characterized by NMR spectroscopy and has been attributed to be the active species under aqueous solution. Therefore when we refer to niobocene dichloride in solution or niobocene in the solid state, we are assuming that the predominant species is $\text{Cp}_2\text{NbCl}_2\text{OH}$.

The inclusion complexes were characterized by a series of physical techniques, including thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), PXRD and solid-state ^{13}C CP-MAS and ^1H NMR spectroscopies.

Table 1 summarizes the thermal properties (TGA and DSC) of the drug, cyclodextrins and the inclusion complexes and Figs. 1S and 2S (Supplementary Material) show representative TGA and DSC thermograms. In general, the α -, β - and γ -cyclodextrins lose 8–11% of mass in the range of 25–108 °C, indicating loss of hydrated water in the cyclodextrins. The TGA and DSC thermograms of the inclusion complexes demonstrated mass loss of 7–9% between 25 and 100 °C, also indicating loss of water from the cyclodextrin inclusion complexes. Although we are aware that elemental analysis of lyophilized sample could be meaningless at some point, we compared the water calculated with elemental analysis and TGA for the new complexes. In fact, there is a discrepancy between the water content calculated by elemental analysis and the water content determined by thermal analysis. This suggests that in the thermal analysis, at low temperature (25–100 °C) only the weakly bound water molecules are released. The tightly bound water molecules are lost at higher temperatures and this process could be overlapped by other thermal events such as melting and decomposition of the inclusion complex. The melting points of the

cyclodextrins are within 284–286 °C, according to the DSC analysis.

Upon analysis of Fig. 1S, it is evident that the TGA and DSC curves of the α -CD-niobocene freeze-dried sample contain features different to the free cyclodextrin or free $\text{Cp}_2\text{NbCl}_2\text{OH}$. PXRD data further clarify this point. Likewise, the thermal behavior of the β - and γ -CD inclusion complexes do not resemble to the free cyclodextrins or the niobocene-CD physical mixtures. From the TGA/DSC curves shown in Fig. 2S, the β -CD, the physical mixture and the inclusion complex lose water between 25 and 100 °C but the calculated dehydration and melting temperatures (from DSC) are different in each case. In all the inclusion complexes, the dehydration temperatures are lower than in the physical mixtures but the melting temperatures are higher for the inclusion complexes. Particularly interesting, the melting points of the α -, β -, and γ -CDs (284–289 °C) and $\text{Cp}_2\text{NbCl}_2\text{OH}$ (230 °C) are higher than the inclusion complexes (195–200 °C) and the physical mixtures (172–183 °C). Also, cyclodextrins and $\text{Cp}_2\text{NbCl}_2\text{OH}$ decompose at 325–330 and 265 °C, respectively and the inclusion complexes decompose at lower temperatures (about 200 °C). These results suggest that α -, β - and γ -CDs might be able to encapsulate $\text{Cp}_2\text{NbCl}_2\text{OH}$ species since the starting materials and the freeze-dried samples behave differently. As we mentioned before, thermal analysis, in many cases, cannot discriminate with certainty between the inclusion complexes and physical mixtures. Therefore, other solid-state analyses were performed to discriminate between inclusion complexes and physical mixtures.

Powder X-ray diffraction analysis was undertaken on the investigated compounds. This is one of the most

Table 1 Thermoanalytical data of inclusion complexes (FD), physical mixtures (PM) and precursors

	Dehydration		MDSC endothermic max (°C)		
	Temperature range (°C)	Mass loss (%)	Temperature range (°C)	Mass loss (%)	
α -CD	25–100	9	245–496	88	76 (D), 108 (D), 136 (D/glass transition), 289 (M), 325 (Decomposition/exothermic)
β -CD	25–80	11	271–496	88	98 (D), 129 (D/glass transition) 288 (M), 325 (Decomposition/exothermic)
γ -CD	25–98	8	276–496	93	104 (D/glass transition), 284 (M), 330 (Decomposition/exothermic)
$\text{Cp}_2\text{NbCl}_2\text{OH}$	25–97	2	188–495	45	230 (M), 265(decomposition/exothermic)
α -CD- $\text{Cp}_2\text{NbCl}_2\text{OH}$ FD	25–100	9	167–496	88	110(D/glass transition), 195(M)
α -CD- $\text{Cp}_2\text{NbCl}_2\text{OH}$ PM	25–100	8	177–496	80	83 (D), 154 (glass transition), 183 (M)
β -CD- $\text{Cp}_2\text{NbCl}_2\text{OH}$ FD	25–70	7	165–496	91	92 (D/glass transition), 200 (M)
β -CD- $\text{Cp}_2\text{NbCl}_2\text{OH}$ PM	25–98	12	185–496	82	126 (D/glass transition), 188 (M)
γ -CD- $\text{Cp}_2\text{NbCl}_2\text{OH}$ FD	25–98	9	182–496	91	95 (D/glass transition), 197 (M)
γ -CD- $\text{Cp}_2\text{NbCl}_2\text{OH}$ PM	25–97	8	170–496	82	123 (D/glass transition), 172 (M)

Note: D = Dehydration, M = Melting, and FD = freeze dried

important methods to characterize inclusion complexes of cyclodextrins in the solid state. Table 2 shows the PXRD peaks of the hosts, guests, and inclusion complexes. Figures 1, 3S, and 4S (Supplementary Material) depict PXRD spectra of CD–niobocene complexes.

Upon analysis of the Figs. 1, 3S, and 4S we can observe that $\text{Cp}_2\text{NbCl}_2\text{OH}$ has a very simple diffraction pattern (spectrum) and the cyclodextrins have well defined diffraction patterns at $2\theta < 30^\circ$. The physical mixtures are only overlapping spectra of the free cyclodextrin and $\text{Cp}_2\text{NbCl}_2\text{OH}$. However, the PXRD spectra of “ α -CD–Niobocene” and β -CD–Niobocene inclusion complexes are

substantially different. The PXRD spectrum of “ α -CD– $\text{Cp}_2\text{NbCl}_2\text{OH}$ FD” (Fig. 3S) contains broad diffraction peaks that can be attributed to free α -CD and $\text{Cp}_2\text{NbCl}_2\text{OH}$. This strongly suggests that α -CD cannot encapsulate efficiently niobocene dichloride. On the other hand, the diffraction pattern of the β -CD– $\text{Cp}_2\text{NbCl}_2\text{OH}$ FD is different to the physical mixture as well as to the free β -CD and $\text{Cp}_2\text{NbCl}_2\text{OH}$ species. The β -CD– $\text{Cp}_2\text{NbCl}_2\text{OH}$ FD PXRD spectrum showed that this solid is more amorphous and has two major peaks at higher angles ($2\theta > 30^\circ$), demonstrating that it is an inclusion complex. Similar behavior was observed for the γ -CD– $\text{Cp}_2\text{NbCl}_2\text{OH}$

Table 2 Powder X-ray diffraction spectral analysis of inclusion complexes (FD), physical mixtures (PM) and precursors

Compound	Major characteristic peaks at 2θ
α -CD	14.4 (strongest), 12.3, 21.8 (secondly), 5.4, 13.7, 15.8, 19.4, 22.9, 31.8, 36.3, 56.6
β -CD	4.7 (strongest), 9.2, 12.7, 25.9 (secondly), 23.1
γ -CD	5.2 (strongest), 12.5, 15.5, 16.5, 18.9, 36.3 (secondly), 31.8
$\text{Cp}_2\text{NbCl}_2\text{OH}$	13.7 (strongest), 15.3, 33.7 (secondly), 19.2, 22.3
α -CD– $\text{Cp}_2\text{NbCl}_2\text{OH}$ FD	19.5 (strongest), 13, 15 (secondly), 23
β -CD– $\text{Cp}_2\text{NbCl}_2\text{OH}$ FD	36.3 (strongest), 31.8 (secondly), 6.3, 14.2, 17.8, 22.6, 34.5, 56.6, 67.9
γ -CD– $\text{Cp}_2\text{NbCl}_2\text{OH}$ FD	36.3 (strongest), 31.8 (secondly), 17.1, 22.6, 34.5, 47.6, 56.6, 67.9

Note: FD = freeze dried, PM = physical mixtures

Fig. 1 PXRD of: (a) $\text{Cp}_2\text{NbCl}_2\text{OH}$, (b) β -CD, (c) β - $\text{Cp}_2\text{NbCl}_2\text{OH}$ FD, and (d) β -CD– $\text{Cp}_2\text{NbCl}_2\text{OH}$ PM

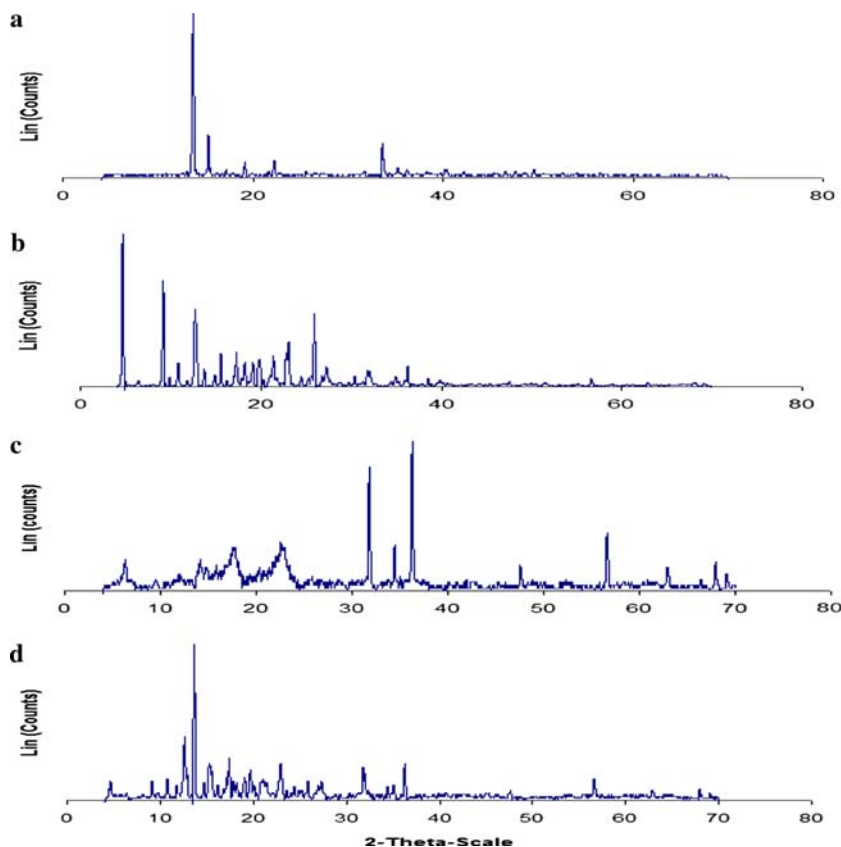
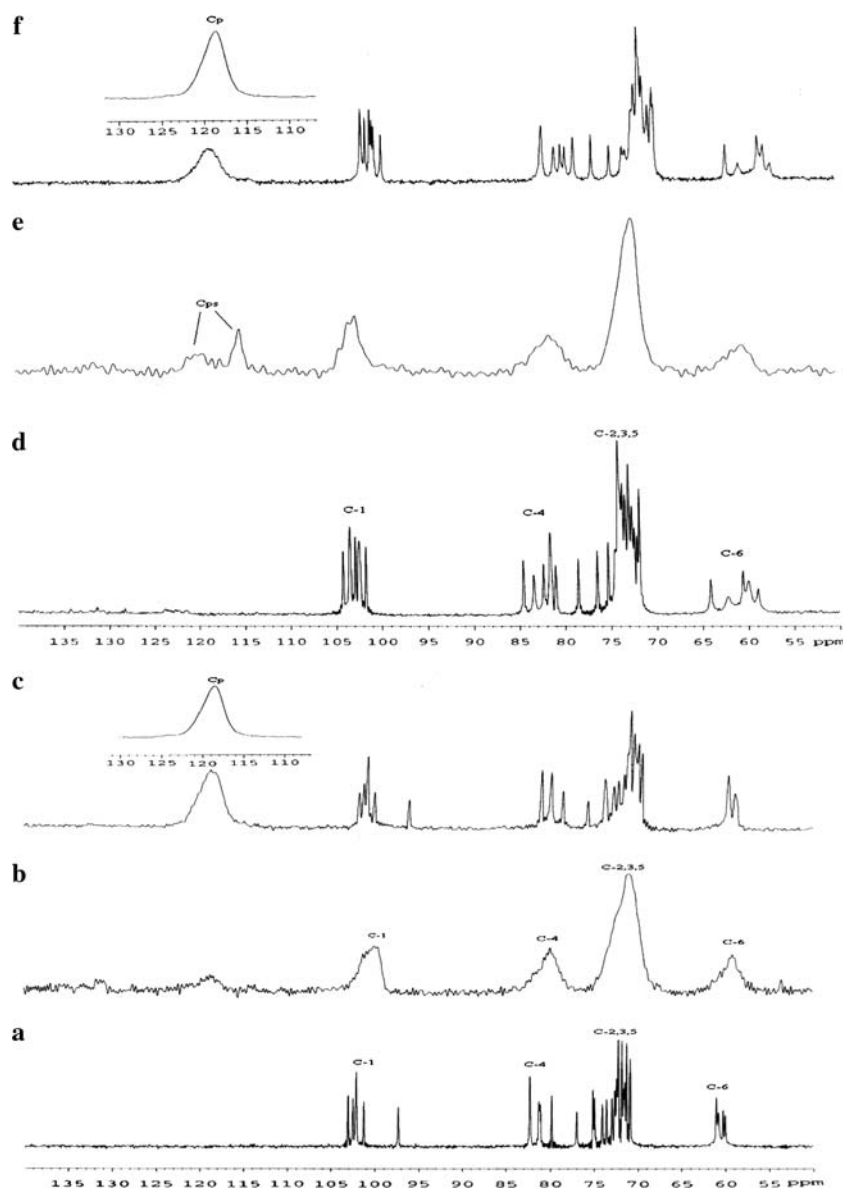


Fig. 2 ^{13}C CP-MAS spectra of (a) α -CD, (b) α -CD- $\text{Cp}_2\text{NbCl}_2\text{OH}$ FD, (c) α -CD- $\text{Cp}_2\text{NbCl}_2\text{OH}$ PM, (d) β -CD, (e) β -CD- $\text{Cp}_2\text{NbCl}_2\text{OH}$ FD, and (f) β -CD- $\text{Cp}_2\text{NbCl}_2\text{OH}$ PM. Top inset: Cp signal of $\text{Cp}_2\text{NbCl}_2\text{OH}$



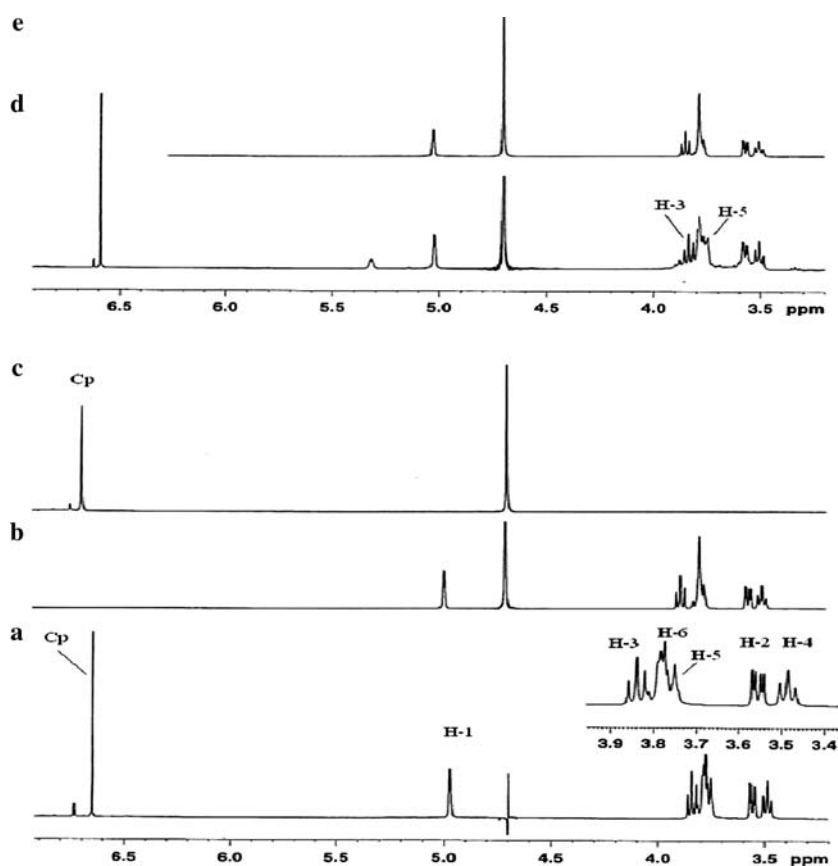
FD spectrum. Therefore based on the PXRD data, only β -CD and γ -CD can form inclusion complexes with CpNbCl_2OH .

The inclusion complexes were further investigated by solid-state ^{13}C CP-MAS NMR spectroscopy. This technique has allowed us to distinguish between the free host and the inclusion complexes, since the chemical environment between these two species are different and as result the ^{13}C chemical shift patterns are different. Figure 2 presents the solid-state ^{13}C CP-MAS NMR spectra of $\text{Cp}_2\text{NbCl}_2\text{OH}$, cyclodextrin, physical mixture and inclusion complex for α - and β -CDs, respectively. The spectra of free α - and β -CDs exhibited very similar pattern, multiple resonances for each type of carbon atom and in the same range of chemical shifts. This behavior has been attributed to the different torsional angles about the C-1 and C-4 linkages

and the orientation of the hydroxyl groups [27–29]. According to previous reports, the carbon resonances are assigned as followed: C-1 (100–105 ppm), C-4 (77–85 ppm), C-2,3,5 (70–75 ppm), C-6 (57–65 ppm).

Upon analysis of Fig. 2 it is evident that the physical mixture spectrum is the combination of two spectra, α -CD and niobocene. The ^{13}C CP-MAS NMR spectrum of the apparent inclusion complex showed peak broadening which may suggest that niobocene might be encapsulated into the α -CD but the Cp signal intensity is too small relative to the α -CD carbon atoms signals. This suggests that the α -CD does not encapsulate niobocene efficiently in a 1:1 ratio. Also in the α -CD-Niobocene ^{13}C CP-MAS NMR spectrum, although broad, on each carbon atom on the cyclodextrin multiple resonances can be identified, which indicates that the glucose units of α -CD have not adopted a

Fig. 3 ^1H NMR spectra of (a) β -CD- $\text{Cp}_2\text{NbCl}_2\text{OH}$ (1:1 inclusion), (b) β -CD, (c) $\text{Cp}_2\text{NbCl}_2\text{OH}$, (d) γ -CD- $\text{Cp}_2\text{NbCl}_2\text{OH}$ (1:1 inclusion), and (e) γ -CD, in D_2O at 25°C



more symmetrical environment as might be expected when a cyclodextrin encapsulates the guest species.

On the other hand, the spectrum of the β -CD- $\text{Cp}_2\text{NbCl}_2\text{OH}$ inclusion complex shows four single broad peaks corresponding to the C-1, C-4, C-2, 3, 5, and C-6 and two additional peaks (one broad and one sharp) corresponding to the $\text{Cp}_2\text{NbCl}_2\text{OH}$. This evidence strongly suggests that $\text{Cp}_2\text{NbCl}_2\text{OH}$ is included in the β -CD and the chemical environment of the glucose units becomes more symmetrical. Since the β -CD adopts a more symmetrical conformation, single resonances for C-1, C-4, C-2, 3, 5, and C-6 atoms are observed. Interestingly, the γ -CD- $\text{Cp}_2\text{NbCl}_2\text{OH}$ inclusion complex spectrum (Fig. 4S) shows four single resonances for the cyclodextrin carbon atoms but different to the β -CD, two sharp peaks can be identified for the $\text{Cp}_2\text{NbCl}_2\text{OH}$ species. It is not clear, using only ^{13}C CP-MAS NMR spectroscopy, if $\text{Cp}_2\text{NbCl}_2\text{OH}$ occupies the γ -CD cavity adopting a different inclusion geometry or conformation as compared to the β -CD.

In order to investigate in more details the nature of the above CD- $\text{Cp}_2\text{NbCl}_2\text{OH}$ interactions, ^1H NMR spectroscopic studies in D_2O were performed. While the α -CD- $\text{Cp}_2\text{NbCl}_2\text{OH}$ spectrum shows no significant change in the protons inside the cavity (H-5 and H-3, Fig. 5S), the spectrum of the β -CD- $\text{Cp}_2\text{NbCl}_2\text{OH}$ (Fig. 3) shows upfield shift on H-3 signal and in a minor extent on H-5. H-3

proton is located inside the cavity on the wider edge of the cone while the H-5 is located inside toward the narrow edge of the cavity [23]. Based on this solution information, we confirm that $\text{Cp}_2\text{NbCl}_2\text{OH}$ has a shallow penetration on the β -CD.

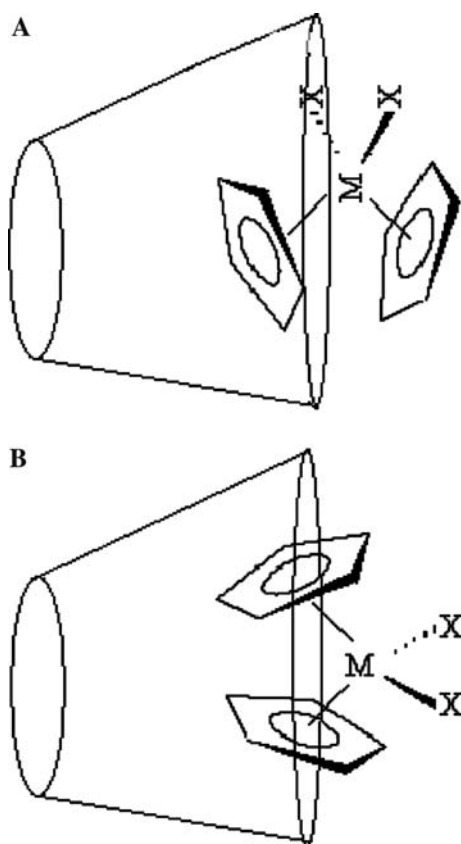
Likewise, the γ -CD-Niobocene system was investigated in solution by ^1H NMR spectroscopy (Fig. 3). ^1H NMR spectra of this system showed that H-3 as well as H-5 signals are shifted upfield significantly. This suggests that, different to the β -CD- $\text{Cp}_2\text{NbCl}_2\text{OH}$, niobocene may possibly have a deeper penetration into the γ -CD cavity as a consequence, H-5 is also perturbed substantially (shifted upfield) by the niobocene [23]. This is the logical result of γ -CD having a cavity with larger diameter than the β -CD.

Conclusion

The host-guest interaction between α -, β -, and γ -CDs and $\text{Cp}_2\text{NbCl}_2\text{OH}$ have been characterized by a combination of solid-state physical methods and solution ^1H NMR spectroscopy. The formation of a true inclusion complex in a 1:1 ratio has been detected for β - and γ -CDs. The evidence suggests that $\text{Cp}_2\text{NbCl}_2\text{OH}$ species is incorporated in the CD cavity but the penetration deepness depends on the CD cavity size. Solution ^1H NMR spectral data suggest that

$\text{Cp}_2\text{NbCl}_2\text{OH}$ species penetrates deeper into the γ -CD than into the β -CD. Two possible geometries for these inclusion complexes can be envisioned, A and B. According to the ^{13}C CP-MAS NMR spectral data, two Cp signals are observed for β - and γ -CDs. This suggests that the Cp rings are on different magnetic and chemical environments and geometry A might explain the observed spectroscopic data. Geometry A has also been found to be the lowest energy conformation for Cp_2MoCl_2 species [25], but geometry B has been proposed by Turel et al. [23]. At the present time, it is not clear which geometry the inclusion complex adopts, but ab initio calculations are being performed to determine the preferred geometry. Other structures for the inclusion complexes can be envisioned such as possible coordination of the C_2 and C_3 secondary hydroxyl groups to Nb(IV) center, as found for Cu(II) center [30] but that will require to create vacant coordination sites by removing the chloride and hydroxo ancillary ligands. In any event, with current spectroscopic data we cannot rule out this possibility with certainty.

Finally, in ^1H NMR experiments we have explored the possibility of 2:1 and 1:2 ratios of cyclodextrin:niobocene but we have found no evidence of host–guest interactions.



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