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Qualitative study of supramolecular assemblies of β-cyclodextrin and cholecalciferol and the cobalt (II), copper (II) and zinc (II) ions

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ABSTRACT

A ^{13}C NMR in DMSO-d_6 as solvent, diffuse reflectance spectra and X-ray powder diagram study of the inclusion of vitamin D in β-cyclodextrin and of the ternary assemblies with β-cyclodextrin, vitamin D and metal ions (*e.g.* Co(II), Cu(II) and Zn(II)) was carried out to determine the structure of these associations in which the molecular ratios (β-cyclodextrin:vitamin D:metal ions) were 5:1:1 or 10:1:1.

1. Introduction

Lipophilic vitamins (A, D, E and K) present very low solubility in water and consequently in biological media. Cyclodextrins, mainly β-cyclodextrins (β-CD) are commonly used as inclusion material (Del Valle, 2004; Pitha, 1981). In fact, cyclodextrins are cyclic oligomers of (1→4) linked α-D-glucose monomers and their main structural feature is their toroidal shape with an hydrophobic cavity (5–8 Å) which can be used in chemical synthesis (Tabushi, Yamamura, Fujita, & Kawakubo, 1979). In the case of β-CD the size of this cavity is 6.0–6.5 Å for its diameter and the height is 7.9 Å (Li & Purdy, 1992). In many cases, the inclusion complexes of vitamin D_3 (VitD) or cholecalciferol do not take into account a third species like metal ion (Delaurent, Siouffi, & Pepe, 1998; Tian & Holick, 1995). Crespo-Biel et al. described a supramolecular complex with β-CD and metal-ion coordinated with ethylenediamine (Crespo-Biel, Lim, Ravoo, Reinhoudt, & Huskens, 2006) while Torri et al. described the complex inclusion of progesterone (Torri et al., 2007; Zoppetti et al., 2007). There are also papers dealing with metals derivatives inclusion but, as far as we know, without any organic molecule (Jiang et al., 2006, 2004; Matsui, Kurita, & Date, 1972; Mochida & Matsui, 1976). In addition, very few articles dealt with ternary complexes associating β-CD, an organic molecule and a metal ion (Fernandes et al., 2006; Thanabal, Omecinsky, Reily, & Cody, 1994; Zheng & Tarr, 2006). The inclusion of metal complexes of VitD should allow them to be more stable in aqueous media. We have previously determined the stability constants of various binary complexes of cholecalciferol (Mercê, Szpoganicz, Khan, Do Thanh, & Bouet, 1999; Mercê et al., 1998; Mercê, Yano, Khan, Thanh, & Bouet, 2003) and we describe in this paper supramolecular assemblies of β-cyclodextrin and cholecalciferol (VitD) and the cobalt (II), copper (II) and zinc (II) ions using ^{13}C NMR, diffuse reflectance UV–visible spectroscopy and X-ray powder diagrams.

2. Experimental

2.1. Reagents

β-Cyclodextrin (Aldrich, USA) and vitamin D_3 or cholecalciferol (Sigma, USA) were analytical grade. Proper mass of the metal salts of $Cu(NO_3)_2{\cdot}3H_2O$, $CoCl_2{\cdot}6H_2O$ (both *p.a.*, Merck, Germany) and $ZnCl_2$ (Janssen Chimica, Belgium) were used to make the initial aqueous solutions of the metal ions. The pH of copper (II) solutions were set with 0.1 mol L^{-1} aqueous solutions of either HCl or KOH p.a. (Merck, Germany) to 3.0 and 7.0, respectively.

2.2. General technique of inclusion complexes

In an Atmosbag™ (AL-211 – Aldrich) under dinitrogen atmosphere (Air Liquide, France) the reagents in water/ethanol solution (50% v/v) were mixed in a glass flask and let to react under magnetic stirring for 4 h. The amounts were in all cases 0.200 mmol of β-CD, 0.02 or 0.04 mmol of cholecalciferol (VitD p.a, Sigma,

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USA) and finally 0.02 or 0.04 mmol of metal ion depending on the ratio β-CD:VitD:M^{2+}, *e.g.*: 10:1:1 or 5:1:1, respectively. A small Schlenk glass flask capped with rubber stop (Saint-Gobain, Verneret, France) was used to weight the VitD outside the Atmosbag™. The solvent was completely removed under vacuum. The obtained solid was kept under N_2 in a sealed small Schlenk glass flask.

2.3. ^{13}C NMR spectra

The spectra were recorded at 20 °C (293 K). An amount of approximately 50 mg of the samples were dissolved in 0.75 mL of DMSO-d_6 (H_2O <0.02%, Eurisotop, France), and added with drops of 1,4-dioxane (99%, Aldrich) as an internal standard. In our experimental conditions, the ^{13}C chemical shift of the singlet of 1,4-dioxane carbon atoms was 66.36 ppm with respect to TMS (Jones, Katritzky, Murrell, & Sheppard, 1962; Thanabal et al., 1994). This compound produces a singlet more accurate than the septuplet at *c.a.* 39.43 ppm of DMSO-d_6. Only ^{13}C spectra were recorded because most of metal complexes are paramagnetic species.

2.4. Diffuse reflectance ultraviolet–visible spectroscopy

An exact mass of 25–30 mg of the solid samples were placed in an aluminum cell of 0.7 cm diameter and analyzed in a Shimadzu UV-2401PC spectrophotometer with an integration sphere (240-52454-01). The absorbance from 190 to 900 nm and the reflectance from 0 to 100 were recorded for all samples.

2.5. X-rays powder diagrams

The X-rays powder diagrams were obtained using a Shimadzu XD-3A diffractometer, with Cu K_α radiation with the samples in a glass holder.

3. Results and discussion

The proportions of the reagents were chosen in order to provide enough quantity of β-CD to encapsulate VitD. We have tested other β-CD–VitD ratios like 3:1, 2:1 and 1:1, respectively, but unsuccessful. The formation of the supramolecular structure was only observed when this ratio was at least 5:1. Finally, all experiments were performed using 5:1 or 10:1 β-CD to VitD ratios for binary systems. Consequently, in the cases of ternary assemblies, the β-CD:VitD:metal ion ratios were 5:1:1 or 10:1:1. When mixing the three compounds without performing the above inclusion technique, no inclusion was observed. The variations of chemical shifts when passing from free components to inclusion complexes are very small because the interaction forces between host and guest molecules are only physical interactions like hydrogen bonding or Van der Waals electrostatic interactions.

3.1. Vitamin D and β-cyclodextrin ^{13}C NMR spectra

The carbon numbering scheme for cyclodextrin used in this paper was first proposed by French and Murphy(1973), Saenger et al. (1998) and Lipkowitz (1998). It is shown in Fig. 1b.

The ^{13}C NMR spectra of β-CD in DMSO-d_6 has been detailed by Jiang et al. (2007). The chemical shifts are 102.58 ppm for C(1); 72.80 for C(2); 73.80 for C(3); 81.55 for C(4); 72.53 for C(5) and finally 63.09 ppm for C(6). Under our experimental conditions (Fig. 2), the chemical shifts are slightly modified: 101.889 C(1); 72.370 C(2); 73.010 C(3); 81.490 C(4); 72.000 C(5) and 59.889 ppm C(6).

The ^{13}C NMR spectra of vitamin D in $CDCl_3$ and of some of its analogues have been published by Berman, Luz, Mazur and Sheves (1977) and in 1996 by Mizhiritskli, Konstantinovskii and Vishkautsan (1996) and are reported in Table 1. In our experimental conditions, the chemical shifts are slightly shifted (Fig. 2): *e.g.* $C_v(1)$ at 32.197, $C_v(2)$ at 35.481, $C_v(3)$ at 67.904 and $C_v(4)$ at 45.947 ppm while $C_v(11)$ appears at 21.871, $C_v(17)$ at 55.953 and $C_v(18)$ at 11.833 ppm, respectively. As expected, the same small variations are observed for all other signals (Table 1).

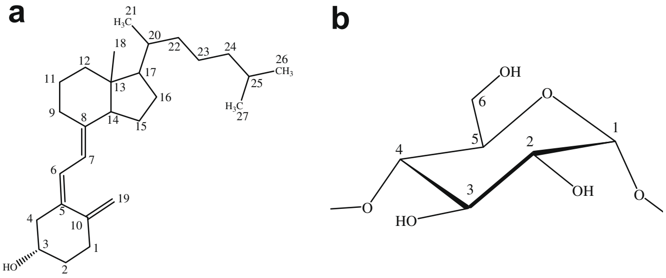

Fig. 1. Chemical structures of vitamin D_3 or cholecalciferol (a) and of one α-D-glucose unit of β-CD, numbering scheme from Lipkowitz (1998) (b).

3.2. Binary assemblies ^{13}C NMR spectra

The chemical shifts of ^{13}C signals of respectively, β-CD and VitD, are not coincidental. So, when β-CD is only mixed with VitD, all signals referring to both compounds are present in the spectrum, while when they have undergone an inclusion reaction, all chemical shifts due to VitD are not present, as shown in Fig. 2. Only two peaks relative to VitD are observed for chemical shifts lower than 60 ppm. These signals correspond to the portion of VitD, which is outside the supramolecular structure (Jiang et al., 2007). The intracavity complexation of the substrate occurs only when the experimental conditions are used as described in Section 2.

In binary assemblies (Table 2), all signals relative to β-CD are slightly shifted (+0.029 to 0.045 ppm). In the mean time, the remaining signals relative to VitD are located at 18.632 and 55.994 ppm corresponding to C(21) and C(17), respectively. When the VitD is included in β-CD, no changes appear in the structure of the cyclodextrin. The hydrophilic moiety (e.g. C(3) with OH) of the cholecalciferol is inside the cavity, while only two lipophilic carbons produce a signal and are located at the outside part of the β-CD cavity. The inner carbon atoms of the β-CD molecules (e.g. $C_v(1)$ to $C_v(4)$) could lead to hydrogen bonding with the hydroxyl of the VitD.

3.3. Ternary complexes ^{13}C NMR spectra

3.3.1. Cobalt complexes

The ^{13}C NMR spectra are given Fig. 3 and the main chemical shifts are reported in Table 3. In binary assemblies with cobalt (II) in both studied ratios (Fig. 3a and b) very few changes are observed in the spectrum of β-CD. A small new signal appeared at *c.a.* 31 ppm, higher for 5:1 ratio than for 10:1 ratio. In ternary inclusion complexes, this new signal remains and some additional signals from VitD are observed at 18.60 and 56.05 for 5:1:1 ratio and 30.74 ppm for 10:1:1 ratio. The other signals corresponding to VitD (not shown on Fig. 3) are located as follows: $C_v(5)$ at 144 ppm, $C_v(11)$ and $C_v(26)$ at 22.4 and 22.7 ppm, respectively, for β-CD–VitD–Co assembled in 10:1:1 ratio (Fig. 3d). So, in this complex, the alkyl moiety of VitD is outside the cavity.

The metal ion complexes have probably twisted the bonds around $C_v(5)$ and $C_v(8)$ in order to fit all rings and alkyl portions inside the cavity of β-CD. To obtain such a space for the whole molecule of VitD, an ion channel structure (Saenger et al., 1998) is

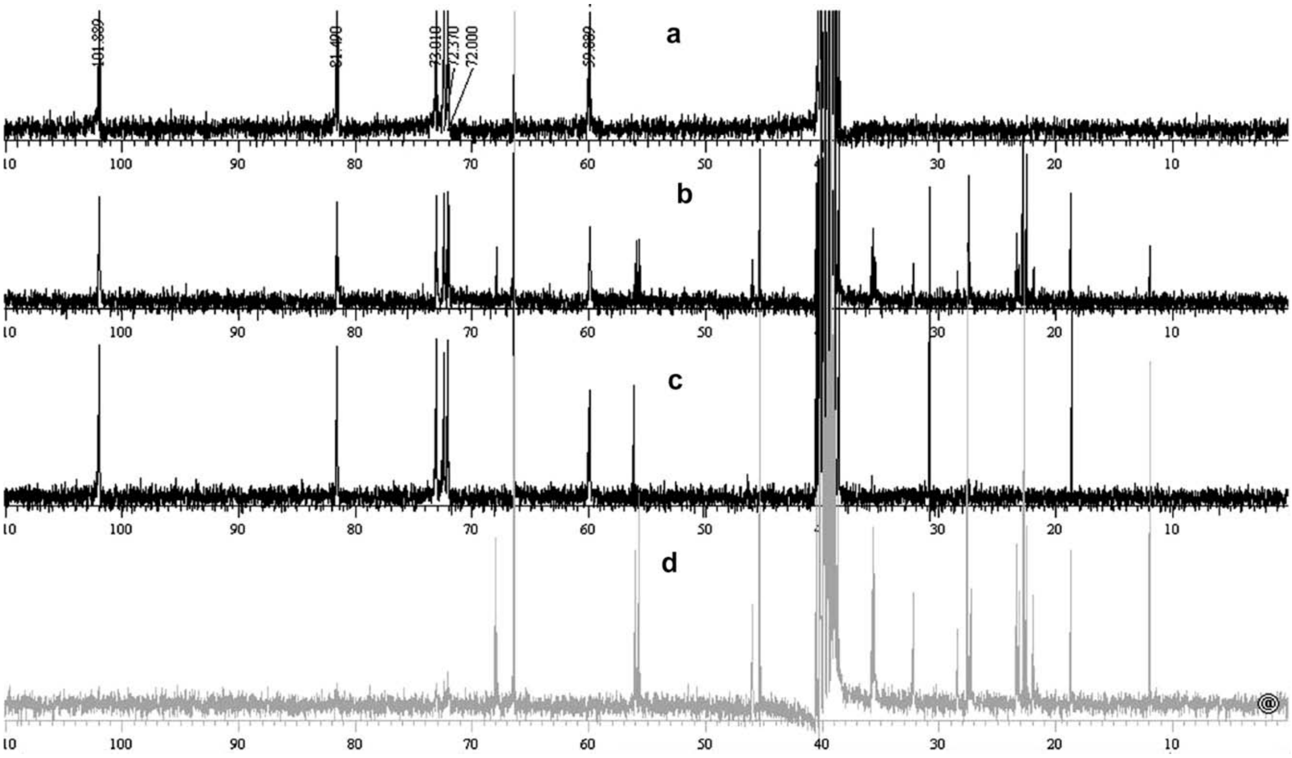

Fig. 2. ^{13}C NMR spectra: (a) β-CD; (b) mixture β-CD + VitD$_3$; (c) reaction between β-CD and VitD; (d) VitD alone.

Table 1
Chemical shifts (^{13}C, ppm) of vitamin D$_3$.

C_v[a]	Ref. (Mizhiritskli et al., 1996)	Ref. (Berman et al., 1977)	This work
1	32.55	32.05	32.197
2	35.78	35.25	35.481
3	69.83	69.20	67.904
4	46.55	46.00	45.947
5	135.69	145.20	145.387
6	123.10	122.40	120.964
7	118.13	117.65	117.434
8	142.90	142.10	140.553
9	29.65	29.10	28.376
10	145.70	135.25	145.395
11	24.21	22.30	21.871
12	41.04	40.65	39.955
13	46.41	45.90	45.262
14	57.06	56.40	55.621
15	22.87	23.60	22.418
16	28.43	27.70	27.463
17	57.08	56.75	55.953
18	12.91	12.05	11.833
19	113.06	112.35	111.797
20	41.04		40.447
21	18.23		18.671
22	136.25		136.335
23	135.28		
24	43.45		
25	34.74		35.401
26	20.29		
27	20.60		
28	21.75		21.871

[a] v Subscript means C atoms from VitD.

probably being formed with cobalt (II) in the 5:1:1 ratio. On the other hand, when cobalt (II) is in the equilibrium in 10:1:1 β-CD + VitD + Co ratio complex, the probably spatial arrangement is herringbone or back-type structures, as C_v(17) till C_v(26) atoms show ^{13}C NMR signals in the supramolecular formed structure.

Table 2
Chemical shifts (^{13}C, DMSO-d$_6$, dioxane as internal reference, ppm) of β-CD and in binary complex.

Compound	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)
β-CD	101.873	72.355	72.994	81.475	71.985	59.874
β-CD + VitD	101.918	72.397	73.029	81.507	72.014	59.905
Δδ	0.045	0.042	0.035	0.032	0.029	0.031

The signals relative to β-CD are shifted to smaller values (Table 3) and the difference is greater in the case of binary complexes with cobalt (II) ions. Some complexes with VitD and cobalt ions have been previously described (Gadais, Khan, Bouet, & Thanh, 1994). In these species, the coordination occurs through the hydroxyl on C(1). As the Co^{II} cation stands in an octahedral geometry, the internal hydroxyl functions from β-CD may be involved in the structure of the ternary complexes as indicated by the signal of C(1) in all spectrum, whatever the complex.

3.3.2. Copper complexes

The ^{13}C NMR spectra for copper derivatives are given Figs. 4 and 5 and the main chemical shifts are reported in Table 4. In binary assemblies with copper (II), in both studied ratios, very few changes are observed in the spectrum of β-CD. A weak singlet appeared at 30.72 ppm for 10:1 ratio (pH 7) but there is no new signal for pH 3 (Fig. 4a and b). In ternary inclusion complexes obtained in the 5:1:1 ratio at pH 3 and pH 7, only the signals due to β-CD are present.

In binary complexes (Fig. 4), only positive shifts are observed for all the signals (Table 4). In the cases of the ternary assemblies obtained at pH 3, the signal corresponding to C(2) is shifted to higher values whatever the ratio of reactants. The most important downfield shift is observed for C(5) at pH 7, with a 5:1:1 ratio. On the opposite side, the shift is identical for this carbon for both ratios. The inner C(2), bearing an hydroxyl functional group is in-

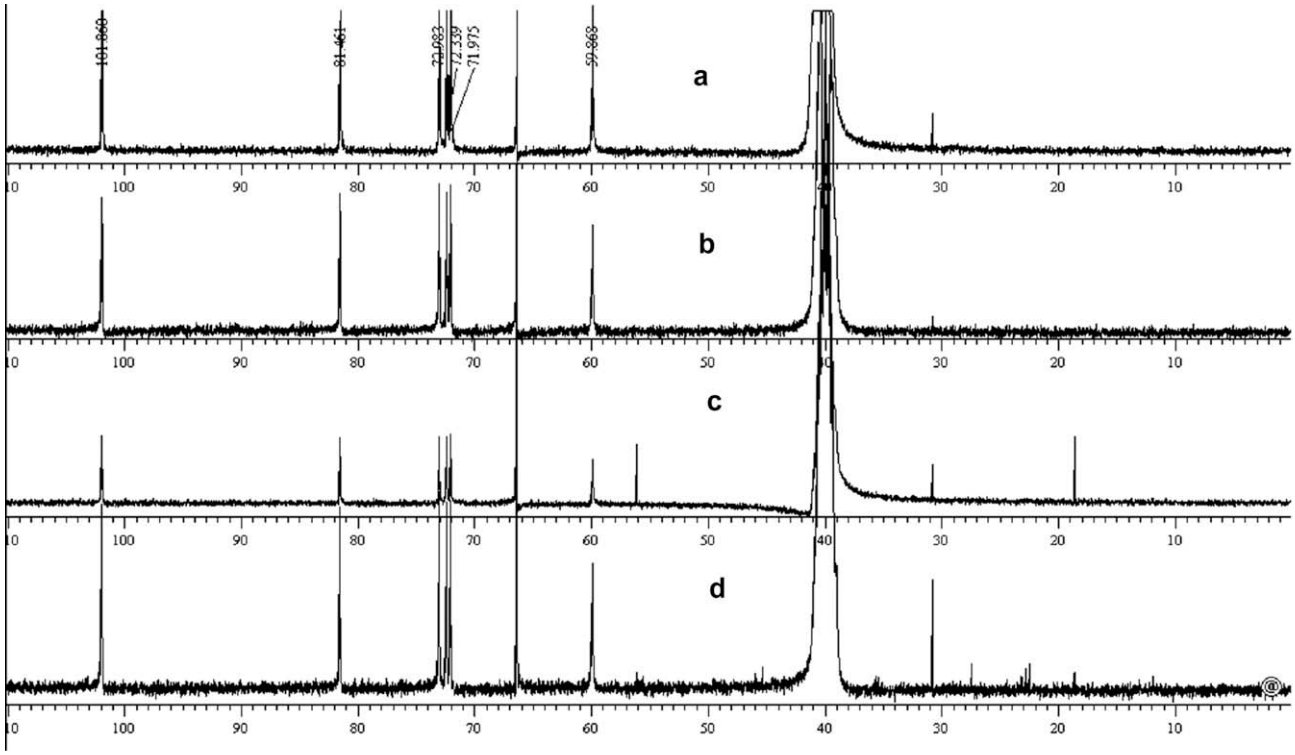

Fig. 3. ^{13}C NMR at pH 7. (a) β-CD + Co^{2+}, 5:1; (b) β-CD + Co^{2+}, 10:1; (c) β-CD + VitD + Co^{2+}, 5:1:1; (d) β-CD + VitD + Co^{2+}, 10:1:1.

Table 3

Chemical shifts in ppm (^{13}C, DMSO-d6, dioxane as internal reference) of β-CD complexes in the supramolecular assemblies with cobalt ions. Δδ means the mathematical differences among the chemical shifts of β-CD alone and the chemical shifts for the supramolecular assembly.

Complex	Ratio	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)
β-CD + Co^{2+}	5:1	101.860	71.975	72.983	81.461	72.339	59.868
Δδ – β-CD		−0.058	−0.039	−0.046	−0.046	−0.058	−0.037
β-CD + Co^{2+}	10:1	101.868	71.987	72.999	81.476	72.358	59.872
Δδ – β-CD		−0.050	−0.027	−0.030	−0.031	−0.039	−0.033
β-CD + VitD + Co^{2+}	5:1:1	101.888	72.000	72.997	81.472	72.367	59.875
Δδ – β-CD + VitD		−0.030	−0.014	−0.032	−0.035	−0.030	−0.030
β-CD + VitD + Co^{2+}	10:1:1	101.900	72.016	73.014	81.504	72.366	59.887
Δδ – β-CD + VitD		−0.018	0.002	−0.015	−0.003	−0.031	−0.018

volved in the chelation of the copper ion together with VitD, inside the cavity of the β-CD.

According to the Irving–Williams series, the copper (II) complexes are more stable than cobalt (II) complexes (Irving & Williams, 1953; Mercê et al., 2003). So, the copper ions (binary species) may coordinate with inner OH groups of β-CD and therefore no changes could be seen in the spectra. In the case of the ternary assemblies, the copper (II) ions are linked to VitD inside the β-CD cavity.

3.3.3. Zinc complexes

The corresponding spectra (pH 7) are given in Fig. 6 and the main chemical shifts are reported in Table 5. In the binary assemblies, a new signal at 30.73 is present with a feeble intensity in the case of 10:1 ratio and with a high intensity for 5:1 ratio.

In the spectra of the ternary assemblies, there is not any signal from VitD carbon atoms and the spectra are quite identical to those obtained in the case of copper assemblies. So, the structure of these ternary assemblies look like those obtained with copper (II) ions.

3.4. Diffuse reflectance spectra

In the diffuse reflectance and UV–visible results, the maximum absorption bands are reported in Table 6 for each solid supramolecular structure obtained from the three metal ions studied. Although the accuracy in reading the maximum wavelength is not the same than with samples in aqueous solutions, it can be seen that the differences among the absorptions due to charge transfer transitions and d–d transitions bands are enough to show that different products were obtained with β-CD and the three studied metal ions in the absence and in the presence of VitD.

The bands appear in the same region than in the case of the spectra of various solutions of cobalt (II) species (Fine, 1962; Lechat, Khan, Bouet, & Vierling, 1993). For instance, when Co^{2+} ion was complexed to β-CD, the maximum absorption wavelengths were 210 and 205 nm in the ultraviolet region and 527 and 536 nm (d–d), for 10:1 and 5:1 β-CD–Co ratios, respectively. These bands are located at 208 and 529 nm (d–d) for both ratios, for the same metal ion in the presence of the supramolecular structure,

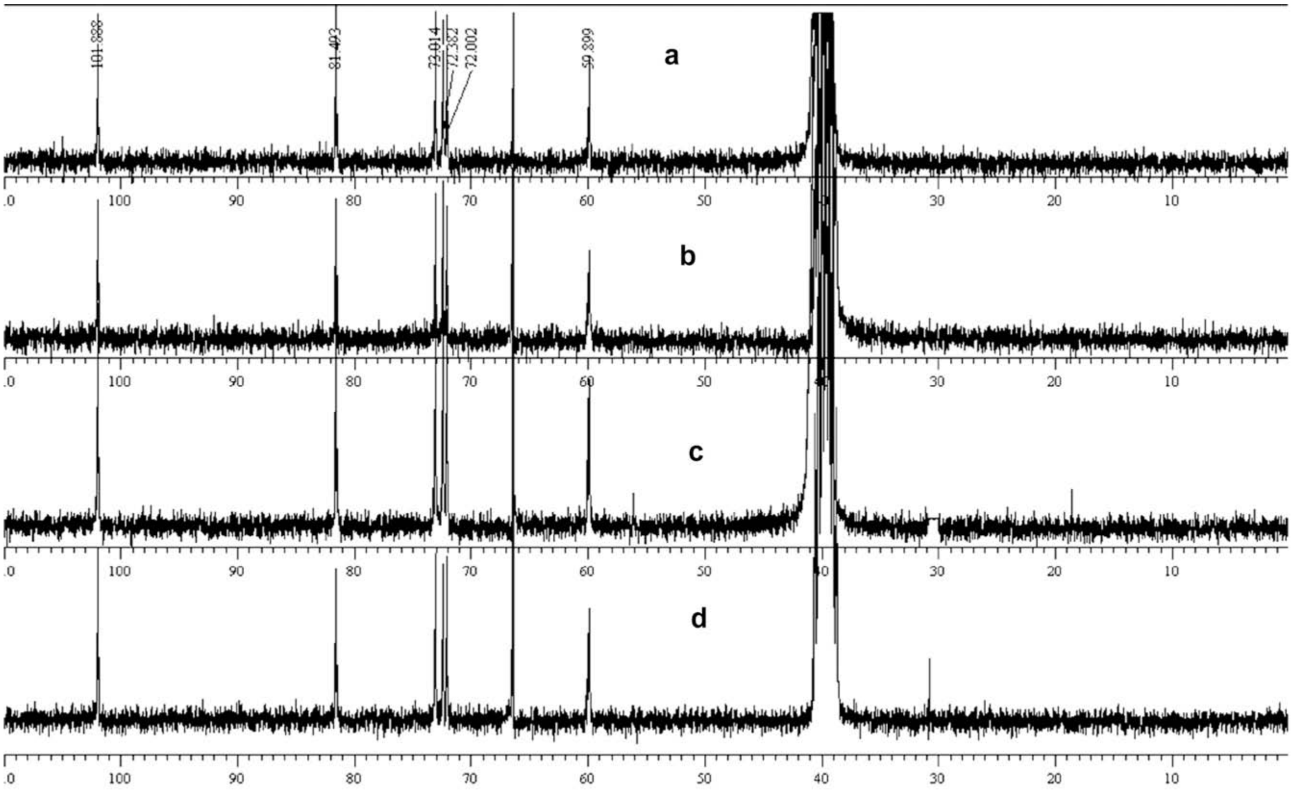

Fig. 4. (a) ^{13}C NMR of β-CD + Cu^{2+} 5:1 L:M, pH 3; (b) β-CD + Cu^{2+} 10:1 L:M, pH 3; (c) β-CD + Cu^{2+} 5:1 L:M, pH 7; (d) β-CD + Cu^{2+} 10:1 L:M, pH 7.

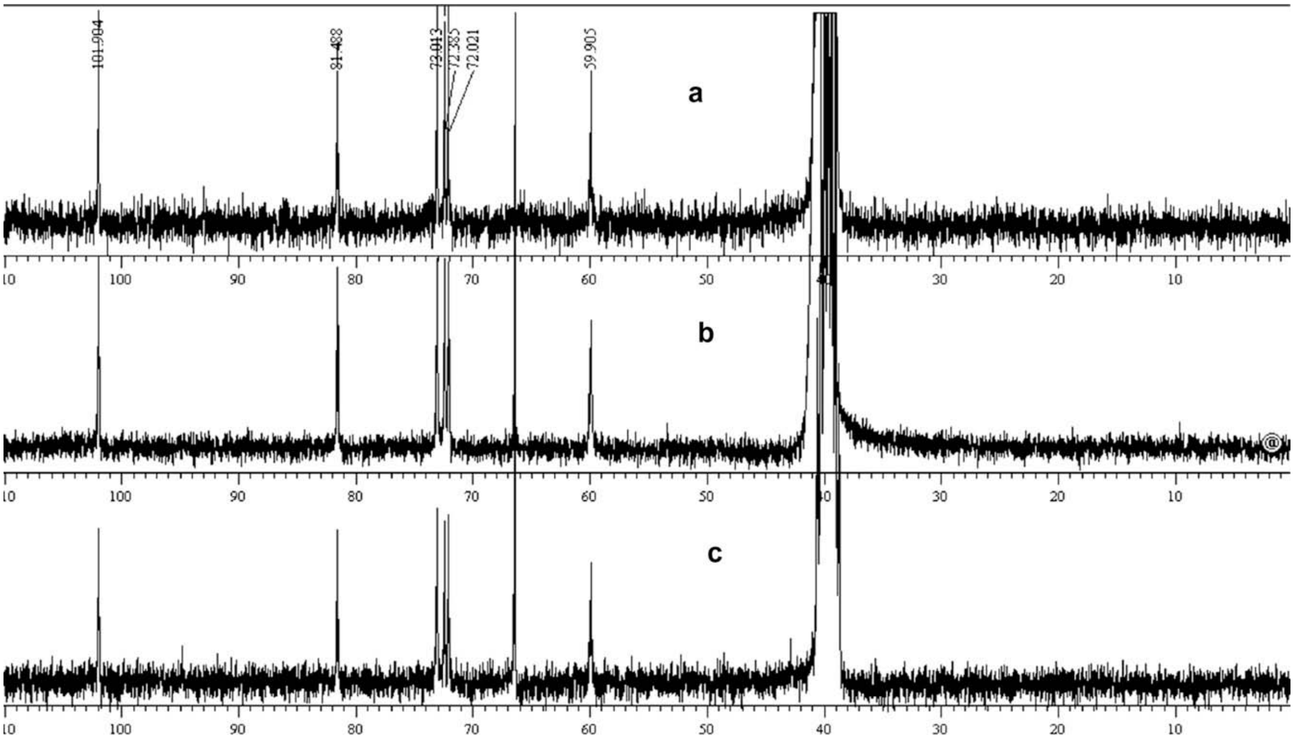

Fig. 5. (a) ^{13}C NMR of β-CD + VitD + Cu^{2+} 5:1:1, pH 3; (b) β-CD + VitD + Cu^{2+} 5:1:1, pH 7; (c) β-CD + VitD + Cu^{2+} 10:1:1, pH 3.

β-CD–VitD. Although the spectra are not well resolved due to the amorphous and not very homogeneous character of the obtained complexes, the differences in the wavelengths are enough to show that the supramolecular assembly was forming different complexes than β-CD alone and the same metal ion. The same kind of difference was observed in the systems β-CD and the supramo-

Table 4
Chemical shifts in ppm (^{13}C, DMSO-d_6, dioxane as internal reference) of β-CD complexes in the supramolecular ternary complexes with copper ions. Δδ means the mathematical differences among the chemical shifts of β-CD alone and the chemical shifts for the supramolecular assembly.

Complex	Ratio	pH	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)
β-CD + Cu^{2+}	5:1	3	101.888	72.002	73.014	81.493	72.382	59.899
Δδ – β-CD			0.015	0.017	0.020	0.018	0.027	0.025
β-CD + Cu^{2+}	10:1	3	101.887	71.999	73.008	81.491	72.373	59.875
Δδ – β-CD			0.014	0.014	0.014	0.016	0.018	0.001
β-CD + Cu^{2+}	5:1	7	101.904	72.018	73.027	81.508	72.384	59.902
Δδ – β-CD			0.031	0.033	0.033	0.033	0.029	0.028
β-CD + Cu^{2+}	10:1	7	101.904	72.019	73.012	81.504	72.382	59.889
Δδ – β-CD			0.031	0.034	0.018	0.029	0.027	0.015
β-CD + VitD + Cu^{2+}	5:1	3	101.904	72.021	73.013	81.488	72.385	59.905
Δδ – β-CD + VitD			−0.014	0.007	−0.016	−0.019	−0.012	0
β-CD + VitD + Cu^{2+}	5:1:1	7	101.885	72.000	73.013	81.491	72.354	59.901
Δδ – β-CD + VitD			−0.033	−0.014	−0.016	−0.016	−0.043	−0.004
β-CD + VitD + Cu^{2+}	10:1:1	3	101.885	72.017	73.013	81.492	72.385	59.887
Δδ – β-CD + VitD			−0.033	0.003	−0.016	−0.015	−0.012	−0.018

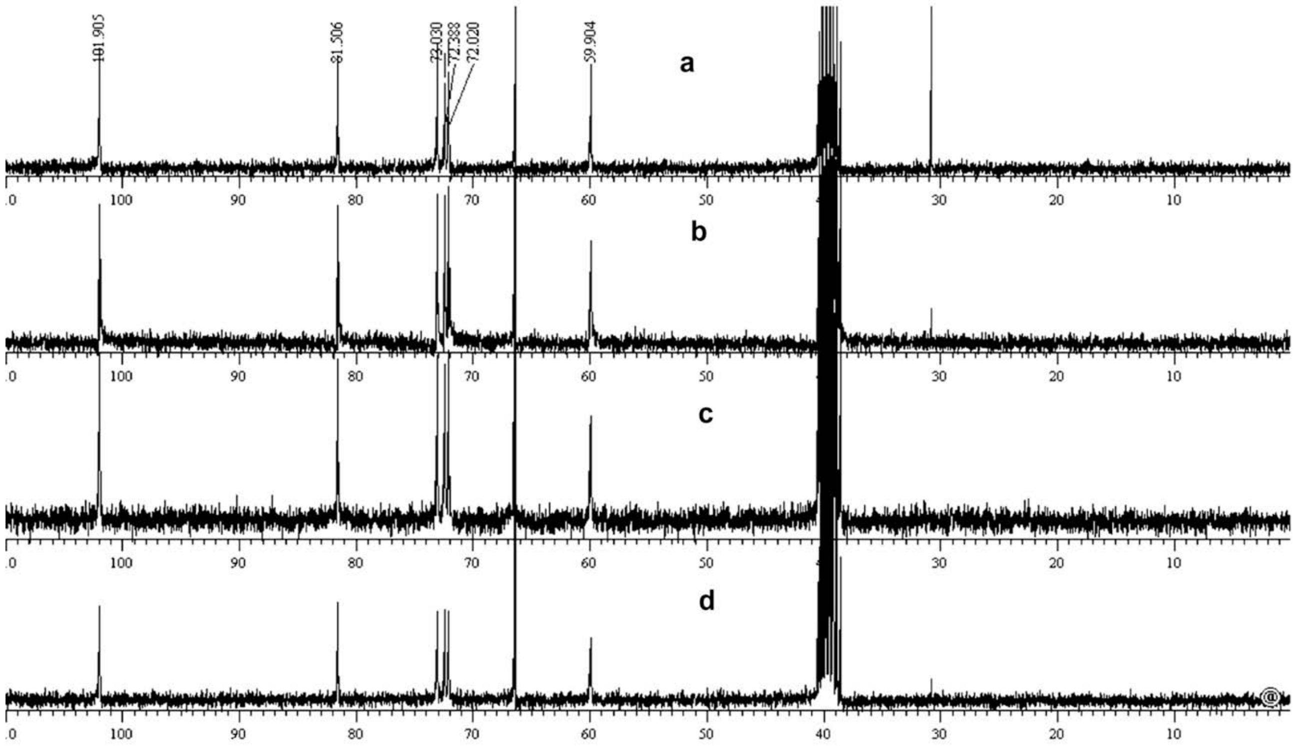

Fig. 6. (a) ^{13}C NMR of β-CD + Zn^{2+} 5:1 L:M, pH 7; (b) β-CD + Zn^{2+} 10:1 L:M, pH 7; (c) β-CD + VitD + Zn^{2+} 5:1 L:M, pH 7; (d) β-CD + VitD + Zn^{2+} 10:1 L:M, pH 7.

Table 5
Chemical shifts in ppm (^{13}C, DMSO-d_6, dioxane as internal reference) of β-CD complexes in the supramolecular ternary complexes with zinc ions. Δδ means the mathematical differences among the chemical shifts of β-CD alone and the chemical shifts for the supramolecular assembly.

Complex	Ratio	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)
β-CD + Zn^{2+}	5:1	101.905	72.020	73.03	81.506	72.388	59.904
Δδ – β-CD		−0.013	0.006	0.001	−0.001	−0.009	−0.001
β-CD + Zn^{2+}	10:1	101.888	72.017	73.014	81.494	72.371	59.887
Δδ – β-CD		−0.030	0.003	−0.015	−0.013	−0.026	−0.018
β-CD + VitD + Zn^{2+}	5:1:1	101.901	72.021	73.03	81.509	72.386	59.905
Δδ – β-CD + VitD		−0.017	0.007	0.001	0.002	−0.011	0
β-CD + VitD + Zn^{2+}	10:1:1	101.902	72.017	73.026	81.509	72.388	59.905
Δδ – β-CD + VitD		−0.016	0.003	−0.003	0.002	−0.009	0

lecular β-CD–VitD and the other two metal ions *e.g.* Cu^{II} and Zn^{II} (not shown in Fig. 7).

Another interesting feature was the complex β-CD–VitD–Co 10:1:1 ratio be a pink solid, showing that Co^{2+} is a six coordinate complex with also water as ligand. The diffuse reflectance and UV–visible spectrum showed transitions in the visible region very similar to all cobalt (II) complexes that presented themselves as blue solids (Fig. 7). However, all visible transitions seem to have

Table 6
Maximum absorptions (nm) for diffuse reflectance ultraviolet–visible spectroscopy for the solid samples of β-CD, VitD, binary and ternary assemblies.

Compound	UV-max 1	UV-max 2	d–d Transitions
β-CD	214 ± 1	–	–
VitD alone	–	328 ± 1	–
β-CD + VitD	216 ± 4	260 ± 4	–
β-CD + Co^{2+} 5:1	205 ± 3	–	536 ± 3
β-CD + Co^{2+} 10:1	210 ± 1	–	527 ± 3
β-CD + VitD + Co^{2+} 5:1:1	208 ± 4	269 ± 4	529 ± 2 628 ± 0
β-CD + VitD + Co^{2+} 10:1:1	208 ± 0	260 ± 2	600 ± 0
β-CD + Cu^{2+} + 5:1 pH 3	210 ± 0	278 ± 0	548 ± 1
β-CD + Cu^{2+} + 10:1 pH 3	212 ± 3	273 ± 6	557 ± 4
β-CD + Cu^{2+} + 5:1 pH 7	212 ± 0	264 ± 2	423 ± 6
β-CD + Cu^{2+} + 10:1 pH 7	212 ± 0	264 ± 2	483 ± 4
β-CD + VitD + Cu^{2+} – 5:1:1 pH 3	216 ± 2	268 ± 2	548 ± 9
β-CD + VitD – Cu^{2+} – 5:1:1 pH 7	207 ± 0	264 ± 0	530 ± 0
β-CD + VitD + Cu^{2+} – 10:1:1 pH 3	207 ± 3	266 ± 3	570 ± 2
β-CD + VitD + Cu^{2+} – 10:1:1 pH 7	207 ± 0	264 ± 0	530 ± 0
β-CD + Zn^{2+} 5:1	212 ± 0	–	–
β-CD + Zn^{2+} 10:1	207 ± 4	–	–
β-CD + VitD + Zn^{2+} 5:1:1	210 ± 9	257 ± 3	–
β-CD + VitD + Zn^{2+} 10:1:1	211 ± 2	260 ± 1	–

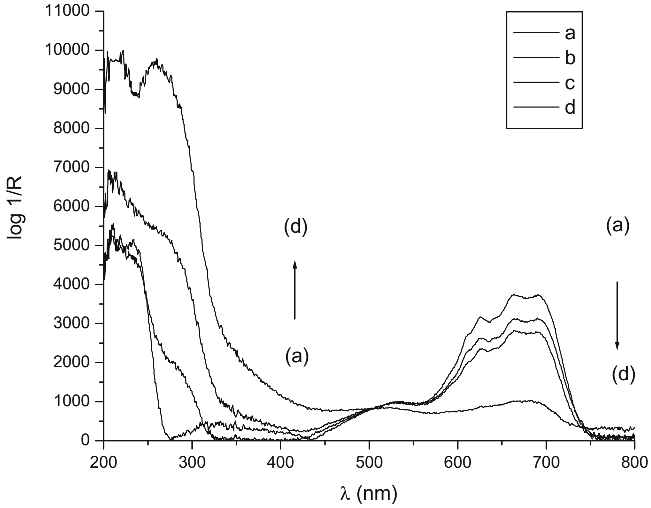

Fig. 7. Diffuse reflectance UV–vis of the samples containing cobalt (II): (a) β-CD + Co^{2+}, 5:1; (b) β-CD + Co^{2+}, 10:1; (c) β-CD + VitD + Co^{2+}, 5:1:1; (d) β-CD + VitD + Co^{2+}, 10:1:1.

disappeared in the pink complex (Fig. 7d). These visible transitions (625, 660 and 690 nm) are compatible with a four coordinate trigonal bipyramidal structure, and at 530 nm, the diffuse reflectance signal is derived from an octahedral structure (Lever, 1984). So the β-CD–VitD–Co (10:1:1 ratio) complexes presented almost only octahedral structure in the obtained complexes. On the other hand, the blue solids presented a great majority of trigonal bipyramidal structure. This feature, once again, showed the importance of the ratio between β-CD and VitD in the experimental step in order to obtain the inclusion compound (refer to Fig. 7d).

3.5. X-ray diffraction

Powder X-ray diffraction patterns afford information about the type of the cyclodextrin: cage, layer or channel type (Okumura, Kawaguchi, & Harada, 2001). The four diffraction patterns of ternary complexes given in Fig. 8 are close to those described for β-CD cage structure (Jiao, Goh, Valiyaveettil, & Zheng, 2003), with main peaks at *ca.* $2\theta = 10.8°$, 12.5°, 15.7° and 19.2°. It is has been shown that, in the case of β-CD inclusion complexes, the cyclodextrin turned into channel type and the number of diffraction peaks is lower than with pure β-CD (Jiao et al., 2003; Li & Yan, 2001; Yan-An et al., 2005). These results show that the crystallinity of β-cyclodextrin is the same no matter if it is alone in aqueous solutions, complexed to the metal ions Co^{2+}, Cu^{2+} and Zn^{2+} or when in the supramolecular assembly with VitD and complexed with the studied metal ions.

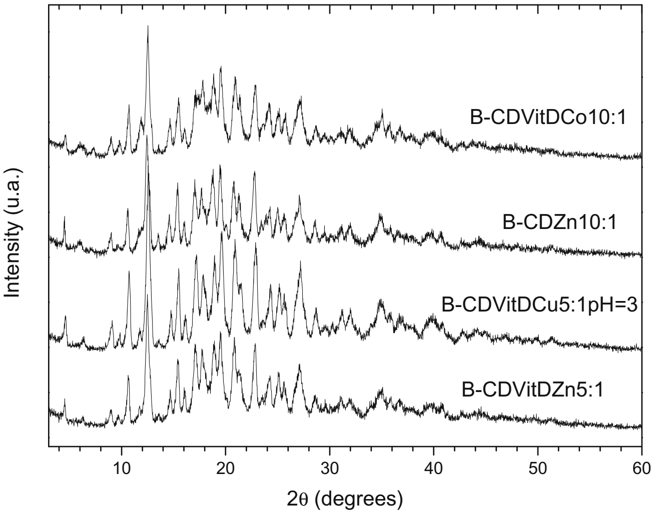

Fig. 8. X-ray diffraction spectra of complexes of β-CD and VitD and metal ions.

These results also show that the arrangement of pure β-CD (Saenger et al., 1998), is not maintained in the supramolecular structure formed in this work.

4. Conclusion

We have synthesized new ternary assemblies gathering β-cyclodextrin, vitamin D_3 (cholecalciferol) and three metal ions: Co(II), Cu(II) and Zn(II). Using ^{13}C NMR, UV–visible diffuse reflectance spectroscopy and powder X-ray diffraction pattern, we have shown that VitD gives metal complexes with these three metal and these complexes are included in the inner cavity of β-CD. These ternary assemblies could only be observed in our experimental conditions when the ratio β-CD:VitD:metal ion was 10:1:1 or 5:1:1 at pH 3 as well as pH 7.

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