



**SYNTHESIS AND STRUCTURAL CHARACTERIZATION OF GALLIUM (III) AND PALLADIUM (II) ON THE FORMATION OF TERNARY SUPRAMOLECULAR ASSEMBLIES.  
PART 1:  $\beta$ -CYCLODEXTRIN - CHOLECALCIFEROL**

**Chemical Science**

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**ABSTRACT**

$\beta$ -cyclodextrin ( $\beta$ CD) has been previously shown to encapsulate cholecalciferol (VitD) and the supramolecular structure, to present chelating abilities. In this work, spectroscopic parameters for the chelating power of supramolecular structures were investigated in the presence of  $Ga^{3+}$  and  $Pd^{2+}$ , two important metal ions especially in the medical science. The ternary obtained complexes, although presenting some degree of insolubility in many organic solvents and in different mixed proportions among them, could be studied by a number of analytical techniques. The binding constants for the complexes in a major aqueous solution were not possible to be determined by Potentiometric Titrations and the possible reasons are addressed. In the complexation, it was inferred that according to the host in the  $\beta$ CD structure and to the binded metal ion, the participating basic sites are different, consequently leading to a different spatial conformation and, in some cases, to a distortion in the 3D structure. Bragg's peak values 2 for all binary and ternary assemblies are given. Near infrared spectroscopy provided some important wavelengths for the differentiation of the metal complexes. The most probable deprotonated -OH groups mainly involved in the complexation in the ternary complexes were of C-3 from VitD and of C-6, C-3 and C-2 from  $\beta$ CD. The analytical techniques of  $^{13}C$  NMR, powder XRD and UV-NIR spectroscopies, characterized the binary and the ternary supramolecular structures.

**KEYWORDS**

Supramolecular assemblies,  $\beta$ -cyclodextrin-cholecalciferol, Gallium, Palladium, ternary complex, XRD,  $^{13}C$  NMR

**1. Introduction**

Beta-cyclodextrins ( $\beta$ CD) has been inspected in many works<sup>1</sup> in the literature as a capable of forming supramolecular structure compound and also of forming ternary complexes with the further complexation with metal ions<sup>2,3</sup>.

Ternary assemblies are more difficult to find in the literature especially with metal ions related to medical applications. Although presenting some toxicity, according to the literature<sup>4,5</sup>, Gallium (III) is employed from the semiconductor industries to the medical applications especially in clinical medicine. Radioactive gallium and gallium nitrate are used as diagnostic and therapeutic agents in cancer and disorders of calcium and bone metabolism as well as imaging agents used in nuclear medicine.<sup>4,6</sup>

It has also been shown some results of gallium compounds presenting some anti-inflammatory and immunosuppressive activity in animal models of human disease. Other studies have shown that gallium compounds may function as antimicrobial agents against certain pathogens.<sup>4</sup>

Palladium (II) has many applications from implantable medical devices as in dentistry for instance<sup>7</sup>, as anticancer drugs<sup>8,9</sup> to catalyst in the pharmaceutical<sup>10</sup> and fine chemical industries<sup>11</sup> to cite a few.

Supramolecular binary and ternary structures involving  $\beta$ CD and cholecalciferol, vitamin D<sub>3</sub> (VitD), were previously studied with some metal ions<sup>1,2</sup> but to our knowledge, no further studies were conducted in the literature so far.

The supramolecular assemblies obtained by encapsulating VitD in  $\beta$ CD made possible to obtain pro-drugs able to deliver VitD whenever needed as well as the metal ion complexed in the assembly. Because of VitD's high lipid solubility and due to its toxicity effect when ingested in large quantities, as an encapsulated form, especially in a non toxic host like  $\beta$ CD, it can be of a very valuable pharmaceutical drug.<sup>12,13</sup>

Therefore, the synthesis and characterization of ternary compounds of this inclusion compound chelated with  $Ga^{3+}$  or  $Pd^{2+}$  for the delivery of this whole system together to specific target organs with the metal ion, was the aim of this work. Analytical tools were employed for the characterization of the complexes in the solid state ( $^{13}C$  Nuclear Magnetic Resonance, powder X-Ray Diffraction, Ultraviolet-Near Infrared) and Potentiometric Titrations, to measure the binding constants for the binary and ternary systems in water/DMSO solutions.

**2. Experimental****2.1. Materials**

All reagents are analytical grade and used as received.  $\beta$ -cyclodextrin ( $\beta$ CD) and palladium nitrate salt were purchased from Sigma, USA; cholecalciferol (Vitamin D<sub>3</sub> - VitD) from Acros Organics, France. Gallium (III) in  $HNO_3$ , ICP standard solution, ethanol absolute and  $KNO_3$  were purchased from Merck, Germany. Gallium (III) standard and Palladium (II) acidic water solutions (HCl, Merck - Germany) were standardized by AAS, SPECTRAA model 220FS, air - acetylene). DMSO- $d_6$  was purchased from Cambridge Isotope Laboratories, Inc., USA.

The total acid concentration of both Pd(II) and Ga(III) aqueous solutions were measured by Gran's plot. Cholecalciferol, always dissolved in absolute ethanol, was manipulated in glove bags under  $N_2$  (White Martins, Brazil) atmosphere using Schlenk glasses and proper procedures. All water employed in the solutions and in the experiments was double - distilled in a quartz bidistillator (Fisatom, Brazil) and deionized in cationic exchange columns and freshly boiled for degassing. All titration experiments were made in triplicate. Standardized KOH (Merck - Germany) 0.1 mol/L was the titrant (0.02  $\pm$  0.01 mL) and  $KNO_3$  or KCl (Merck - Germany) used to maintain the ionic strength set at 0.100 mol/L. All titrations were made in 10%v/v DMSO (Vetec, Brazil) and 90% water, in which  $pK_a$  is still equal to 10.78<sup>14</sup>.

**2.2. Apparatus and procedure for Synthesis**

Binary and ternary systems were synthesized according to the literature<sup>2,3</sup> in pH values of 4.5 - 5.5 in ligand:metal ratios of 5:1 and 10:1. All solid materials were completely removed from the solvent under vacuum. The obtained solid was kept under  $N_2$  in sealed small Eppendorf flasks. The supramolecular assembly and the ternary complexes were obtained in 5:1:1 and 10:1:1 molar ratios of  $\beta$ CD:VitD:metal ion, respectively.<sup>2,3</sup>

**2.3. Physical measurements**

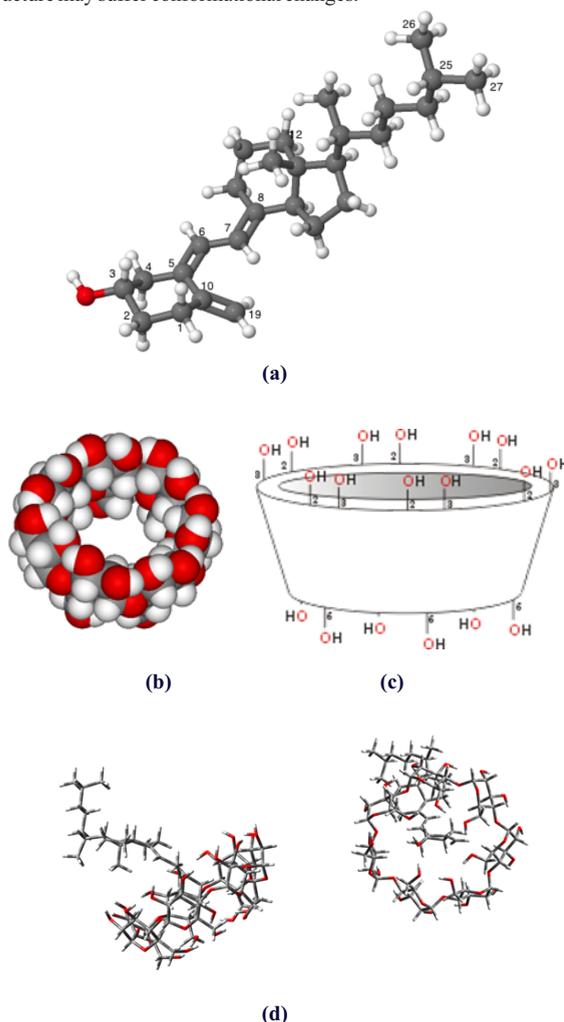
Electronic absorption spectra in the 200-2000 nm range were recorded on a Perkin-Elmer Lambda 750 spectrophotometer in quartz cuvettes of 1 mL in DMSO.  $^{13}C$  NMR were recorded in a Bruker AC-200 NMR spectrometer, operated at 60 MHz, DMSO- $d_6$  as solvent and recording the chemical shifts using this same solvent as internal standard (= 39.51 ppm). Mestrenova® was the employed software. D2 Phaser X-ray diffraction Bruker instrument using  $Cu-K_{\alpha}$  ( $\lambda=1.5406 \text{ \AA}$ ) radiation

collected the data for the powder samples in the  $2\theta$  ranges of 0 to  $50^\circ$  with a step of  $0.06^\circ$  with a 2 sec collection time.

Potentiometric titrations were made in a Metrohm automatic titrator model Titrando 806 (Switzerland) software Tiamo<sup>®</sup> with  $H^+$  and  $Ag/AgCl$  electrodes calibrated with 3 fresh buffer solutions (4.01; 7.00 and 10.01 Orion, USA) prior to each experiment. A proper mass of the ligand was dissolved in 10%v/v DMSO till its complete solubilization and after this time, the other reagents were added to 90%v/v water. The  $pK_w$  employed was 13.78.  $KNO_3$  or  $KCl$  (for all titrations with  $Pd^{2+}$ ) proper masses were added to adjust the ionic strength to 0.100 mol/L in a final volume in the reaction vessel of 50.0 mL, thermostated by a circulating water bath to  $298.15 \pm 0.10$  K (MQBTC 99-22, Microquímica, Brazil). The  $pK_{w,DMSO}$  employed in the calculations was 13.78<sup>14</sup>. The stability constants were calculated using Hyperquad<sup>15</sup> and the species diagram, HYSS<sup>16</sup> computer programs.

### 3. Results and discussion

In Fig. 1(a) it is possible to see a spatial representation of VitD and of  $\beta$ CD in (b) and (c). The supramolecular assembly for  $\beta$ CDVitD is represented in Fig. 1(d) in two different perspectives where, taking among other parameters into account, the width of the structure of VitD and the cavity size of  $\beta$ CD, the optimized structure was obtained. This structure is maintained by hydrogen bonding or van der Waals electrostatic interactions. However, when a metal ion is present in the equilibrium, complexation (or coordination) is obtained, and the structure may suffer conformational changes.



**Fig. 1:** (a) 3D sticks structure of cholecalciferol (Vitamin D3 - VitD) average maximum width - 16.95 Å; average minimum width 6.06 Å from C8 to C27 not counting C10 and C19<sup>2</sup>; and of (b)  $\beta$ CD inner cavity - 6.0 - 6.5 Å.<sup>17</sup> (Structure reproduction a courtesy of JMOL development team) and (c) numbering of -OH in the C atoms of  $\beta$ CD; (d) optimized  $\beta$ CDVitD supramolecular assembly in different perspectives, employing the Gaussview software<sup>18</sup>.

#### 3.1. <sup>13</sup>C NMR

The complete numbering of the structure of either  $\beta$ CD or VitD is reported in the literature, (also in Fig. 1 (a)), as well as the <sup>13</sup>C chemical shifts for  $\beta$ CD and VitD and  $\beta$ CDVitD.<sup>2</sup> Different instruments and slightly different experimental conditions change the chemical shift values in NMR determinations, so Table 1 presents the <sup>13</sup>C NMR data for  $\beta$ CD and Table 2, for  $\beta$ CDVitD for the sake of further comparison. Values for the difference among the chemical shifts of pure and complexed or encapsulated assemblies were considered significant in the range of 0.01 ppm since the binding forces involved in the supramolecular structures are weak, like van der Waals and hydrogen bonds.<sup>19</sup>

Table 1 presents the chemical shifts for  $\beta$ CD alone and complexed with Pd(II). Taking a look at the values of the chemical shifts and the differences among the values ( $\Delta\delta$ ) it is seen that the complexation deshielded the host, being the complex rate of 5:1 the most deshielded, hence forming the most stable structure. The most affected C atoms in the Pd(II)  $\beta$ CD complexes are for the ratio 5:1, C-2 > C-6 > C-3, and for the ratio 10:1, C-6 > C-3 > C-2, although all 6 carbons were affected in both ratios.

The initial shielding effect presented in  $\beta$ CDVitD structure when comparing the chemical shifts for  $\beta$ CD alone confirms the proposed structure in Fig. 1(d), in which C-3 portion from cholecalciferol is inside  $\beta$ CD cavity. Table 2 shows the chemical shifts for the supramolecular assembly  $\beta$ CDVitD and Pd(II). The ternary systems with the metal ion  $Pd^{2+}$  showed a deshielding effect not so pronounced as the complexes with  $\beta$ CD and  $Pd^{2+}$  presented, but nonetheless, presenting significative numbers. The order in the  $\Delta\delta$  values being C-6 > C-3 > C-5 in the ratio 5:1:1 and C-6 > C-3 > C-4 in the ternary structure in the ratio 10:1:1. As C-6 and C-3 -OH groups are pointing outside the  $\beta$ CD cavity, although in opposite direction, they are the most affected, probably because they are the preferred binding sites when deprotonated, for  $Pd^{2+}$  in the supramolecular assembly. C-5 is also affected due to the 3-D arrangement in the complexation through C-3 and C-6 deprotonated -OH groups. On the other hand, the internal C-4 are also affected due to the complexation through the -OH groups of  $\beta$ CD and because C-3 -O<sup>-</sup> from VitD is probably also involved in the complexation with  $Pd^{2+}$ . Since this VitD molecule is inside  $\beta$ CD's cavity, once a complexation is established in the former molecule, the whole assembly is affected.

**Table 1:** Chemical shifts ( $\delta$ ) of <sup>13</sup>C NMR of  $\beta$ CD and  $\beta$ CD Pd(II) complexes and comparative  $\Delta\delta$  values.

	C1	C2	C3	C4	C5	C6
$\beta$ CD (a)	102.188	72.614	73.308	81.766	72.283	60.190
$\beta$ CD_Pd (b) 5:1	102.324	72.779	73.467	81.917	72.431	60.352
$\beta$ CD_Pd (c) 10:1	102.270	72.715	73.436	81.862	72.382	60.330
(a) - (b) = $\Delta\delta$ 5:1	-0.136	-0.165	-0.159	-0.151	-0.148	-0.162
(a) - (c) = $\Delta\delta$ 10:1	-0.082	-0.101	-0.128	-0.096	-0.099	-0.140

**Table 2:** Chemical shifts ( $\delta$ ) of <sup>13</sup>C NMR of  $\beta$ CD,  $\beta$ CDVitD and  $\beta$ CDVitD Pd(II) complexes and comparative  $\Delta\delta$  values.

	C1	C2	C3	C4	C5	C6
$\beta$ CD (a)	102.188	72.614	73.308	81.766	72.283	60.190
$\beta$ CDVitD (b) 5:1	102.105	72.561	73.223	81.689	72.198	60.095
$\beta$ CDVitD_Pd (c) 5:1:1	102.151	72.619	73.303	81.745	72.260	60.178
$\beta$ CDVitD_Pd (d) 10:1:1	102.180	72.632	73.327	81.776	72.280	60.206
(a) - (b) = $\Delta\delta$	0.083	0.053	0.085	0.077	0.085	0.095
(b) - (c) = $\Delta\delta$	-0.046	-0.058	-0.080	-0.056	-0.062	-0.083
(b) - (d) = $\Delta\delta$	-0.075	-0.071	-0.104	-0.087	-0.082	-0.111

**Table 3:** Chemical shifts ( $\delta$ ) of <sup>13</sup>C NMR of  $\beta$ CD and  $\beta$ CD Ga(III) complexes and comparative  $\Delta\delta$  values.

	C1	C2	C3	C4	C5	C6
$\beta$ CD (a)	102.188	72.614	73.308	81.766	72.283	60.190
$\beta$ CD_Ga (b) 5:1	102.121	72.548	73.293	81.697	72.225	60.155
$\beta$ CD_Ga (c) 10:1	102.250	72.642	73.425	81.803	72.347	60.265
(a) - (b) = $\Delta\delta$ 5:1	0.067	0.066	0.015	0.069	0.058	0.035
(a) - (c) = $\Delta\delta$ 10:1	-0.062	-0.028	-0.117	-0.037	-0.064	-0.075

**Table 4:** Chemical shifts ( $\delta$ ) of  $^{13}\text{C}$  NMR of  $\beta\text{CD}$ ,  $\beta\text{CDVitD}$  and  $\beta\text{CD}_2\text{VitD}$  Ga(III) complexes and comparative  $\Delta\delta$  values.

	C1	C2	C3	C4	C5	C6
$\beta\text{CD}$ (a)	102.188	72.614	73.308	81.766	72.283	60.190
$\beta\text{CDVitD}$ (b) 5:1	102.105	72.561	73.223	81.689	72.198	60.095
$\beta\text{CDVitD}$ Ga (c) 5:1:1	102.269	72.680	73.405	81.844	72.362	60.282
$\beta\text{CD}_2\text{VitD}$ Ga (d) 10:1:1	102.157	72.580	73.321	81.704	72.246	60.154
(a) - (b) = $\Delta\delta$	0.083	0.053	0.085	0.077	0.085	0.095
(b) - (c) = $\Delta\delta$	-0.164	-0.119	-0.182	-0.155	-0.164	-0.187
(b) - (d) = $\Delta\delta$	-0.052	-0.019	-0.098	-0.015	-0.048	-0.059

From Table 3, it is seen that for the same complexes with  $\beta\text{CD}$  and  $\text{Ga}^{3+}$ , when comparing the chemical shifts with those with  $\text{Pd}^{2+}$  (refer to Table 1), this effect is different in the former complexes, showing that  $\text{Ga}^{3+}$  binds differently from  $\text{Pd}^{2+}$  with  $\beta\text{CD}$ . Even different  $\text{Ga}^{3+}$  concentrations in the assemblies suggest different kinds of complexation. In the ligand to metal ratio of 5:1, the Ga(III) complexes are shielded when compared to  $\beta\text{CD}$  alone. On the contrary, in the ligand to metal ratio of 10:1, there is a deshielding effect. Somehow, when  $\text{Ga}^{3+}$  is in higher concentrations in the solution, the complexed generated in the solid state, is less stable than when there is less  $\text{Ga}^{3+}$  in the same experimental conditions. So much so, that the more influenced carbon atoms in the 5:1 ratio are  $\text{C4} > \text{C1} > \text{C2} > \text{C5} > \text{C6} > \text{C3}$  (shielding effect), showing the contrary order to the deshielding effect in the 10:1 ratio,  $\text{C3} > \text{C6} > \text{C5}$ . The  $\text{Ga}^{3+}$  concentration in the synthesis method<sup>2</sup> influenced the final ternary metal complexes obtained. The less  $\text{Ga}^{3+}$  in solution, the strongest the complexes obtained, following the experimental conditions in this work. The existence of a limiting value for this trend was not tested in this work.

On the contrary, when the ternary assembly is concerned (refer to Table 4), the deshielding effect is the highest for the supramolecular assemblies of  $\beta\text{CDVitD}$  and  $\text{Ga}^{3+}$ . It is also seen from Table 4 that in the ternary assembly with  $\text{Ga}^{3+}$  both proportions showed a deshielding effect, being the most affected  $\text{C-6} > \text{C-3} > \text{C1} = \text{C5}$  for the 5:1:1 ratio, and for the 10:1:1 proportion,  $\text{C-3} > \text{C-6} > \text{C-1}$ . When  $\text{Ga}^{3+}$  complexes with the supramolecular assembly, the binding driving force is almost the same no matter the ligand to metal proportion employed in the synthesis. The difference seen in the  $\Delta\delta$  comparative values in Table 4 shows that the deprotonated  $-\text{OH}$  groups of C-3 and C-6 are the preferred sites for the complexation of both metal ions studied.

For the sake of comparison, in fact, shielding and deshielding effects were observed in  $\beta\text{CD}$  and  $\beta\text{CDVitD}$  and  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Al}^{3+}$  previous results. For the supramolecular ternary assembly of  $\beta\text{CDVitD}$ , deshielding effects were mainly observed with  $\text{Co}^{2+}$  and  $\text{Pd}^{2+}$ , both shielding and deshielding values for C atoms in the complexes with  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  as well as for  $\text{Ga}^{3+}$ , and shielding effects for  $\text{Al}^{3+}$  complexes.<sup>2,3</sup> So, no trend can be assigned to the values obtained for the metal ions studied, showing that this ternary assembly is influenced by each particular metal ion studied so far. No correlation can be established based neither on Pearson's hard acid and base theory nor with the oxidation numbers of the metal ions studied,<sup>2,3</sup> suggesting the ternary assembly  $\beta\text{CDVitD}$ , as a very unique binding compound.

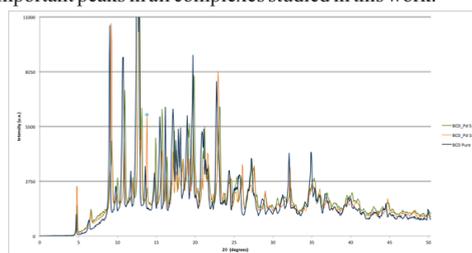
### 3.2. Powder x-ray diffraction (XRD)

The powder XRD analytical tool provides the crystallinity of a compound including supramolecular assemblies like  $\beta\text{CDVitD}$ . It was used then to assess the differences in the crystallinity by the inclusion of VitD in  $\beta\text{CD}$  and in the complexation of the metal ions chosen in this work.

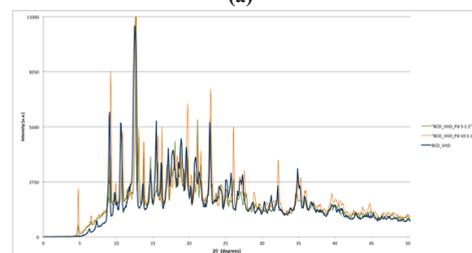
In Fig. 2(a),  $\beta\text{CD}$  diffractogram is depicted along with the two ligand to metal ratios for Pd(II). It is seen that the binding of the metal ion to  $\beta\text{CD}$  does not alter much the crystallinity of  $\beta\text{CD}$  alone. That is not the case in Fig. 3(a) where it can be seen a greater change in the crystallinity pattern of  $\beta\text{CD}$ , when complexed to the metal ion Ga(III), for the two ligand to metal ratios studied. Those results are indications that the binding of  $\beta\text{CD}$  is better when Ga(III) is the Lewis acid present when compared to Pd(II).

In Fig. 2(b), when the proportion of Pd(II) is the least studied, there is an improvement in the crystallinity of the supramolecular assembly structure when Pd(II) is the complexed metal ion ( $\beta\text{CDVitD}$ , Fig. 2(c)), whereas in the 5:1:1 Pd(II) complex proportion, the crystallinity of  $\beta\text{CDVitD}$  is decreased. The same effect in the crystallinity of  $\beta\text{CDVitD}$  is observed when Ga(III) is the complexed metal ion (Fig. 3(b)). The XRD results for the ternary supramolecular assemblies showed that the concentration of both the metal ions studied are important in

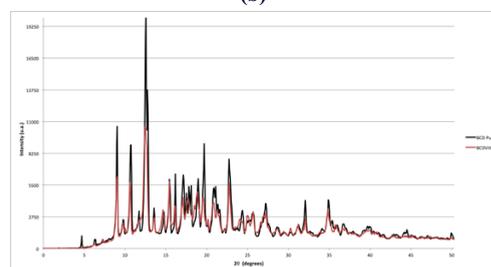
improving the crystallinity through better arrangements of the final obtained structure. The crystallinity of  $\beta\text{CD}$  is maintained at a certain degree when VitD is encapsulated in it (Fig. 2(c)) in accordance with the structure proposition in Fig. 1(d). Table 5 gives the 2 values for the most important peaks in all complexes studied in this work.



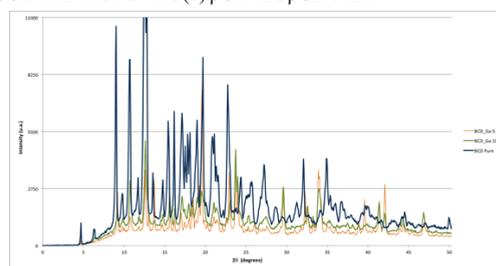
(a)



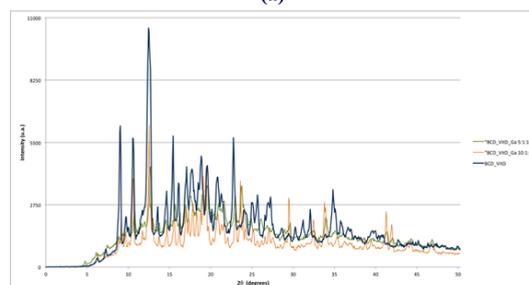
(b)



(c)

**Fig. 2:** X-Ray diffraction of (a)  $\beta\text{CD}$  and  $\beta\text{CD}$  with  $\text{Pd}^{2+}$ ; (b)  $\beta\text{CDVitD}$  and  $\beta\text{CDVitD}$  and  $\text{Pd}^{2+}$  and (c)  $\beta\text{CD}$  and  $\beta\text{CDVitD}$ .

(a)



(b)

**Fig. 3:** X-Ray diffraction of (a)  $\beta\text{CD}$  and  $\beta\text{CD}$  with  $\text{Ga}^{3+}$  and of (b)  $\beta\text{CDVitD}$  and  $\beta\text{CDVitD}$  and  $\text{Ga}^{3+}$ .

This is in agreement with the  $^{13}\text{C}$  NMR results that showed that  $\beta\text{CDPd}$  is a more stable structure than with  $\text{Ga}^{3+}$ , as the loss of crystallinity by complexing with the latter, may imply a deformation in the 3D structure of  $\beta\text{CD}$ .<sup>21-24</sup>

**Table 5:** Main peaks (2 $\theta$ ) obtained by powder XRD of the studied ligands and of the binary and ternary metal complexes in the two different ligand:metal ratios studied.

$\beta$ CD (Lit.)	$\beta$ CDPd 5:1	$\beta$ CDPd 10:1	$\beta$ CDGa 5:1	$\beta$ CDGa 10:1	$\beta$ CDVitD 5:1	$\beta$ CDVitDPd 5:1:1	$\beta$ CDVitDPd 10:1:1	$\beta$ CDVitDGa 5:1:1	$\beta$ CDVitDGa 10:1:1
4.67	4.73	4.67	4.61			4.61	4.73		
6.19	6.43	6.43			6.01		6.43		6.25
					7.16				
8.98 (9.06)	9.16	9.10	9.10	9.10	8.98	9.10	9.10		9.10
10.62	10.80	10.74	10.68	10.68	10.50	10.68	10.74	10.68	10.50
11.65	11.77	11.77		11.71		11.77	11.77		11.65
12.44 (12.62)	12.62	12.56	12.56	12.56	12.38	12.56	12.50	12.54	12.44
12.69	12.99	12.93	12.68			12.99	12.93		12.87
14.69	14.75	14.63		14.69	14.57	14.57	14.57	14.44	14.63
15.36	15.60	15.54	15.60	15.54	15.36	15.60	15.60	15.58	15.48
16.08	16.21	16.08	16.08	16.14	16.02	16.08	16.14	16.19	16.08
			16.93			16.87		16.87	16.93
17.06	17.12	17.00		17.06	17.00		17.00		
17.72	17.84		17.91	17.84	17.54	17.91	17.84	17.40	17.72
18.03	18.21	18.21	18.21	18.21	18.76	18.63	18.21	18.21	18.21
18.88		18.82	18.45				18.70	18.82	
			19.12	19.06		19.12			19.00
19.60	19.67	19.60	19.48	19.42	19.42	19.60	19.67	19.46	19.36
								20.80	20.76
	21.06	21.06			21.18	21.00	21.00	21.41	
22.64 (22.54)	22.94	22.76	22.82	22.94	22.64	22.70	22.76	22.82	22.88
			23.61	23.61				23.59	23.49
			23.92	23.67					
24.22					24.82			24.76	
		25.86	25.86		25.55	25.86	25.92	25.90	25.01
									26.89
27.07 (26.96)		27.38			27.13		27.38		
					28.34				
			29.50	29.44	30.05			29.42	29.32
31.93 (31.91)		31.99	32.11	32.35	31.93	31.93	31.99		32.30
			32.41						32.96
			33.08	33.08					
			33.75						
			33.81	33.81				33.75	
34.72 (34.70)					34.66		34.66		
		35.75			35.57		35.02		35.08
36.48									
			38.67						38.54
			39.40						39.27
			41.21	41.16				41.07	41.10
			41.88	41.82					41.76
		44.49	44.19	44.13					
			46.62	46.62				46.44	46.44*

(Lit.)= reference 20

\* = specific peaks presented only in the metal complexes determined in this work

### 3.3. UV-NIR

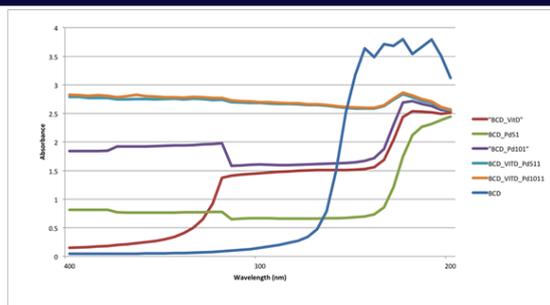
The ultraviolet and near infrared spectra (UV-NIR) were gathered in three different regions: from 200 to 400 nm, 400 to 700 nm and 700 to 2000nm, according to the different employed lamp to provide the different wavelengths. NIR was chosen because of the very little data in the literature for  $\beta$ CD and its complexes. All obtained spectra in the visible region of 400 to 700 nm did not show any important event, as expected. Figs. 4 and 5 show the UV-NIR spectra of  $\beta$ CD and  $\beta$ CDVitD and of the binary and ternary systems with Pd<sup>2+</sup> (Fig. 4) and Ga<sup>3+</sup> (Fig. 5).

In Figs. 4 and 5(a), the UV region of the spectra shows the charge transfer absorption band of  $\beta$ CD alone as a large band from 200 to 270 nm which is much less extensive and intense in Pd<sup>2+</sup> complexes (from 200 to 240 nm - Fig. 4(a)), while for Ga<sup>3+</sup> complexes, they are displaced to a region of higher wavelengths, starting from 270 nm till 340 nm - bathochromic shift). The band around 1200 nm in the spectra of  $\beta$ CD alone, assigned to -CH<sub>2</sub> groups disappears in all metal complexes. Also taking a look at the spectrum of  $\beta$ CD in Figs. 4 and 5 in the region around 1400 nm, it is seen the assigned 2v to all types of not bonded -OH bands. Following this same region for the obtained complexes, it is seen that these bands are displaced towards a higher wavelength for the two metals studied, between 1450 to 1600 nm, a second bathochromic shift. Band displacement, disappearance or changing in intensity are clear indications of complexation of the metal ion in both binary and ternary systems.<sup>21,25-26</sup>

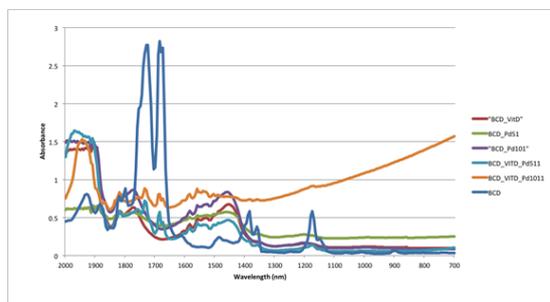
Overtone in the NIR for C-H aliphatic groups are: The first at 1800 to 1700 nm; the second at 1210 to 1150 nm and the third at 915 to 857 nm<sup>27</sup>. All three can be seen in the spectra of  $\beta$ CD alone (Fig. 4(b)). The very existence of overtones requires vibrational anharmonicities, and their intensities increase with increased anharmonicity. The overtone absorptions get gradually less intense as the number of the set gets higher, as expected for this set of absorptions. As the third overtone is the less intense, when there is the host in  $\beta$ CD or there are already binded the host and metal ions, all these sets of overtones either become very less intense or either disappear. The second overtone, at 1210 to 1150 nm, in the complexed systems, has also decreased visibly. But no doubt is to be raised when considering the first aliphatic C-H overtone at 1800 to 1700 nm. When looking at the  $\beta$ CDVitD spectrum, in Fig. 4(b), all set of overtones have almost disappeared and in Fig. 4(c), the overtone in 1800 to 1700 nm for this supramolecular binary assembly, has completely disappeared.

When the metal ions are analyzed in the system, (Fig. 5(b) and (c)) it seems that when the 3+ metal ion is in low concentration, it presents a more stable 3D structure with VitD in the supramolecular structure, as its spectra in 1800 - 1700 nm shows more the profile of  $\beta$ CD alone. On the other hand, that seems not to be the case when the metal ion is the 2+, Pd, once in Fig. 4(c), it can be seen that when the ternary system is analyzed in both studied ratios, the overtone at 1800 - 1700 nm disappears completely, as when the supramolecular system presented itself undisturbed by a third element (the metal ions).

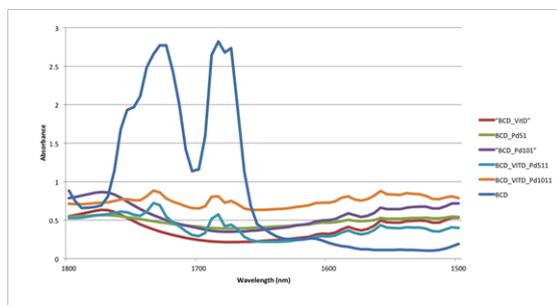
The low solubility of the assembly  $\beta$ CDVitD originated the descendent line in high absorptions seen in Fig. 4 and 5(b), from 700 to 1400 nm. Also, the noisy part of the IR spectra in Fig. 5(b) above 1900 nm is also due to low solubility especially for Ga<sup>3+</sup> ternary complex.



(a)

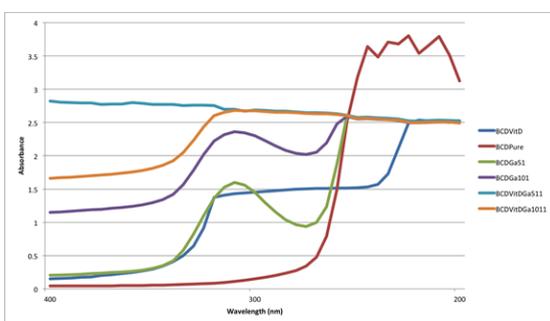


(b)

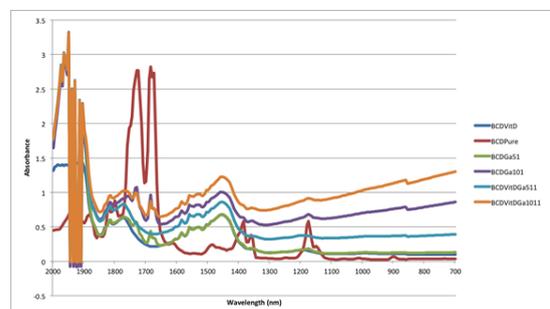


(c)

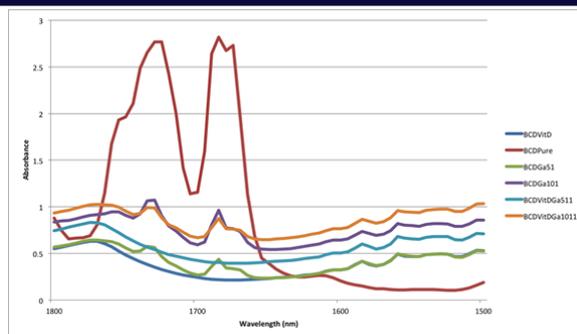
**Fig. 4:** UV (a) – NIR (b) region spectra of  $\beta$ CD and  $\beta$ CDVitD and the complexes with  $\text{Pd}^{2+}$  in different ligand to metal ratios and (c) Detailed region of the occurrence of the first C-H overtone in  $\beta$ CD.



(a)



(b)



(c)

**Fig. 5:** UV (a) – NIR (b) region spectra of  $\beta$ CD and  $\beta$ CDVitD and the complexes with  $\text{Ga}^{3+}$  in different ligand to metal ratios; (c) Detailed region of the occurrence of the first C-H overtone in  $\beta$ CD.

### 3.4. Potentiometric titration

Potentiometric titration experiments were tirelessly conducted but no result came from the effort. The mathematical model was made including the use of the protonation constants of  $\beta$ CD<sup>14</sup> and the hydrolysis constants for both metal ions<sup>28</sup> and Cl<sup>-</sup> association constants (when  $\text{Pd}^{2+}$  was the metal ion titrated). All complexed species tried in the mathematical models did not alter the pH profiles and in the end, the only constants needed to fit the experimental and the calculated curves were the protonation constants of the ligand used. The high water insolubility of the supramolecular assembly, on one hand rendered it possible to be synthesized and isolated from aqueous solution, but on the other hand prevented the detection in solution of the complexed species even in the tried system composed of water 90%v/v, 10%v/v DMSO.

The very small number of binding constants published in the literature so far for cyclodextrin inclusion complexes comprising electrochemistry methods not in the presence of any metal ion, are reported elsewhere<sup>29,30</sup> and in the presence of metal ions, in other papers including reviews<sup>31-32</sup>. So, it is important to know that to determine the stability constants of these kinds of complexes in water systems, one has to assay the solubility of the final supramolecular assembly and the chemical nature of the metal ions employed in the formation of the metal complexes.

## 4. CONCLUSIONS

Ternary supramolecular assemblies of  $\beta$ CDVitD and the metal ions  $\text{Pd}^{2+}$  and  $\text{Ga}^{3+}$  were obtained in the synthesis employed in this work, and precisely because they presented low or no solubility in water, it can be a promising slow-release Pd(II) or Ga(III) nanopharmaceutical for specific targets in the body. Due to the lipophilicity of VitD, its insolubility in water is an advantage to prevent the toxicity of this vitamin in the blood. When encapsulated in  $\beta$ CD, this supramolecular assembly, comprising the metal ions, can more safely reach the target organs in a body taking the metal ion in question with them.

The best solvent to deal with the complexes was DMSO. In the solution experiments and for the Potentiometric Titrations, DMSO 10% and 90% v/v water was the best solvent, although not obtaining a satisfactory solubility for the detection of the species to the calculation of the binding constants in the ternary systems studied.

The <sup>13</sup>C NMR analytical tool continues to be the most precise to identify the formation of supramolecular assemblies and in the identification of ternary compounds. It could also be inferred that the C atoms which were the most affected in the host-guest structure followed the trend: C-6, C-3 and C-2 from  $\beta$ CD and C-3 from VitD. Also the ligands to metal ratio, mainly the concentration of the metal ion, influenced the complexed species obtained. So, the proposed structure for the binary supramolecular assembly  $\beta$ CDVitD in Fig. 1 (d) is in agreement with the spectroscopic data obtained.

The obtained molecular spectra of the structures confirmed the formation of the binary and the ternary complexes and also confirmed the suggestion of the complexation sites by <sup>13</sup>C NMR, for the ternary compounds.

The loss or gain in crystallinity of a certain structure can also say about

some characteristics of the assembly, showing that  $\beta$ CD has its preferences when binding to certain metal ions and this can be changed when  $\beta$ CD is hosting a different guest. This work showed that parameters such as pH, concentration of the metal ion and liposolubility are the ones which this kind of complexes are dependent as drug delivery assemblies<sup>33-37</sup> and it is not easy to make predictions based only on previously known theories in this particular cases.

Other encapsulated products comprising molecules of composed amino acids are being synthesized along with these two studied metal ions in order to elucidate the results in different supramolecular systems.

## Notes

The authors declare no competing financial interest.

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