PART 1: β- CYCLODEXTRIN - CHOLECALCIFEROL

1. Introduction

Beta-cyclodextrins (βCD) have been inspected in many works in the literature as a capable of forming supramolecular structure compound and also of forming ternary complexes with the further complexation with metal ions. 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, 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.

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.

Palladium (II) has many applications from implantable medical devices as in dentistry for instance, as anticancer drugs to catalyst in the pharmaceutical and fine chemical industries to cite a few. Supramolecular binary and ternary structures involving βCD and cholecalciferol, vitamin D (VitD), were previously studied with some metal ions but to our knowledge, no further studies were conducted in the literature so far.

The supramolecular assemblies obtained by encapsulating VitD in βCD 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 βCD, it can be of a very valuable pharmaceutical drug.

Therefore, the synthesis and characterization of ternary compounds of this inclusion compound chelated with Ga4+ or Pd2+ 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 (13C 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. β-cyclodextrin (βCD) and palladium nitrate salt were purchased from Sigma, USA; cholecalciferol (Vitamin D3, VitD) from Acros Organics, France. Gallium (III) in HNO3, ICP standard solution, ethanol absolute and KNO3 were purchased from Merck, Germany. Gallium (III) standard and Palladium (II) acid water solutions (HCl, Merck - Germany) were standardized by AAS, SPECTRAA model 220FS, air - acetylene). DMSO-d6, 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 N2 (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 ± 0.01 ml) and KNO3 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 (Vetc, Brazil) and 90% water, in which pKw is still equal to 10.7814.

2.2. Apparatus and procedure for Synthesis

Binary and ternary systems were synthesized according to the literature 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 N2 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 βCD:VitD:metal ion, respectively.

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. 13C NMR were recorded in a Bruker AC-200 NMR spectrometer, operated at 60 MHz, DMSO-d6, as solvent and recording the chemical shifts using this same solvent as internal standard (δ = 39.51 ppm). Mesenova® was the employed software. D2 Phaser X-ray diffraction Bruker instrument using Cu-Kα (λ=1.5406 Å) radiation.
collected the data for the powder samples in the 20 ranges of 0 to 50' with a step of 0.06' with a 2 sec collection time.

Potentiometric titrations were made in a Metrohm automatic titrator model Titrando 806 (Switzerland) software Tiamo® 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 pKₐ employed was 13.78. KNO₃ or KCl (for all titrations with Pd²⁺) and after this time, the other reagents were added to 90%v/v water. The pKₐ employed was 13.78. KNO₃ or KCl (for all titrations with Pd²⁺) and after this time, the other reagents were added to 90%v/v water. The pKₐ employed was 13.78. KNO₃ or KCl (for all titrations with Pd²⁺) and after this time, the other reagents were added to 90%v/v water. The pKₐ employed was 13.78. KNO₃ or KCl (for all titrations with Pd²⁺)

3. Results and discussion

In Fig. 1(a) it is possible to see a spatial representation of VitD and of βCD in (b) and (c). The supramolecular assembly for βCDVitD 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 βCD, 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.

3.1. ¹³C NMR

The complete numbering of the structure of either βCD or VitD is reported in the literature, (also as in Fig. 1(a)), as well as the ¹³C chemical shifts for βCD and VitD and VitD supramolecular assembly (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 βCD, 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.

<p>| Table 1: Chemical shifts (δ) of ¹³C NMR of βCD and βCD Pd(II) complexes and comparative ∆δ values. |</p>
<table>
<thead>
<tr>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
</tr>
</thead>
<tbody>
<tr>
<td>βCD (a)</td>
<td>102.188</td>
<td>72.614</td>
<td>73.308</td>
<td>81.766</td>
<td>72.283</td>
</tr>
<tr>
<td>βCD Pd·5:1 (b)</td>
<td>102.324</td>
<td>72.779</td>
<td>73.467</td>
<td>81.917</td>
<td>72.431</td>
</tr>
<tr>
<td>βCD Pd·10:1 (c)</td>
<td>102.270</td>
<td>72.715</td>
<td>73.436</td>
<td>81.862</td>
<td>72.382</td>
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<tr>
<td>(a) - (b) = ∆δ 5:1</td>
<td>-0.136</td>
<td>-0.165</td>
<td>-0.159</td>
<td>-0.151</td>
<td>-0.148</td>
</tr>
<tr>
<td>(a) - (c) = ∆δ 10:1</td>
<td>-0.082</td>
<td>-0.101</td>
<td>-0.128</td>
<td>-0.096</td>
<td>-0.099</td>
</tr>
</tbody>
</table>

<p>| Table 2: Chemical shifts (δ) of ¹³C NMR of βCD, βCDVitD and βCDVitD Pd(II) complexes and comparative ∆δ values. |</p>
<table>
<thead>
<tr>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
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</thead>
<tbody>
<tr>
<td>βCD (a)</td>
<td>102.188</td>
<td>72.614</td>
<td>73.308</td>
<td>81.766</td>
<td>72.283</td>
</tr>
<tr>
<td>βCDVitD (b) 5:1</td>
<td>102.105</td>
<td>72.561</td>
<td>73.223</td>
<td>81.689</td>
<td>72.198</td>
</tr>
<tr>
<td>βCDVitD Pd(c) 5:1</td>
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<td>72.619</td>
<td>73.303</td>
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<td>72.260</td>
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<td>βCDVitD Pd(d) 10:1</td>
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<td>(a) - (b) = ∆δ</td>
<td>0.083</td>
<td>0.053</td>
<td>0.085</td>
<td>0.077</td>
<td>0.085</td>
</tr>
<tr>
<td>(b) - (c) = ∆δ</td>
<td>-0.046</td>
<td>-0.058</td>
<td>-0.080</td>
<td>-0.056</td>
<td>-0.062</td>
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<tr>
<td>(d) - (c) = ∆δ</td>
<td>-0.075</td>
<td>-0.071</td>
<td>-0.104</td>
<td>-0.087</td>
<td>-0.082</td>
</tr>
</tbody>
</table>

<p>| Table 3: Chemical shifts (δ) of ¹³C NMR of βCD and βCD Ga(III) complexes and comparative ∆δ values. |</p>
<table>
<thead>
<tr>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
</tr>
</thead>
<tbody>
<tr>
<td>βCD (a)</td>
<td>102.188</td>
<td>72.614</td>
<td>73.308</td>
<td>81.766</td>
<td>72.283</td>
</tr>
<tr>
<td>βCD Ga (b) 5:1</td>
<td>102.121</td>
<td>72.548</td>
<td>73.293</td>
<td>81.697</td>
<td>72.225</td>
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<tr>
<td>βCD Ga (c) 10:1</td>
<td>102.250</td>
<td>72.642</td>
<td>73.425</td>
<td>81.803</td>
<td>72.347</td>
</tr>
<tr>
<td>(a) - (b) = ∆δ 5:1</td>
<td>0.067</td>
<td>0.066</td>
<td>0.015</td>
<td>0.069</td>
<td>0.058</td>
</tr>
<tr>
<td>(a) - (c) = ∆δ 10:1</td>
<td>-0.062</td>
<td>-0.028</td>
<td>-0.117</td>
<td>-0.037</td>
<td>-0.064</td>
</tr>
</tbody>
</table>
Table 4: Chemical shifts (δ) of 13C NMR of βCD, βCDVitD and βCD_VitD Ga(III) complexes and comparative Δδ values.

<table>
<thead>
<tr>
<th></th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
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<tr>
<td>βCD (a)</td>
<td>0.021801</td>
<td>72.614</td>
<td>73.308</td>
<td>81.766</td>
<td>72.283</td>
<td>60.190</td>
</tr>
<tr>
<td>βCDVitD (b) 5:1</td>
<td>0.02107</td>
<td>72.561</td>
<td>73.225</td>
<td>81.680</td>
<td>72.198</td>
<td>60.095</td>
</tr>
<tr>
<td>βCDVitD Ga (c) 5:1:1</td>
<td>0.02869</td>
<td>72.670</td>
<td>74.405</td>
<td>81.844</td>
<td>72.362</td>
<td>60.282</td>
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<tr>
<td>βCD VitD Ga (d) 10:1:1</td>
<td>0.02157</td>
<td>72.580</td>
<td>73.325</td>
<td>81.704</td>
<td>72.246</td>
<td>60.154</td>
</tr>
<tr>
<td>(a) - (b) = Δδ</td>
<td>0.0083</td>
<td>0.053</td>
<td>0.085</td>
<td>0.057</td>
<td>0.085</td>
<td>0.095</td>
</tr>
<tr>
<td>(b) - (c) = Δδ</td>
<td>-0.164</td>
<td>-0.119</td>
<td>-0.182</td>
<td>-0.155</td>
<td>-0.164</td>
<td>-0.187</td>
</tr>
<tr>
<td>(b) - (d) = Δδ</td>
<td>-0.052</td>
<td>-0.019</td>
<td>-0.098</td>
<td>-0.015</td>
<td>-0.048</td>
<td>-0.059</td>
</tr>
</tbody>
</table>

From Table 3, it is seen that for the same complexes with βCD and Ga**, when comparing the chemical shifts with those with Pd** (refer to Table 1), this effect is different in the former complexes, showing that Ga binds differently from Pd** with βCD. Even different Ga** 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 βCD alone. On the contrary, in the ligand to metal ratio of 10:1, there is a deshielding effect. Somehow, when Ga** is in higher concentrations in the solution, the complexed generated in the solid state, is less stable than when there is less Ga** in the same experimental conditions. So much so, that the more influenced carbon atoms in the 5:1 ratio are C4>C1>C2>C5>C6>C3 (shielding effect), showing the contrary order to the deshielding effect in the 10:1 ratio, C3>C6>C5. The Ga** concentration in the synthesis method** influenced the final ternary metal complexes obtained. The less Ga** 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 βCDVitD and Ga**. It is also seen from Table 4 that in the ternary assembly with Ga**, both proportions showed a deshielding effect, being the most affected C-6>C-3>C-5 for the 5:1:1 ratio and for the 10:1 proportion, C-3>C-6>C-5. When Ga** 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 Δδ comparative values in Table 4 shows that the deprotonated –OH groups of C-3 and C-6 are the most important peaks in all complexes studied in this work. The existence of a limiting value for this trend was not tested in this work.

For the sake of comparison, in fact, shielding and deshielding effects were observed in βCD and βCDVitD and Cu**, Co**, Zn** and Al** previous results. For the supramolecular ternary assembly of βCDVitD, deshielding effects were mainly observed with Co** and Pd**, both shielding and deshielding values for C atoms in the complexes with Cu** and Zn** as well as for Ga**, and shielding effects for Al** complexes. So, no trend is 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, suggesting the ternary assembly βCDVitD, 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 βCDVitD. It was used then to assess the differences in the crystallinity by the inclusion of VitD in βCD and in the complexation of the metal ions chosen in this work.

In Fig. 2(a), βCD 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 βCD does not alter much from the structure of βCD. That is not the case in Fig. 3(a) where it can be seen a greater change in the crystallinity pattern of βCD, when complexed to the metal ion Ga(III), for the two ligand to metal ratios studied. Those results are indications that the binding of βCD 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 (βCDVitD, Fig. 2(c)), whereas in the 5:1:1 Pd(II) complex proportion, the crystallinity of βCDVitD is decreased. The same effect in the crystallinity of βCDVitD is observed for the Ga(III) 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 βCD 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.
shift. Band displacement, disappearance or changing in intensity are two metals studied, between 1450 to 1600 nm, a second bathochromic seen that these bands are displaced towards a higher wavelength for the OH bands. Following this same region for the obtained complexes, it is around 1400 nm, it is seen the assigned 2

taking a look at the spectrum of 

bathochromic shift). The band around 1200 nm in the spectra of 

to a region of higher wavelengths, starting from 270 nm till 340 nm –

200 to 240 nm - Fig. 4(a)), while for Ga complexes, they are displaced 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.

But no doubt is to be raised when considering the first aliphatic C-H overtone at 1800 to 1700 nm; the second at 1210 to 1150 nm and the third at 915 to 857 nm. All three can be seen in the spectra of βCD 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 βCD or there are already bonded 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.

Overtones 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. All three can be seen in the spectra of βCD 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 βCD or there are already bonded 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 βCDVitD 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 βCD 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 βCDVitD 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 ternary complex.

### Table 5: Main peaks (20) obtained by powder XRD of the studied ligands and of the binary and ternary metal complexes in the two different ligand:metal ratios studied.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>3+</th>
<th>βCD</th>
<th>βCDPd</th>
<th>βCDGa</th>
<th>βCDVitD</th>
<th>βCDVitDpD</th>
<th>βCDVitDGa</th>
<th>βCDVitDGa</th>
</tr>
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<tbody>
<tr>
<td>ICD (Lit.)</td>
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<td>34.66</td>
<td>35.75</td>
<td>36.48</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICDPd 5:1</td>
<td>35.02</td>
<td>33.81</td>
<td>33.75</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ICDPd 10:1</td>
<td>34.66</td>
<td>33.81</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>ICDGa 5:1</td>
<td>34.66</td>
<td>33.81</td>
<td></td>
<td></td>
<td></td>
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<td>ICDGa 10:1</td>
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<td></td>
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</tr>
</tbody>
</table>

(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 βCD 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 βCD and βCDVitD and of the binary and ternary systems with Pd" (Fig. 4) and Ga" (Fig. 5).

In Figs. 4 and 5(a), the UV region of the spectra shows the charge transfer absorption band of βCD alone as a large band from 200 to 270 nm which is much less extensive and intense in Pd" complexes (from 200 to 240 nm - Fig. 4(a)), while for Ga" 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 βCD alone, assigned to CH, groups disappears in all metal complexes. Also taking a look at the spectrum of βCD 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. **21,25-26**
Fig. 4: UV (a) – NIR (b) region spectra of βCD and βCD/VitD and the complexes with Pd²⁺ in different ligand to metal ratios and (c) Detailed region of the occurrence of the first C-H overtone in βCD.

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 BCDD and the hydrolysis constants for both metal ions and CT association constants (when Pd²⁺ 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 and in the presence of metal ions, in other papers including reviews. 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 βCD/VitD and the metal ions Pd²⁺ and Ga³⁺ 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 βCD, 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 ¹³C 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 βCD 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 βCD/VitD 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 ¹³C 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 βCD has its preferences when binding to certain metal ions and this can be changed when βCD 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 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**

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