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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 13C NMR in DMSO-d6 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; Pitla, 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 D₃ (VitD) or cholecalciferol do not take into account a third species like metal ion (Delauerau, Siouffi, & Pepe, 1998; Tian & Holick, 1992). In many cases, the inclusion complexes of vitamin D₃ (VitD) or cholecalciferol do not take into account a third species like metal ion (Delauerau, Siouffi, & Pepe, 1998; Tian & Holick, 1992).

2. Experimental
2.1. Reagents
β-Cyclodextrin (Aldrich, USA) and vitamin D₃ or cholecalciferol (Sigma, USA) were analytical grade. Proper mass of the metal salts of Cu(NO₃)₂·3H₂O, CoCl₂·6H₂O (both p.a., Merck, Germany) and ZnCl₂ (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, 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:M²⁺, 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 N₂ in a sealed small Schlenk glass flask.

2.3. 13C 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-d₆ (H₂O <0.02%, Eurisotop, France), and added with drops of 1,4-dioxane (99%, Aldrich) as an internal standard. In our experimental conditions, the 13C chemical shift of the singlet of 1,4-dioxane carbon atom 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-d₆. Only 13C 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-ray powder diagrams were obtained using a Shimadzu XD-3A diffractometer, with Cu Kα 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 5:1, 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 13C 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 13C NMR spectra of β-CD in DMSO-d₆ 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 13C NMR spectra of vitamin D in CDCl₃ and of some of its analogues have been published by Berman, Luz, Mazur and Sheves (1977) and in 1996 by Mizhiritski.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(1) at 32.197, C(2) at 35.481, C(3) at 67.904 and C(4) at 45.947 ppm while C(11) appears at 21.871, C(17) at 55.953 and C(18) at 11.833 ppm, respectively. As expected, the same small variations are observed for all other signals (Table 1).

3.2. Binary assemblies 13C NMR spectra

The chemical shifts of 13C 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(1) to C(4)) could lead to hydrogen bonding with the hydroxyl of the VitD.

3.3. Ternary complexes 13C NMR spectra

3.3.1. Cobalt complexes

The 13C 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 ppm for 5:1:1 ratio and 30.74 ppm for 10:1:1 ratio. The other signals corresponding to VitD (not shown in Fig. 3) are located as follows: C(5) at 144 ppm, C(11) and C(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(5) and C(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
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_2v(17) till C_{2v}(26) atoms show 13C NMR signals in the supramolecular formed structure. 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 CoII 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.

### Table 1

| C_v | Ref. (Mizhiritskii 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   | 142.00                         | 141.10                    | 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                          | 40.44                     | 40.447    |
| 21  | 18.23                          | 18.67                     | 18.671    |
| 22  | 136.25                         | 136.335                   | 136.335   |
| 23  | 135.28                         |                           |           |
| 24  | 43.45                          |                           |           |
| 25  | 34.74                          |                           |           |
| 26  | 20.29                          |                           |           |
| 27  | 20.60                          |                           |           |
| 28  | 21.75                          |                           |           |

* v Subscript means C atoms from VitD.

### Table 2

| Compound | C(1) | C(2) | C(3) | C(4) | C(5) | C(6) |
|----------|------|------|------|------|------|------|
| β-CD     | 101.87 | 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 |

ΔH = 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 CoII 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 13C 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, 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-
involved 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 Co2+ 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.
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-
Another interesting feature was the complex b-CD–VitD–Co 10:1:1 ratio be a pink solid, showing that Co²⁺ 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 4

Chemical shifts in ppm (13C, DMSO-d₆, 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 + Cu²⁺ | 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 + Cu²⁺ | 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 + Cu²⁺ | 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 + Cu²⁺ | 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 + Cu²⁺ | 5:1 | 3 | 101.904 72.021 73.013 81.488 72.385 59.905 | | | | | |
| Δδ – β-CD | -0.014 | 0.007 | -0.016 | -0.019 | -0.012 | 0 | | |
| β-CD + VitD + Cu²⁺ | 5:1:1 | 7 | 101.885 72.000 73.013 81.491 72.354 59.901 | | | | | |
| Δδ – β-CD | -0.033 | -0.014 | -0.016 | -0.016 | -0.043 | -0.004 | | |
| β-CD + VitD + Cu²⁺ | 10:1:1 | 3 | 101.885 72.017 73.013 81.492 72.385 59.887 | | | | | |
| Δδ – β-CD | -0.033 | 0.003 | -0.016 | -0.015 | -0.012 | -0.018 | | |

Table 5

Chemical shifts in ppm (13C, DMSO-d₆, 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    | pH | C(1)           | C(2)           | C(3)           | C(4)           | C(5)           | C(6)           |
|---------|----------|----|----------------|----------------|----------------|----------------|----------------|----------------|
| β-CD + Zn²⁺ | 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 + Zn²⁺ | 10:1 | 101.888 72.017 73.014 81.494 72.371 59.887 | | | | | | |
| Δδ – β-CD | -0.030 | 0.003 | 0.005 | 0.012 | 0.013 | 0.018 | | |
| β-CD + VitD + Zn²⁺ | 5:1:1 | 101.901 72.021 73.03 81.509 72.386 59.905 | | | | | | |
| Δδ – β-CD | -0.017 | 0.007 | 0.001 | 0.002 | 0.012 | 0 | | |
| β-CD + VitD + Zn²⁺ | 10:1:1 | 101.902 72.017 73.026 81.509 72.388 59.905 | | | | | | |
| Δδ – β-CD | -0.016 | 0.003 | -0.003 | 0.002 | 0.009 | 0 | | |

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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(II), Cu(II) and Zn(II) or when in the supramolecular assembly with VitD and complexed with the studied 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 D₃ (cholecalciferol) and three metal ions: Co(II), Cu(II) and Zn(II). Using ¹³C 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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Table 6

| 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 + Co²⁺ 5:1 | 205 ± 3  | –        | 536 ± 3         |
| β-CD + Co²⁺ 10:1 | 210 ± 1  | –        | 527 ± 3         |
| β-CD + VitD + Co²⁺ 5:1:1 | 208 ± 4  | 269 ± 4  | 529 ± 2         |
| β-CD + VitD + Co²⁺ 10:1:1 | 208 ± 0  | 260 ± 2  | 600 ± 0         |
| β-CD + Cu²⁺ + 5:1 pH 3 | 210 ± 0  | 278 ± 0  | 548 ± 1         |
| β-CD + Cu²⁺ + 10:1 pH 3 | 212 ± 3  | 273 ± 6  | 557 ± 4         |
| β-CD + Cu²⁺ + 5:1 pH 7 | 212 ± 0  | 264 ± 2  | 423 ± 6         |
| β-CD + Cu²⁺ + 10:1 pH 7 | 212 ± 0  | 264 ± 2  | 483 ± 4         |
| β-CD + VitD + Cu²⁺ 5:1:1 pH 7 | 216 ± 2  | 268 ± 2  | 548 ± 9         |
| β-CD + VitD – Cu²⁺ 5:1:1 pH 7 | 207 ± 0  | 264 ± 0  | 530 ± 0         |
| β-CD + VitD + Cu²⁺ + 10:1 pH 7 | 207 ± 3  | 263 ± 6  | 570 ± 2         |
| β-CD + VitD + Cu²⁺ + 10:1:1 pH 7 | 207 ± 0  | 264 ± 0  | 530 ± 0         |
| β-CD + Zn²⁺ 5:1 | 212 ± 0  | –        | –               |
| β-CD + Zn²⁺ 10:1 | 207 ± 4  | –        | –               |
| β-CD + VitD + Zn²⁺ 5:1:1 | 210 ± 9  | 257 ± 3  | –               |
| β-CD + VitD + Zn²⁺ 10:1:1 | 211 ± 2  | 260 ± 1  | –               |

Fig. 7. Diffuse reflectance UV–vis of the samples containing cobalt (II): (a) β-CD + Co²⁺ 5:1; (b) β-CD + Co²⁺ 10:1; (c) β-CD + VitD + Co²⁺ 5:1:1; (d) β-CD + VitD + Co²⁺ 10:1:1.

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

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, & Zhong, 2003), with main peaks at ca. 2θ = 10.8°, 12.5°, 15.7° and 19.2°. It has been
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).
