Inclusion compound of vitamin B6 in β-CD. Physico-chemical and structural investigations

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Abstract. Structural and physico-chemical characterization of supramolecular assembly of vitamin B6 with β-cyclodextrin (β-CD) prepared by different methods (kneading, co-precipitation and freeze-drying) has been performed by using several spectroscopic techniques (FTIR, 1H NMR, UV-Vis), powder X-ray diffraction and DSC in order to evidence the inclusion compound formation. An analysis of the chemical shifts observed in the 1H-NMR spectra and of the vibrational frequency shifts led to the tentative conclusion that the vitamin B6 probably enters the cyclodextrin torus when forming the β-CD–vitamin B6 inclusion complex.

1. Introduction
A bioactive compound act orally, parentherally or locally depending on the administration form (solid or liquid); in function of the auxiliary substances, its biodisponibility is influenced [1]. In order to solve this problem, the most employed method is the complexation with cyclodextrins (CD) [2-4]. Cyclodextrins are cyclic oligosaccharides having a hydrophobic external surface and an inner hydrophilic surface. There are different CD types depending on the glucopyranose unit number [α-six; β-seven, γ-eight, etc.] (see figure 1a). Cyclodextrins and their derivatives are used to increase the solubility and the solving rate of the bioactive substances.

![Figure 1. (a) β-Cyclodextrin molecule. (b) Pyridoxine molecule.](image)

They are capable to form more soluble guest-host systems through non-covalent bonds, Van der Waals interactions, hydrophobic effect, solvent molecules reorganization and hydrogen bonds [5].
Vitamin B6 (pyridoxine) (figure 1b) is a water-soluble vitamin and is part of the vitamin B complex group. Pyridoxal phosphate (PLP) is the active form and is a cofactor in many reactions of amino acid metabolism, including transamination, deamination, and decarboxylation. PLP is necessary also for the enzymatic reaction governing the release of glucose from glycogen. The primary role of vitamin B6 is to act as a coenzyme to many other enzymes in the body that are involved predominantly in metabolism. Vitamin B6 is involved in the following metabolic processes: amino acid, glucose and lipid metabolism, neurotransmitter synthesis, histamine synthesis, hemoglobin synthesis and function and gene expression.

The aim of this paper was to investigate the molecular encapsulation of the vitamin B6 in β-CD through different spectroscopic methods (FTIR, NMR, UV-Vis), DSC and powder X-ray diffraction.

2. Experimental
The preparation of the inclusion compounds was done by kneading (kn), co-precipitation (co) and freeze-drying (fd) methods. Also, physical mixtures (pm) were prepared in order to be compared with the inclusion compounds.

To evidence the formation of the inclusion compounds, FTIR, DSC and X-ray powder diffraction (XRD) were employed. FTIR measurements were performed with JASCO 6100 spectrometer in the 4000 to 400 cm\(^{-1}\) spectral region with a resolution of 4 cm\(^{-1}\) using KBr pellet technique. DSC thermograms were obtained with DSC 60 Shimadzu calorimeter and X-ray diffraction patterns were obtained with a Bruker D8 Advance diffractometer in the 2θ = 2-50° angular domain using Cu K\(_{\text{a}1}\) radiation.

The inclusion complexes of β-CD (named host, H) with the active substance as guest (G) were prepared in aqueous solutions starting from millimolar “mother solutions” of G and H in D\(_2\)O for \(^1\)H NMR and in H\(_2\)O for UV-Vis, respectively. \(^1\)H NMR spectra were collected with Bruker Avance 500 spectrometer after 15 min. of thermal equilibration at 25°C. The spectrometer was operated at 500.1325 MHz with the following parameters: 32000 data points, pulses of 90º, 2 seconds delay between scans (16 scans) and a digital resolution of 0.588 Hz/point. The chemical shifts (in ppm) are relative to the HOD signal located at 4.6897 ppm. UV-Vis spectra were obtained with JASCO V-550 spectrometer.

3. Results and discussion
In order to evidence the vitamin B6–β-CD inclusion compound formation, several methods were employed: FTIR, DSC and XRD. As concerned FTIR spectroscopy, the spectra of pure vitamin B6, of β-CD, their 1:1 physical mixture and of the products obtained by co-precipitation, kneading and freeze-drying procedures were compared.

3.1. FTIR

Figure 2. FTIR spectra of: vitamin B6, β-CD, their 1:1 pm and the products obtained by kn, co, and fd procedures, 4000-2500 cm\(^{-1}\) (left) and 1900-1400 cm\(^{-1}\) (right) spectral ranges.
As concerned the O–H stretching vibration, its maximum, located at ~3373 cm$^{-1}$ in β-CD FTIR spectrum (see figure 2), is shifted to 3402 cm$^{-1}$ for $kn$ and $co$ and to 3413 cm$^{-1}$ for $fd$ products spectra, respectively. The frequency shift of the O–H bending vibration located at 1645 cm$^{-1}$ in pure β-CD, is shifted to 1636 cm$^{-1}$ for $kn$ and $co$ products and to 1633 cm$^{-1}$ for $fd$ product. This frequency shift can be related to the appearance of hydrogen bonds during the complexation process, especially for the $fd$ product. This complexation mechanism must be taken into account for the $kn$ and $co$ products, also.

3.2. X-ray powder diffraction
X-ray powder diffraction patterns of vitamin B6, β-CD and of the products obtained by $co$, $kn$ and $fd$ procedures were compared (figure 3a). From the powder diffraction patterns one can see that inclusion compounds are formed in different degree. Both crystalline and amorphous phases are present in the obtained compounds. The difference between these patterns is due to different ratios between crystalline and amorphous phases. The highest degree of formation is obtained for kneading procedure.

![Figure 3.](image)

*Figure 3.* (a) X-ray patterns of: vitamin B6, β-CD, and the products obtained by $kn$, $co$ and $fd$ procedures; (b) DSC thermograms of the vitamin B6, β-CD and of the obtained inclusion compounds.

3.3. DSC
DSC thermograms of vitamin B6, β-CD and of the products obtained by $kn$, $co$ and $fd$ procedures were recorded (figure 3b). DSC trace of vitamin B6 presents a sharp endothermic melting peak at 212°C, followed by an exothermic peak, possibly melt degradation. The thermogram of the β-CD showed an endothermic transition between 70-110°C, due to the loss of water molecules and another endothermic peak at around of 300°C indicating the degradation of cyclodextrin. By comparing the thermograms of the pure compounds with the thermograms of products the decreasing of dehydration endothermic peak of cyclodextrin was observed, as well as a disappearance of the melting peak of the vitamin B6 in the inclusion compounds, but the exothermic peak of melt degradation remains for all inclusion compounds.

3.4. $^1$H NMR
The preliminary NMR measurements are presented in the figure 4 where we can see the $^1$H NMR spectra of both molecules, B6 vitamin and β-CD, separately, and of the 1:1 mixture of the two components, all as aqueous solutions in D$_2$O. The aim of these measurements was to notice if the mixture spectrum is or not an algebraic sum of the components spectra. This fact can give us information about the complex formation during the preparation procedure. A very careful
examination of the mixture spectrum’ points out some chemical shifts variation for any protons belonging both to B6 vitamin and β-CD. For instance, we can notice a modification of the chemical shift corresponding to H5 and H3 protons of guest and of the B6 molecule ethyl protons.

**Figure 4.** $^1$H NMR spectra of the inclusion complex of vitamin B6 and β-CD. Bottom: vitamin B6; middle: β-CD; top: inclusion complex of vitamin B6:β-CD (1:1).

**Figure 5.** UV-Vis spectra of the inclusion complex of vitamin B6 (pyridoxine) with β-CD ([B6] = 0.1 mM in H$_2$O, [β-CD]=variable).

All these allowed us to conclude that the inclusion complex of these two molecules is formed, and the NMR measurements are in good accordance with the other measurements presented in this paper. Further NMR measurements connected with the possibilities to determine the stoichiometry and the complexation constant of the complex are in progress.

3.5. UV-Vis
The vitamin B6 has two absorption maxima, at 292 and 324 nm (figure 5: the arrows suggest the absorption maxima variations with the increase of the β-CD concentration). With increasing concentration of β-CD, the absorption intensity at 292 nm gradually increased, whereas the absorption intensity at 324 nm decreased gradually. The isosbestic point was observed at 307 nm. This can confirm the supramolecular inclusion complex formation between vitamin B6 and β-CD.

4. Conclusions
Based on FTIR, X-ray powder diffraction and DSC data it was evidenced that an inclusion compound between vitamin B6 and β-CD was formed. The intensity diminishing or the disappearance of the melting endothermic peak characteristic to pure substance and the diminishing of the endothermic peak characteristic to bonded and non bonded cyclodextrin water elimination show the inclusion complex formation, also. All the preparation methods employed were successfully in obtaining these supramolecular assemblies. The changes in the chemical shift corresponding to H5 and H3 protons of guest and of the B6 ethyl protons demonstrate the formation of B6–β-CD inclusion complex in aqueous solution. The isosbestic point of the UV-Vis spectrum demonstrates also the inclusion complex formation in aqueous solution.

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References
[1] Leucuta S E 1989 *Farmacocinetica și biodisponibilitatea în terapia medicamentoasă* (București: Editura Medicală)
[2] Albers E and Muller B W 1992 *J. Pharm. Sci.* 81 756-61
[3] Ficarra R, Ficarra P and Rameri D 2000 *J. Pharm. Biomed. Anal.* 23 231-6
[4] Acerbi D, Bovis G, Carli F, Pasini M, Pavesi L and Peveri T 1990 *Drug Investigation* 2, 29-36
[5] Lopata A, Darvas F, Stadler-Szőke A and Szejtli J *J. Pharm. Sci.* 1995 74 211-3.