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To cite this article: Irina Kacso et al 2009 J. Phys.: Conf. Ser. 182 012009

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Inclusion compound of vitamin B13 in β-Cyclodextrin. Structural investigations

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Abstract. Structural characterization of inclusion compound of vitamin B13 (orotic acid) with β-cyclodextrin (β-CD) prepared by different methods (kneading, co-precipitation and freeze-drying) has been performed by using FTIR spectroscopy, powder X-ray diffraction and DSC, FTIR data being compared with molecular modeling of these supramolecular architectures.

1. Introduction
A bioactive compound acts depending on the administration form (solid or liquid); in consequence, its biodisponibility can be 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). 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].

![Figure 1](https://example.com/figure1.png)

**Figure 1.** (a) β-Cyclodextrin molecule. (b) Orotic acid molecule.

Vitamin B13 (orotic acid) (figure 1b) is not really recognized as a vitamin, since it is manufactured by the body intestinal flora. The orotic acid plays a central role in the metabolism of folic acid and vitamin B12. It assists and may enhance the transportation and absorption of essential nutrients, especially calcium and magnesium, across cell membranes and helps the production of genetic material. It may be beneficial after a heart attack and has been used in conditions such as multiple sclerosis and chronic hepatitis. It is also reported to prevent liver-related complications and premature aging. Found naturally in whey and root vegetables, such as beets, turnips, and carrots. Orotic acid is
easily destroyed by water and sunlight. Deficiency of vitamin B13 may be cause of liver disorders, cell degeneration, premature aging.

The investigation of the molecular encapsulation of the vitamin B13 in β-CD through different techniques (FTIR spectroscopy, DSC and X-ray diffraction) and the comparison of this information with that obtained by molecular modeling is the purpose of this paper.

2. Experimental
The inclusion compounds were prepared by kneading, co-precipitation and freeze-drying methods. Also, physical mixtures were prepared in order to be compared with the so prepared inclusion compounds.

2.1. Experimental investigation methods
To evidence the inclusion compounds formation, several techniques 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 2\(\theta\) = 2-50° angular domain using Cu K\(\alpha_1\) radiation.

2.2. Molecular modeling
Guest molecules can have interaction with the hosting CD via the latters OH groups, by Van der Waals forces, electrostatic forces and hydrogen bonding [6]. The interaction may be external but also internal. In the latter case, the guest enters the CD cavity, thus forming an inclusion complex. To assess the precise type of complex, a starting model was built by positioning the orotic acid molecule at the larger side of the β-CD cavity. The geometry of the complex was optimized in vacuum using the molecular- mechanics algorithm of the HyperChem software [7]; the details are given elsewhere [8]. The well-known MM+ (with the Polak-Ribière conjugate gradient) method was used to minimize the energy of the structures until a RMS gradient lower than 0.015 kcal mol\(^{-1}\) Å\(^{-1}\) was obtained.

3. Results and discussion
3.1. FTIR
As concerned the C=O stretching band (see figure 2), its maximum located at ~1709 cm\(^{-1}\) is diminished strongly in intensity after complexation with β-CD, the same occurs with C=C stretching band at 1674 cm\(^{-1}\) [9].

![Figure 2](image_url)

**Figure 2.** FTIR spectra of: vitamin B13, β-CD, their 1:1 physical mixture (pm) and the products obtained by kneading (kn), co-precipitation (co), and freeze-drying (fd) procedures, 4000-2500 cm\(^{-1}\) (left) and 1900-1400 cm\(^{-1}\) (right) spectral ranges.
The intensity diminishing can be related with the mobility hindering of the ring, an indirect argument of the inclusion process. This frequency shift can be related to the appearance of hydrogen bonds during the complexation process, especially for the \( fd \) product. The frequency of the O-H bending vibration, located at 1640 cm\(^{-1}\) in pure \( \beta\)-CD is not shifted for \( kn \), \( co \) and \( fd \) products. This already mentioned complexation mechanism must be taken into account for the \( kn \) and \( co \) products, also.

3.2. X-ray powder diffraction

![Figure 3](image)

(a) X-ray patterns of: vitamin B13, \( \beta\)-CD, and the products obtained by \( kn \), \( co \) and \( fd \) procedures. (b) DSC thermograms of vitamin B13, \( \beta\)-CD and of the obtained inclusion compounds.

From powder diffraction patterns (see figure 3a) one can see that inclusion compounds are formed in different degree depending on the preparation method. The difference between these patterns is due to different crystalline/amorphous ratios. The highest degree of formation is obtained for freeze-drying procedure.

3.3. DSC

The thermogram of the \( \beta\)-CD showed an endothermic transition between 70-110°C, due to the loss of water molecules and other endothermic peak at around 300°C indicating the degradation of cyclodextrin. DSC trace of vitamin B13 presents a sharp endothermic melting peak at 146°C. By comparing the thermograms of the pure compounds with that of the obtained products, the decreasing of dehydration endothermic peak of cyclodextrin was observed, as well as a decreasing until disappearance of the melting peak of the vitamin B13 in the inclusion compounds obtained by different methods (see figure 3b). One can conclude that the inclusion compound formation depends of the preparation method employed.

3.4. Molecular modeling

An analysis of the vibrational frequency shifts based on FTIR spectra (C=O and C=C stretching modes) led to the tentative conclusion that the orotic acid probably enters the cyclodextrin torus when forming the \( \beta\)-CD - orotic acid inclusion compound. Molecular mechanics calculations are consistent with this model, but a more detailed interpretation is not possible due to the MM+ approximations used.
More work needs to be done to establish the nature [6] of the forces implied in the inclusion process and in particular the expulsion processes of the intracavity water molecules [10] must be taken into account.

4. Conclusions
The inclusion compound between vitamin B13 and β-CD was confirmed based on FTIR, X-ray powder diffraction and DSC data. All preparation methods employed were successfully in obtaining these supramolecular assemblies. Molecular modeling with Hyperchem software based on MM+ molecular mechanics method was successfully used to determine the geometry of this supramolecular assembly: the orotic acid molecule enters the cyclodextrin torus when forming the inclusion compound.

Acknowledgments
This work received financial support from the Romanian Research and Education Ministry under the Core Project PN-09-44 02 01.

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