ORIGINAL ARTICLE

Host–guest system of Vitamin B10 in *β*-cyclodextrin: characterization of the interaction in solution and in solid state

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Abstract Conventional drugs are usually formulated for the immediate release of the medicinal substances and for obtaining the desired therapeutic effect. The aim of this paper was to investigate the possible interactions between Vitamin B10 and *β*-cyclodextrin (*β*-CD), to determine the physical-chemical characteristics and the interactions present in the corresponding inclusion compound. The so-obtained compounds were characterized by X-ray diffraction, DSC and FTIR spectroscopy. ^{1}H NMR and UV–vis spectroscopic methods were employed to study the inclusion process in aqueous solution. The X-ray powder diffraction patterns demonstrate the inclusion compound formation, especially for the lyophilized product where the amorphous phase dominates. The existence of the inclusion compounds obtained by different methods was confirmed by comparing with DSC and FTIR data of the pure compounds and the (1:1) Vitamin B10:*β*-CD physical mixture (*pm*). ^{1}H NMR measurements on aqueous solutions of Vitamin B10 and *β*-CD in D_2O allowed us to establish the corresponding Vitamin B10's and cyclodextrin's protons implied in the complexation process. 2D NMR spectroscopy established the geometry of the inclusion complex. ^{1}H NMR, UV–Vis and fluorescence data were used to obtain the stoichiometry and the stability constant of the complex.

Introduction

A series of researches will conduct in the field of controlled drug release (release of Vitamin included in cyclodextrin). Para-amino benzoic acid (PABA) or Vitamin B10 (see Fig. 1a), is an intermediate in bacterial synthesis of folate [1], the microorganisms in the intestines are capable of synthesizing folic acid (an important factor in the protein use) from this compound, and humans lack this ability. Vitamin B10 is sometimes marketed as an essential nutrient for use whenever normal Vitamin B10 synthesis by intestinal bacteria is insufficient. The compound is used as a UV filter in sunscreen formulations [2], as a drug against fibrotic skin disorders, in treating irritable bowel syndrome [3, 4].

CDs are cyclic oligosaccharides consisting of six, seven or eight units of *α*-D-(+)-glucopyranose, referred to as *α*-, *β*- and *γ*-CD respectively [5] (see Fig. 1b) obtained from starch by enzymatic reaction, that may encapsulate a wide variety of guest molecules (completely, or at least partially) in their hydrophobic cavity.

Through controlled release, these systems ensure control of the release and of the absorption of the medicinal substances from the respective system.

The aim of this paper was to obtain an inclusion compound (IC) of Vitamin B10 with *β*-cyclodextrin (*β*-CD) and to characterize it by FT IR, X-ray powder diffraction, DSC, ^{1}H NMR, UV–Vis and spectrofluorimetry. Molecular modeling technique was employed to obtain the spatial architecture of this supramolecular assembly.

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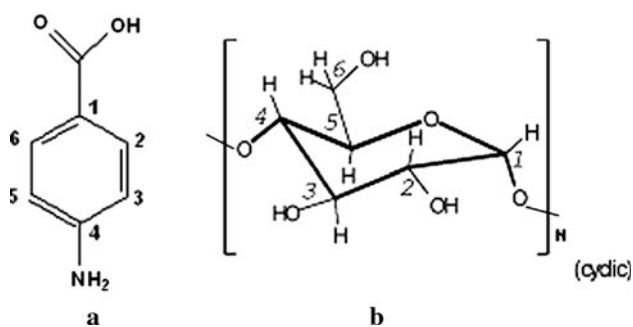Senda del Rey 9, 28040 Madrid, Spain

Fig. 1 **a** *p*-Aminobenzoic acid (PABA, Vitamin B10); **b** cyclodextrins: for *β*-CD, *N* = 7

Experimental

The solid state inclusion compounds were prepared in 1:1 molar ratio of the *β*-cyclodextrin (host) and Vitamin B10 (guest). The kneaded compound (*kn*) was prepared by mixing in an agate mortar the mixture of both components, using distilled water, for 1 h. The co precipitated product (*co*) was prepared by mixing the equimolar aqueous solution of the Vitamin B10 and *β*-cyclodextrin, and stirring this mixture for 24 h at room temperature. An identical solution was frozen, and then freeze-dried in an Alpha 1-2 LD type freeze dryer to obtain the lyophilized product (*fd*).

FTIR measurements were performed with a FTIR JASCO 6100 spectrometer in the in 4,000–400 cm^{-1} spectral range with a resolution of 4 cm^{-1} using KBr pellet technique.

X-ray powder diffraction patterns were collected with Bruker D8 Advance diffractometer in the $2\theta = 2$–$50°$ angular domain using Cu Kα_1 radiation. In order to increase the resolution a monochromator was used to eliminate Kα_2 radiation.

DSC thermograms were registered with a DSC60 Shimadzu differential scannning calorimeter by heating the samples from room temperature up to 350 °C, in a crimped aluminium pan, under flowing nitrogen flux, the heating rate being 10 °C/min. For data collection the Shimadzu TA-WS60 and TA60 2.1 software were employed.

UV–vis spectra were registered with a double beam JASCO V-550 spectrophotometer in the 190–400 nm spectral range with a resolution of 0.2 nm. The absorption spectra were obtained using $1 \times 1 \times 4$ cm^3 quartz cells. The spectrophotometer was equipped with a thermostat unit, the temperature in the cells was maintained at 20°C, during the experiments. In the reference cell the employed solvent was introduced, in the same volume ratio as the used for solving the samples. The solutions were prepared in bidistiled water, the concentration of the bioactive

substance was constant (10^{-4} mol/L) and the *β*-cyclodextrin concentration was increased progressively (10^{-3}–10^{-2} mol/L) (pH $\cong$ 5). The solutions were keept for 12 h at room temperature in order to equilibrate them.

Fluorescence spectroscopy measurements were performed using a 1.0 cm quartz cell with a JASCO FP6500 spectrofluorimeter. The excitation wavelength of 266 nm was employed, and the emission spectra were recorded from 300 to 450 nm and 600 to 730 nm, respectively. The Vitamin B10 concentration was fixed at 0.05 mM while the CDs concentration was varied from 0 to 8 mM. All aqueous solutions were shaken for 12 h.

^{1}H NMR spectra were obtained with *Bruker Avance 500* spectrometer after at least 15 min of thermal equilibration at 25 °C. The spectrometer was operated at 500.1325 MHz, with the following parameters: 32,000 data points, pulses of 90°, 2 s delay between scans (16 scans) and a digital resolution of 0.588 Hz/point. The chemical shifts were expressed in parts per milion (ppm) relativ to the chemical shift of HOD signal located at 4.6897 ppm. The inclusion complexes of *β*-CD (host, H) with the active substance (G) were prepared in aqueous solutions starting from a "mother" millimolar concentration of H and G in D$_2$O. *β*-CD (having $\leq$ 15% water in weight) was obtained from CYCLOLAB Hungary being used without further purification. The aqueous solutions were prepared taking into account the molecular water present in *β*-CD. Two starting solutions, of *β*-CD and of guest molecule, of 10×10^{-3} mol/dm^3 concentration in D$_2$O (ROMAG Turnu Severin, Romania), were prepared. Starting from these "mother" solutions, several mixtures having different molar ratios at constant volume of G and *β*-CD were prepared. The sum of the total concentration M = ([*β*-CD]$_t$ + [G]$_t$) (where the total index refers to the total concentration, being equal with 10×10^{-3} mol/dm^3. The molar ratio of the guest G, $r_G = [G]_t/([β\text{-CD}]_t + [G]_t)$ varies between 0 and 1 with an interval of 0.1.

The two-dimensional NMR spectrum at 500 MHz was obtained on a solution with $r_G = 0.7$ through standard Bruker software. The conditions for ROESY phase-sensitive spectra via time proportional phase incrementation (TPPI) were: presaturation of residual HDO signal, spectral widths of 6.8 ppm in both dimensions with a resolution of 0.83 and 1.66 Hz/point in f$_2$ and f$_1$ respectively and a mixing time of 200 ms. The experiment was performed using 4,096 data points in f$_2$ and 2,048t$_1$ increments with 16 scans per t$_1$ value and a relaxation period of 2 s. A sine function (SSB = 2) was applied in f$_1$ and f$_2$ before Fourier transformation.

Molecular modeling computations were performed using MM$^+$ molecular mechanics (Hyperchem software).

Results and discussion

FTIR

The following vibrational modes of Vitamin B10 molecule (assigned elsewhere [6]) were affected (see Fig. 2) by complexation process:

- $v_{as}(C=O)$ located at 1,680 and 1,662 cm^{-1} in pure drug spectrum is shifted to 1,685 cm^{-1} in the spectra of inclusion compounds obtained by different methods. A similar effect was observed by the analysis of a *co* product spectrum [7]. The splitting of the $v_{as}(C=O)$ band can be assigned to the coexistence of different association type species present in solid state. The higher frequency shift can be ascribed to the destruction of strong hydrogen bonding structure in uncomplexed drug upon inclusion compound formation with *β*-CD.
- $\delta_s(NH_2)$ vibrational mode, centered on 1,635 cm^{-1} in pure drug spectrum, is shifted to 1,630 cm^{-1} in the spectra of inclusion compounds obtained by different methods.

These two vibrational bands changes certify the inclusion compound formation and offer an idea about the mechanism of the inclusion compound formation.

XRD

The crystal structure of the Vitamin B10 was already reported [8]. For *kn* (kneaded product) and *fd* (freeze-dried product) inclusion compounds products, mixtures of amorphous and crystalline phases with very small crystallites were obtained, whereas a new quite crystalline

Fig. 2 FTIR spectra of Vitamin B10 and of inclusion compound with *β*-CD, 1,800–1,300 cm^{-1} spectral range

inclusion compound was obtained for the *co* product, see Fig. 3. For *co* (co precipitated one) inclusion compound a new crystalline compound was obtained having a crystalline structure distinct of that for the initial pure compounds. The positions of the diffraction lines for the *co* inclusion compound are different as compared to the corresponding ones for Vitamin B10 and *β*-CD, i.e. the parameters of the elementary cell for the new compound are different as compared with the initial compounds' ones.

DSC

DSC reveals some information on solid-state interactions between drug and cyclodextrin. The DSC thermograms of pure components and of Vitamin B10—*β*-cyclodextrin inclusion compounds are presented in Fig. 4. The curve for the *β*-cyclodextrin revealed a broad endothermic signal from 74 to 118 °C, with $\Delta H = 200$ kJ/mol, that corresponds to the loss by evaporation of the water molecules existing as residual humidity (t < 100 °C) as well as those included in the cavity (t > 100 °C) [9, 10]. From 290 °C onwards there is a new endothermic succeeded of the exothermic, corresponding to the melting, respectively the degradation of the *β*-cyclodextrin.

The DSC curve of Vitamin B10 presents a sharp endothermic peak at 191 °C, with $\Delta H = 15$ kJ/mol, corresponding to the melting of the drug, following the degradation of the substance. In the case of the physical mixture and the inclusion compounds of Vitamin B10 with *β*-cyclodextrin obtained by kneading, co precipitation and freeze-drying methods, the strong decreasing of dehydration endothermic peak of cyclodextrin was observed, as well as a disappearance of the melting peak of the Vitamin B10 in the inclusion compounds. Around 270 °C the decomposition process begins [7]. The DSC thermogram for the physical mixture shows a broad endothermic from 63 to 123 °C, with $\Delta H = 78$ kJ/mol, from 265 °C beginning the decomposition process. The thermogram of the kneaded inclusion compound presents a small broad endothermic from 56 to 120 °C, with $\Delta H = 66$ kJ/mol, the decomposition beginning from 276 °C. The inclusion compound obtained by co precipitation shows a small endothermic from 58 to 117 °C, $\Delta H = 93$ kJ/mol and the start of decomposition increased at 284 °C. The thermogram of the freeze-dried inclusion compound shows a very small broad endothermic from 60 to 105 °C with $\Delta H = 50$ kJ/mol, followed by a decomposition exothermic started at 288 °C. The decreasing of dehydration endothermic peak of cyclodextrin, the disappearance of Vitamin B10 melting peak and the increasing of the decomposition temperature are the indications for the inclusion process between Vitamin B10 and *β*-cyclodextrin.
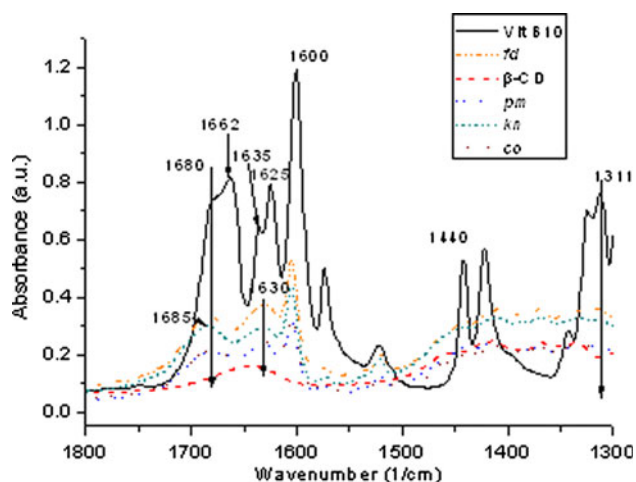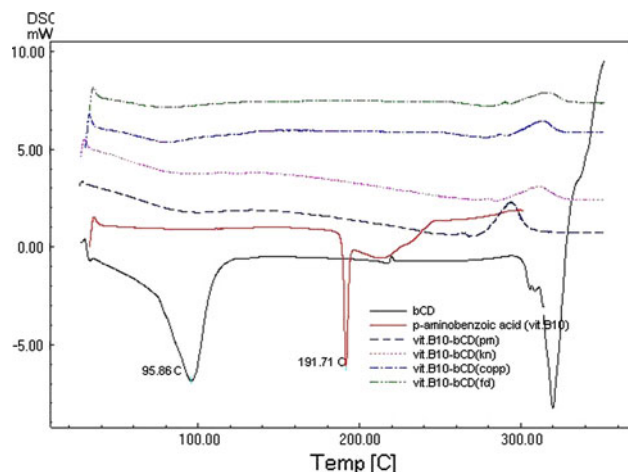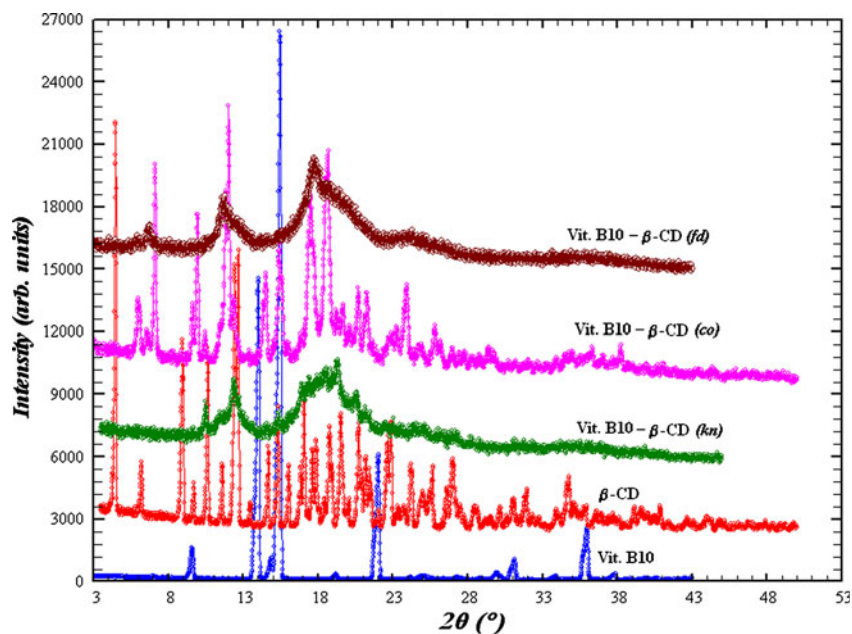
Fig. 4 DSC thermograms of Vitamin B10, β-cyclodextrin and of the corresponding inclusion compounds

¹H NMR

p-Amino benzoic acid has seven protons in its structure; among them, four aromatic protons equivalent each two (H2–H6; H3–H5), see Fig. 5, present two signals as two doublets, at 7.63, respectively at 6.56 ppm in the ¹H NMR spectrum [11].

The observed changes in the chemical shifts values of the Vitamin B10 and of β-CD as compared to the chemical shifts corresponding to the inclusion complex (see Figs. 5, 6 and Table 1) suggests a total inclusion of the guest molecule in the cyclodextrin cavity.

In order to establish the orientation of the Vitamin B10 inside CD cavity, 2D NMR experiments were performed. Two portions of the 2D ROESY spectrum for the Vitamin
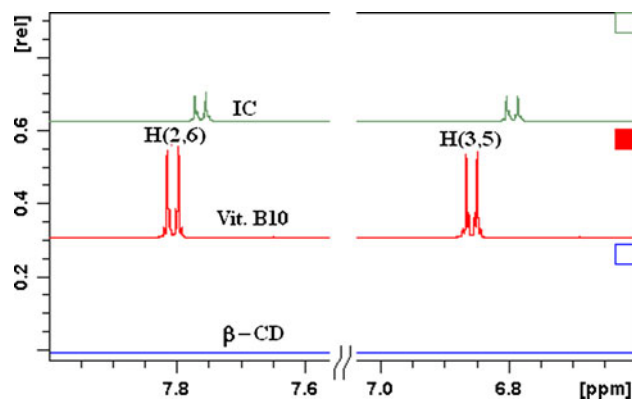

Fig. 5 The chemical shifts in the ¹H NMR spectra of cyclodextrin (β-CD), Vitamin B10 and of the inclusion complex (IC) formed at r = 0.5, 7.9–6.6 ppm domain
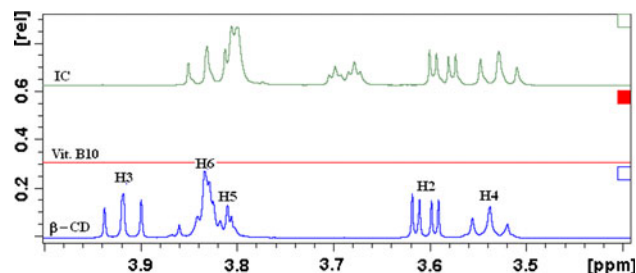

Fig. 6 The chemical shifts in the ¹H NMR spectra of cyclodextrin (β-CD), Vitamin B10 and of the inclusion complex (IC) formed at r = 0.5, 4–3.4 ppm domain

B10—β-CD complex in D₂O are presented in the Figs. 7 and 8.

The cross-peaks between the aromatic hydrogens of Vitamin B10 (located at 6.83 and 7.79 ppm) and the

Table 1 The chemical shifts of the protons belonging to Vitamin B10, β-cyclodextrin and to the inclusion complex (IC)

Proton	Vitamin B10 δ (ppm)	β-CD δ (ppm)	IC (1:1) δ (ppm)	$\Delta\delta$ (ppm)
H2,6	7.8047		7.7619	0.0428
H3,5	6.8565		6.7935	0.063
H3′		3.9183	3.8307	0.0876
H5′		3.8096	3.6850	0.1246
H2′		3.6043	3.5829	0.0214
H6′		3.8331	3.8054	0.0277
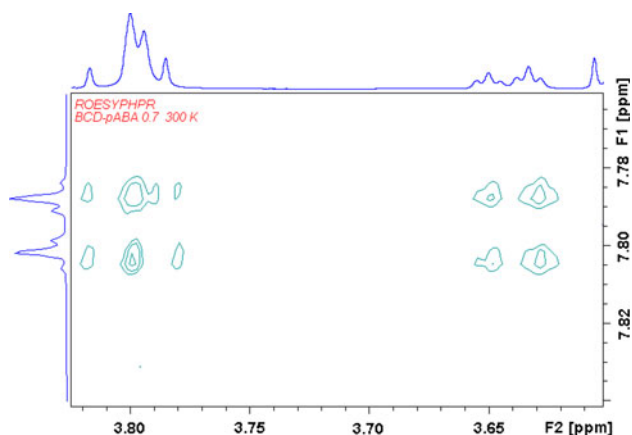
Fig. 7 A section of 500 MHz 2D ROESY symmetrized spectrum of Vitamin B10—β-CD complex showing the interaction between H2,6 of Vitamin B10 and H3′, H5′ of β-CD
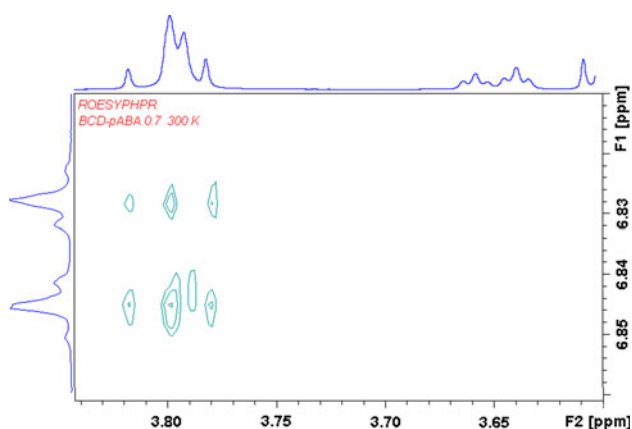

Fig. 8 A portion of 500 MHz 2D ROESY symmetrized spectrum of Vitamin B10—β-CD complex showing the interaction between H3,5 of Vitamin B10 and H3′, H5′ of β-CD

hydrogens H3′ and H5′ (3.77 and 3.63 ppm) indicate that H2,6 (see Fig. 1) interact with H3′ and H5′ whereas the hydrogens H3,5 interact only with H3′. One can conclude that Vitamin B10 molecule enters CD cavity with the carboxylic group near to its narrower rim. This result is in agreement with the data published before [12].

By using the well-known Job plots for different protons of Vitamin B10 and of β-CD, an 1:1 stoichiometry of the inclusion complex was obtained, see Fig. 9.

UV–Vis

The UV–vis absorption spectra of Vitamin B10 are influenced by the solvent nature and by the pH of the solution [12]. By increasing the solution pH, the absorption spectra present a blue shift. At pH = 1, when protonated form dominates in the solution, $\lambda_{abs} \cdot 282$ nm; by increasing pH values till 4–5.5, then the deprotonation of the carboxyl group process begins. Besides neutral species, monoanions appeared which determined a decrease of the absorption maximum till a 265 nm value for the wavelength [13]. The absorption and emission spectra of Vitamin B10 in aqueous solutions, at different pH values and various β-CD concentrations were also studied. At pH = 1 value Vitamin B10 exists as monocation in aqueous solutions, presenting an absorption maximum at 270 nm whereas at pH = 7 when the substance exists as monoanion in aqueous
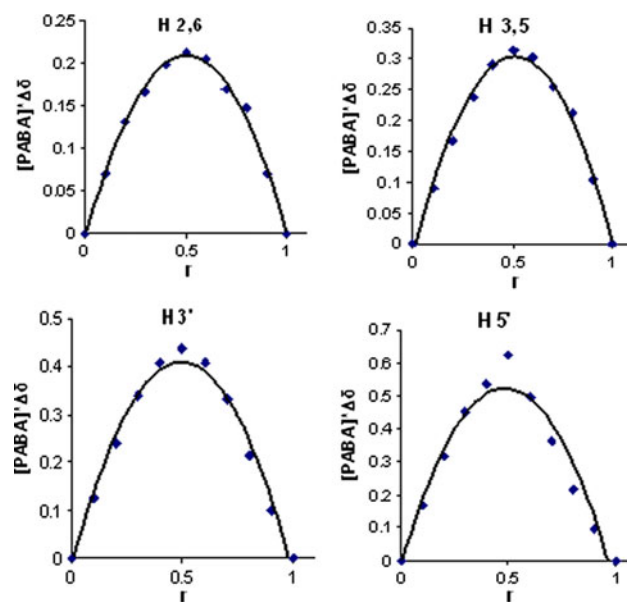
Fig. 9 Job plots for different protons of Vitamin B10 and β-CD

solution, the absorption peak is located at 285 nm [12, 14]. For both situations an isosbestic point is observed. When β-CD is present a weak blue shift (271–269 nm) is observed at pH = 1 and a red shift (266–284 nm) at pH = 7. The absorption intensity increases as the β-CD concentration is increased.

By measuring the absorbance of some aqueous solutions of Vitamin B10 (5×10^{-5} mol/L) and β-CD (10^{-4} –10^{-3} mol/L) at pH ≈ 5 a batochromic shift is observed, see Fig. 10 and Table 2, as compared to that for pure Vitamin B10 (266 nm). The appearance of two isosbestic points is also observed, at 243 and 269 nm.

Fluorescence

The fluorescence of the Vitamin B10 depends on pH value, also: at acid pH value small changes of the emission spectra (with maximum at 350 nm) are observed whereas at pH = 7 a small red shift (340–347 nm) is observed [12]. A diminishing of the intensity as the β-CD quantity is increased is also observed. The red shift of the emission maximum suggests a localization of the COOH group inside β-CD cavity and also the interaction of the NH_2 group of the Vitamin B10 molecule with the OH groups of the β-CD.

The fluorescence measurements were done with a set of aqueous solutions similar to those used for UV–vis absorption ones. The excitation was performed at 269 nm isosbestic point. Two emission maxima were obtained, at 338 and 662 nm. The second maximum can be assigned to the existence of intermolecular hydrogen bridges [15].

By increasing the β-CD excess, an intensity decreasing of the emission maximum and a red shift are observed. At a certain excess level both emission intensity and maximum wavelength remain constant, see Fig. 11 and Table 3.
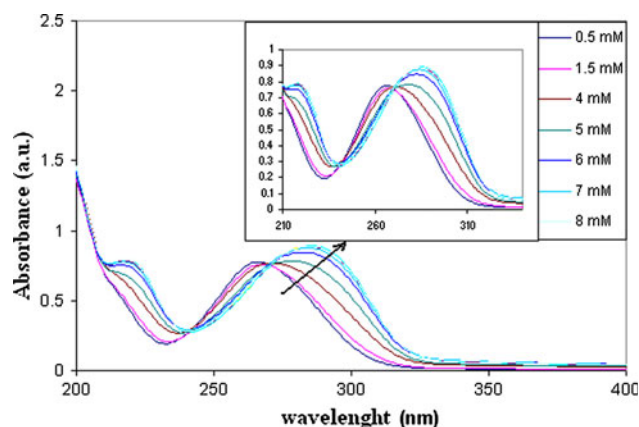
Table 2 Batochromic shift of the absorption maximum

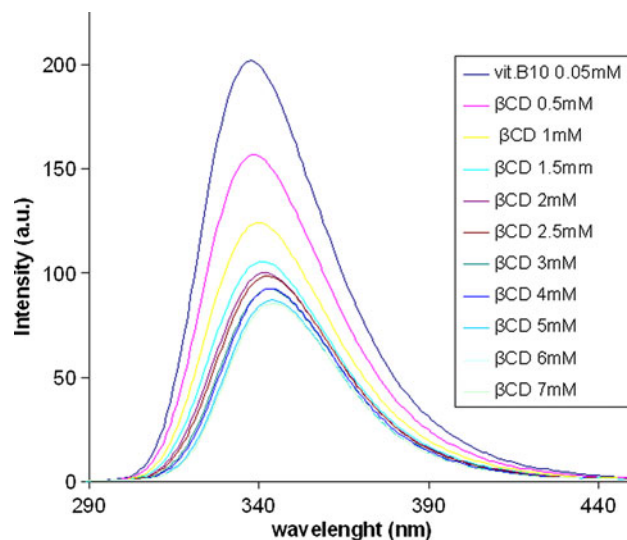| [β-CD] (mM)	λ (nm)	A ($u.a.$)
8	286	0.888
7	284	0.876
6	282	0.846
5	278	0.782
4	271	0.768
1.5	267	0.758
0.5	266	0.776

Fig. 11 Fluorescence spectra of Vitamin B10 and the inclusion complexes with β-CD

Table 3 Batochromic shift of the emission maximum

Sample	λ (nm)	F
[Vit.B10] (0.05 mM)	338	201
[Vit.B10] + [β-CD] (0.5 mM)	339	156
[Vit.B10] + [β-CD] (1 mM)	340	124
[Vit.B10] + [β-CD] (1.5 mM)	341	105
[Vit.B10] + [β-CD] (2 mM)	342	100
[Vit.B10] + [β-CD] (2.5 mM)	343	97
[Vit.B10] + [β-CD] (3 mM)	343	93
[Vit.B10] + [β-CD] (4 mM)	343	92
[Vit.B10] + [β-CD] (5 mM)	344	86
[Vit.B10] + [β-CD] (6 mM)	344	85
[Vit.B10] + [β-CD] (7 mM)	344	85

The decreasing of the fluorescence intensity and the blue shifted fluorescence maximum suggest the formation of an inclusion complex between Vitamin B10 and β-CD. The linearity in the plot reveals the formation of 1:1 complex between Vitamin B10 and β-CD (Fig. 12). The stability

Fig. 10 UV–Vis spectra of the inclusion complexes of Vitamin B10 with β-cyclodextrin ([B10] = 0.05 mM, [β-CD] = variable)

constant K can be determined [11, 16] by using Benesi–Hildebrand equation:

$$\frac{1}{F - F_0} = \frac{1}{K(F_\infty - F_0)[\beta\text{-CD}]_0} + \frac{1}{F_\infty - F_0}$$

where: K, the inclusion constant; F_0, the fluorescence intensity of Vitamin B10 without β-CD; F, the fluorescence intensity with β-CD; F_∞, the fluorescence intensity of Vitamin B10 with the highest concentration of β-CD.

The association constant, obtained from the first emission maximum, is 986 M^{-1} (log $K \approx 2.99$). This value is comparable with the ones obtained by ^{1}H NMR (2.8 ± 0.6) [11] or circular dichroism (2.7) [17].

Molecular modeling

Molecular mechanics computations have been carried out with the HyperChem software [18, 19] to optimize the geometry of p-amino benzoic acid and β-CD in vacuum. The β-CD model was taken from CSD Entry with ref. code BCDEXD03 [20]. p-Amino benzoic acid molecule was obtained from geometrical optimization of the HyperChem software. In the starting models the p-amino benzoic acid molecules were positioned at the larger side of the β-CD cavity. The well-known MM+ method was used with the Polak–Ribière conjugate gradient to minimize the energy of the structures of p-amino benzoic acid molecule and β-CD jointly until a RMS gradient lower than 0.015 kcal/mol/Å was obtained. Details of the algorithms are given elsewhere [19]. Molecular modeling by molecular mechanics established the geometry of the inclusion compound, see Fig. 13, in agreement with the experimental (2D NMR), PM3 and AM1 molecular modeling data [21].

As a result of this process, it was established that whole Vitamin B10 molecule is included inside CD cavity, with
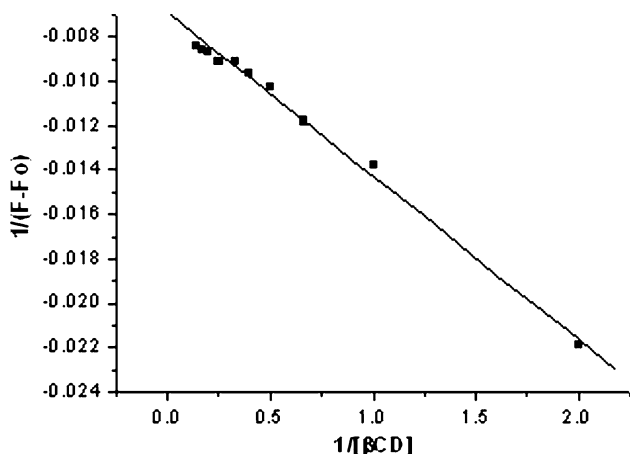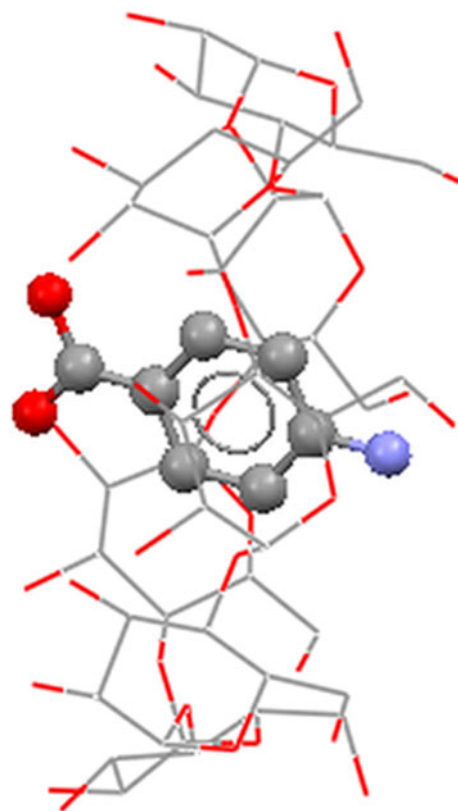

Fig. 13 Molecular modeling of Vitamin B10—β-cyclodextrin inclusion compound

COOH group oriented toward secondary rim, whereas the NH$_2$ group is oriented to the primary rim of β-cyclodextrin.

Conclusions

– v_{as}(C=O) and δ_s(NH$_2$) FTIR vibrational modes changes certify the inclusion compound formation.
– For kn and fd inclusion compounds products a mixture of amorphous and crystalline phases with very small crystallites were obtained, whereas a new quite crystalline inclusion compound was obtained by co product.
– The decreasing of dehydration endothermic peak of cyclodextrin and disappearance of the melting peak of the Vitamin B10 in the DSC thermograms certify the inclusion compounds formation.
– The chemical shifts of the Vitamin B10 and cyclodextrin protons demonstrate the both the formation of an inclusion complex between Vitamin B10 and β-cyclodextrin with an 1:1 stoichiometry.
– 2D NMR experiments showed that Vitamin B10 is included with the carboxylic group inside β-CD cavity near to its narrower rim.

Fig. 12 Benesi–Hildebrand plot for the complexation of Vitamin B10 with β-CD

– The UV–vis and fluorescence data allowed determining the stability constant value of 986 M^{-1}, in agreement with literature data.
– Based on molecular modeling using MM+ approximation the spatial architecture of the inclusion compound was obtained in agreement with FTIR and NMR data.

References

1. Vasilieva, S.: Para-aminobenzoic acid inhibits a set of SOS functions in *E. coli* K12. Mutat. Res. **496**, 89–95 (2001)
2. Bruze, M., Gruvberger, B., Thulin, I.: PABA, benzocaine and other PABA esters in sunscreens and after-sun products. Photodermatol. Photoimmunol. Photomed. **7**, 106–108 (1990)
3. Mackie, B.S., Mackie, L.E.: The PABA story. Aust. J. Dermatol. **40**, 51–53 (1999)
4. Schmidt, T.C., Petersmann, M., Kaminski, L., Loew, V., Stork, E., Fresenius, G.: Analysis of aminobenzoic acids in waste water from a former ammunition plant with HPLC and combined diode array and fluorescence detection. J. Anal. Chem. **357**, 121–126 (1997)
5. Szejtli, J., Osa, T.: Comprehensive Supramolecular Chemistry: vol. 3 Cyclodextrins. Pergamon, copyrighted material. Elsevier Science Ltd, Oxford (1996)
6. Sanchez de la Blanca, E., Nuñez, J.L., Martinez, Y.P.: Espectros de vibraciones algunos acidos benzoicos p-substituidos. An. Quím. **82**, 480–489 (1986)
7. Lu, C.S., Hu, C.J., Yu, Y., Meng, Q.J.: The inclusion compounds of β-cyclodextrin with 4-substituted benzoic acid and benzaldehyde drugs studied by proton nuclear magnetic resonance spectroscopy. Chem. Pharm. Bull. **48**, 56–59 (2000)
8. Lai, T.F., Mash, R.E.: The crystal structure of *p*-amino benzoic acid. Acta Crystallogr. **22**, 885–893 (1967)
9. Castro-Hermida, J.A., Gomez-Couso, H., Ares-Mazas, M.E., Gonzales-Bedia, M.M., Castaneda-Cancio, N., Otero-Espinar, F.J., Blanco-Mendez, J.: Anticryptosporidial activity of furan derivative G1 and its inclusion complex with beta-cyclodextrin. J. Pharm. Sci. **93**, 1197–1206 (2004)
10. Rotich, M.K., Brown, M.E., Glass, B.D.: Thermal studies on mixtures of benzoic and salicylic acids with cyclodextrins. J. Therm. Anal. **73**, 671–686 (2003)
11. Terekhova, I.V., Kumeev, R.S.: Inclusion complex formation of α- and β-cyclodextrins with amino benzoic acids in aqueous solution studied by ^{1}H NMR. J. Incl. Phenom. Macrocycl. Chem. **59**, 301–306 (2007)
12. Stalin, T., Shanthi, B., Vasantha Rani, P., Rajendiran, N.: Solvatochromism, prototropism and complexation of para-aminobenzoic acid. J. Incl. Phenom. Macrocycl. Chem. **55**, 21–29 (2006)
13. Dey, J.K., Dogra, S.K.: Solvatochromism and prototropism of 2-(3′-hydroxyphenyl)benzoxazole and 2-(4′-hydroxyphenyl)benzoxazole in the excited singlet state. J. Photochem. Photobiol. A: Chem. **59**, 307 (1991)
14. Shaomin, S., Yu, Y., Jinghao, P.: Study on molecular recognition of para-aminobenzoic acid species by α-, β- and hydroxypropyl-β-cyclodextrin. Anal. Chim. Acta **458**, 305–310 (2002)
15. Wang, Z.P., Tang, X.D., Ding, Z.J.: Raman and photoluminescence spectroscopy study of benzoic acid at high pressures. J. Phys. Chem. Solids **66**, 895–901 (2005)
16. Benesi, H.A., Hildebrand, J.H.: A spectrophotometric investigation of the interaction of iodine with aromatic hydrocarbons. J. Am. Chem. Soc. **71**, 2703–2707 (1949)
17. Harata, K.: Induced circular dichroism of cycloamilose complexes with meta- end para-disubstituted benzenes. Bioorg. Chem. **10**, 255–265 (1981)
18. HyperChem™ Release 4. Hypercube Inc., Waterloo, ON, Canada (1994)
19. Computational Chemistry. Hypercube Inc., Waterloo, ON, Canada (1994)
20. Steiner T., Koellner G.: Crystalline β-cyclodextrin hydrate at various humidities: fast, continuous, and reversible dehydration studied by X-ray diffraction. J. Am. Chem. Soc. **116**(12), 5122–5128 (1994)
21. Fatiha, M., Djameleddine, K., Leila, L.: Molecular modeling study of para amino benzoic acids recognition by β-cyclodextrin. Orbital **1**, 26–37 (2009)