Scientific paper

Complexation of Amlodipine Besylate with β-Cyclodextrin

Otilia Bradea,[1] Iren Kacso,[2] Gheorghe Borodi,[2] Attila Bende[2] and Ioan Bratu[2,*]

[1] *University of Medicine and Pharmacy, Cluj-Napoca, Romania*

[2] *National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania*

** Corresponding author: E-mail: ibratu@gmail.com*
Phone: (40) 264 584037; Fax: (40) 264 420042

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Abstract

In this paper the procedure for the preparation of inclusion compounds of bioactive substance 2-[(2-aminoetoxy)-methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine dicarboxylic acid 3-ethyl-5-methyl esterbenzene sulfonate, called amlodipine besylate with β-cyclodextrin (β-CD) and their structural characterization was described. Molecular inclusion compound of amlodipine besylate is obtained by different preparation method: kneading, co-precipitation and freeze-drying. The so obtained compounds were investigated by FTIR spectroscopy, X-ray diffraction method and differential scanning calorimetric measurements (DSC) to evidence their formation. Molecular modeling (using DFT theoretical computations) shows the spatial architecture of the inclusion compound in good agreement with FTIR experimental data: the drug is included with dihydropyridine dicarboxylate part inside β-cyclodextrin cavity. The inclusion of amlodipine besylate in β-cyclodextrin increases the stability and bioavailability of the drug.

Keywords: FTIR, X-ray powder diffraction, DSC, molecular modeling, inclusion compounds, β-cyclodextrin

1. Introduction

Amlodipine besylate, (*RS*)-3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate monobenzenesulphonate, (AML) is a relatively new potent long-acting calcium channel blocking agent with slow onset of vasodilatory action.[1] AML block the inward movement of calcium by binding to L-Thype calcium channels in the heart and in smooth muscle of the coronary and peripheal vasculature relaxing the smooth muscle and dilating arterioles.[2,3] The molecular formula of this compound is presented in Fig. 1.

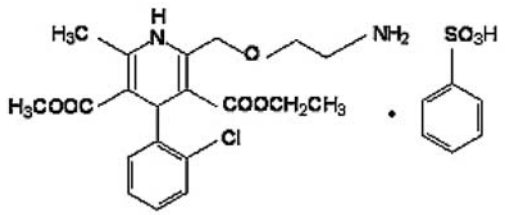

Fig. 1: Amlodipine besylate molecule

It is know that the solubility in water, photostability and the bioavailability of the drug is increased when is this incorporated in cyclodextrins.[4,5,6]

Cyclodextrin (CD) are cyclic (α-1,4)-linked oligosaccharides of α-D-glucopyranose, containing a relatively hydrophobic central cavity and hydrophilic outer surface. The cyclodextrin are not perfectly cylindrical molecules but the toroidal or cone shaped. The primary hydroxyl groups are located on the narow side of the cone shape,

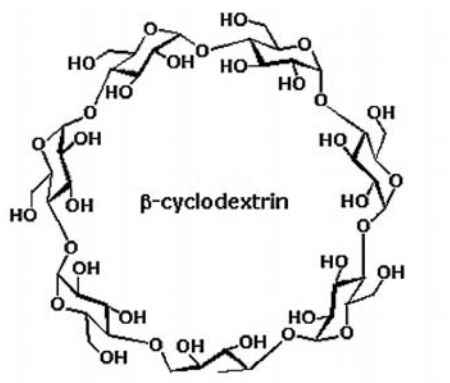

Fig. 2: β-Cyclodextrin molecule

while the secondary hydroxyl groups are located on the wider edge, see Fig. 2. Cyclodextrin and their derivatives have been of considerable interest in the pharmaceutical field because of their potential to form complexes with a variety of drug molecules.[7,8]

Cyclodextrin are used to increase the solubility of water insoluble drug through inclusion complexes formulation.[9,10,11,12] Advantages of drugs complex with cyclodextrin have been reported in scientific literature which includes-increased solubility, enhanced bioavailability, improved stability, masking of bad test or odor, reduced volatility.[13,14,15,16]

The inclusion compounds of AML were prepared by different methods: physical mixture, kneading, co precipitation and freeze-drying. Several experimental methods (such as FTIR spectroscopy, X-ray diffraction, DSC and fluorescence spectroscopy) together with molecular modeling were employed to confirm inclusion compound formation. The architecture of these inclusion compounds was proposed.

2. Materials and Methods

The cardiovascular bioactive substance, AML was obtained from Labormed Pharma, Bucharest (Romania), β-CD (having ≤10% in water weight) was purchased from Sigma Aldrich Chemie GmbH and was used without further purification.

A physical mixture (*pm*) of AML and β-CD (1:1 molar ratio) was obtained by a gentle grinding of the mixture in the agate mortar. The inclusion compound was obtained also by the kneading method (*kn*) using distilled wather as the wetting agent. AML and β-CD were mixed in 1:1 molar ratio and then grounded in an agate mortar. The wetting agent was then added to the mixture and kneaded for at least one hour. The paste thus obtained was dried at 38 °C. The inclusion compound was obtained by co-precipitation (*co*) as follows: by mixing the amounts of AML and β-CD (5 mM aqueous solution) in 1:1 molar ratio, stirring for several days, succeded by evaporation and drying at 38 °C.

The freeze-dried product (*fd*) was obtained by freeze-drying by immersion the congelated 1:1 aqueous solution in a Alpha 1-2 LD plus freezer-drier for over 24 h. Both processes were performed in darkness in order to protect AML from photodegradation.

FTIR spectra were obtained with a JASCO 6100 FTIR spectrometer in the 4000–400 cm^{-1} spectral range with a resolution of 2 cm^{-1}, using the KBr pellet technique.

Differential scanning calorimetry (DSC) was carried out by means of a Shimadzu DSC-60 calorimeter, the sample was heated in the range of 30–350 °C with a heating rate of 10 °C/min in crimped aluminum sample cell. The purge gas was a nitrogen flow of 60 mL/min. For data collection the Shimadzu TA-WS60 and TA60 2.1 software were employed.

X-ray diffraction measurements were performed with a Bruker D8 Advance diffractometer in the 2θ = 2–50° angular domain using Cu $K\alpha_1$ radiation. In order to increase the resolution a monochromator was used to eliminate the $K\alpha_2$ radiation.

The fluorescence spectra due to the interactions between drugs and AML were studied on a ABLE & JASCO FP 6500 recording spectrofluorometer, applying an excitation wavelength of 366 nm and monitoring the emission wavelength over the 375–625 nm range. Both excitation and emission slit openings were set at bandwidths of 3nm respectively 5 nm.

Molecular modeling computations have been carried out at DFT level of theory using the M06-2X[17] exchange-correlation functional implemented in the NWChem[18] program suite. For the geometry optimization the def2-SVP[19] basis set was used, while the intermolecular interaction energy was computed using the def2-TZVP[19] basis set. In the starting model the AML was positioned at the larger side of the β-CD cavity.

3. Results and Discussion

3. 1. FTIR Spectroscopy

FTIR spectra of the so-obtained inclusion compounds are presented in the Fig. 3.

FTIR spectrum of the 1:1 physical mixture (*pm*), see Figs. 3a and b, contains the absorbtion bands of each component, so no inclusion compound was obtained in this case. For the kneaded, co precipitated and freeze-dried products the corresponding FTIR spectra show several differences as follows:

In the 4000–3000 cm^{-1} spectral region (see Fig. 3a), where O–H stretching vibrations appear, one can observe shifts of this broad band, especially for *co* and *fd* products. They are due to the water expelling during the complexation process. In the medium IR range, see Fig. 3b, the spectra of *kn*, *co* and *fd* products differ as compared to the AML spectrum, *i.e.* some interactions appear that can justify the inclusion compounds formation. In a previous paper it was demonstrated that hydrated and anhydrous crystal forms of this drug can be distinguished through their FTIR spectra.[20] If the starting AML compound is an anhydrous product, in the *kn* and *co* products' spectra the monohydrate compound can be observed mostly as an un reacted product, whereas for the *fd* product one can observe a real inclusion compound formation. FTIR experiments show that the dihydropyridine carboxylate part is included inside CD cavity during the inclusion process, the N–H bending vibration located at 1492 cm^{-1} in AML spectrum being shifted to 1484 cm^{-1} in the *fd* spectrum. The hydrogen bonds is implied severely in the inclusion compound formation.

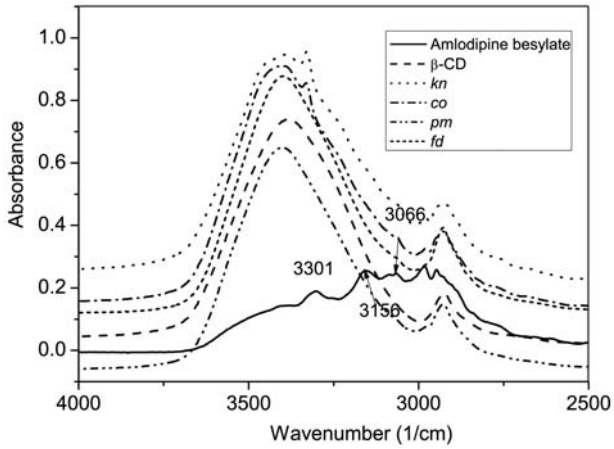

Fig. 3a: FTIR spectra of of β-CD, of pure AML and of its inclusion compounds, 4000–2500 cm^{-1} spectral region.

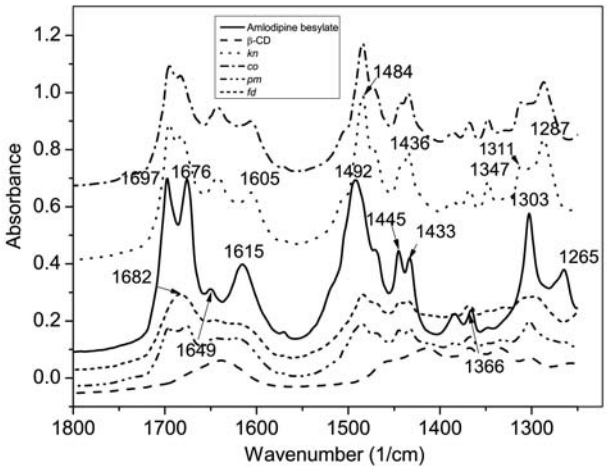

Fig. 3b: FTIR spectra of β-CD, of pure AML and of its inclusion compounds, 1800–1250 cm^{-1} spectral region.

3. 2. X-ray Diffraction

The powder diffraction patterns of AML, β-CD and of the inclusion compounds are shown in the Fig. 4.

To evidence the inclusion compound formation between AML and β-CD, the X-ray diffraction patterns of β-CD and of AML were compared with the diffraction pattern of the inclusion compounds.

X-ray diffraction patterns for kneaded and co precipitated products are different as compared to the corresponding one for physical mixture. In the case of freeze-dried product the amorphous character of the pattern demonstrated the inclusion compound formation, also.

3. 3. DSC

DSC reveals some information on solid-state interactions between drug and cyclodextrin. The DSC thermograms of pure components and of AML– β-CD inclusion compounds are presented in Fig. 5. The curve for the β-cyclodextrin revealed a broad endothermic signal from 74 to 118 °C, 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).[21] From 290ºC onwards there is a new endothermic succeeded of the exothermic, corresponding to the melting, respectively the degradation of the β-CD.

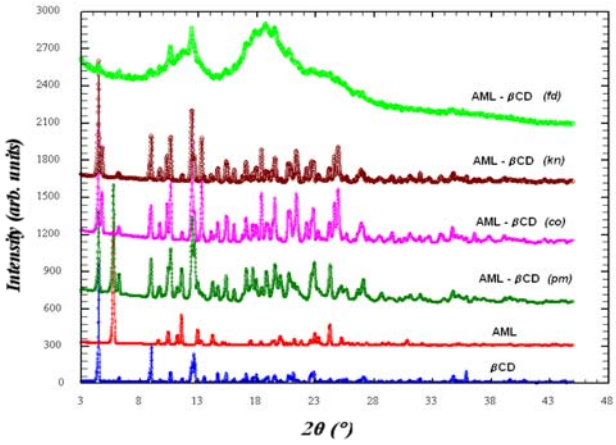

Fig. 4:. X-ray powder diffraction patterns of AML, β-CD, physical mixture of AML and β-CD (*pm*), inclusion compound of AML and β-CD

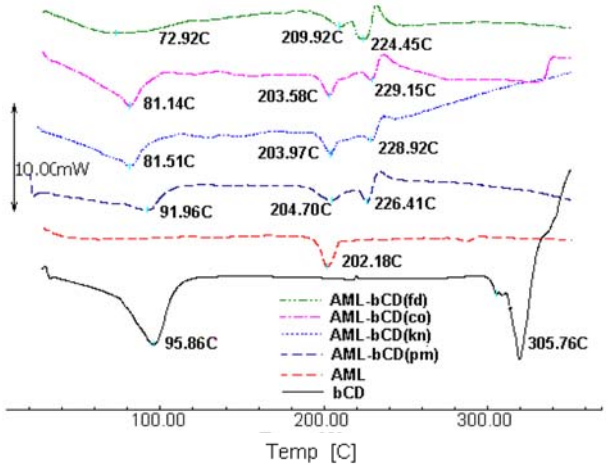

Fig. 5: DSC curves of the AML, β-CD and their inclusion compound, obtained by different methods

The DSC curve of AML presents a sharp endothermic peak at 202 °C, corresponding to the melting of the drug. Thermal behavior of the components' physical mixture shows thermal events characteristics to AML and β-cyclodextrin: the water loss, the melting endotherm and the decomposition signal. In the case of the inclusion compounds of AML with β-cyclodextrin obtained by

kneading and co precipitation methods, a small decreasing of dehydration endothermic peak of cyclodextrin was observed, as well as a decreasing of the endothermic melting peak of the AML followed of an exothermic signal, probable due to the decomposition of the sample, around. The decreasing of this signal was stronger in the case of freeze-dried compound, where the complexation process shows higher efficiency.

3. 4. Fluorescence

The fluorescence measurements were done with a set of aqueous solutions where the concentration of the AML was constant (5×10^{-5} mol/L) and the β-CD concentration was increased progressively ($10^{-4} \div 7 \times 10^{-3}$ mol/L). The solutions were kept for 12 h at room temperature in order to equilibrate them. The fluorescence spectra due to the interactions between AML and β-CD were recorded applying an excitation wavelength of 366 nm and monitoring the emission wavelength over the 375–625 nm range.[22] The emission maximum was obtained at 452 nm. The increase of the fluorescence intensity with the concentration of the β-CD (as other authors demonstrated before[23]) suggests the formation of the inclusion complex between AML and β-CD (see Fig. 6).

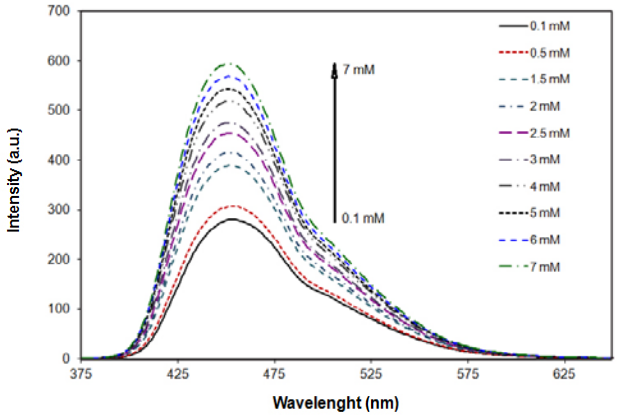

Fig. 6: Fluorescence spectra of the inclusion complexes between AML and β-CD

The linearity in the Benesi-Hildebrand plot reveals the formation of 1:1 complex between AML and β-CD (Fig. 7).

The stability constant K can be determined by using Benesi–Hildebrand equation:[24]

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

Where: K – the inclusion constant; F_0 – the fluorescence intensity of AML without β-CD; F– the fluorescence intensity with β-CD; F_∞ – the fluorescence intensity of AML with the highest concentration of β-CD.

Table 1: Increasing of emission intensity with β-CD concentration

[β-CD] mM	F (a.u)
0.1	279.998
0.5	306.763
1.5	388.529
2	414.878
2.5	454.279
3	476.109
4	518.5
5	542.616
6	567.521
7	593.31

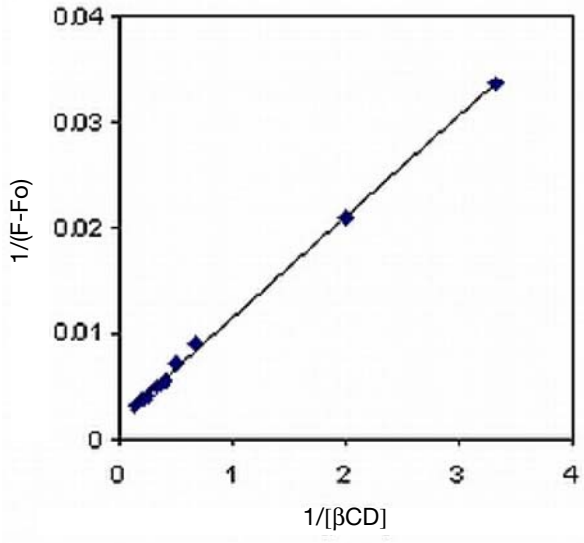

Fig. 7: Benesi–Hildebrand plot for the complexation of AML with β-CD

The association constant value, obtained from the emission maximum, is 210 M^{-1}.

3. 5. Inclusion Compound Geometry

Molecular modeling established the geometry of the inclusion compound (see Fig. 8) in agreement with the experimental (FTIR) data.

As a result of this study, it was found that AML molecule is included inside β-CD cavity, with the ethyl-acetate and 2-ethoxy-1-ethanamine parts, chlorophenyl group oriented toward primary rim of β-cyclodextrin. The two fragments of ethyl-acetate and of 2-ethoxy-1-ethanamine show repulsion between them and therefore the β-CD cavity is also slightly distorted. We have calculated the intermolecular interaction energy in the host-guest complex. The result shows a binding of –49.16 kcal/mol for the AML molecule. The deformation energy of the AML guest molecule (= 9.04 kcal/mol) is mostly due to the straitened cavity of the β-CD.

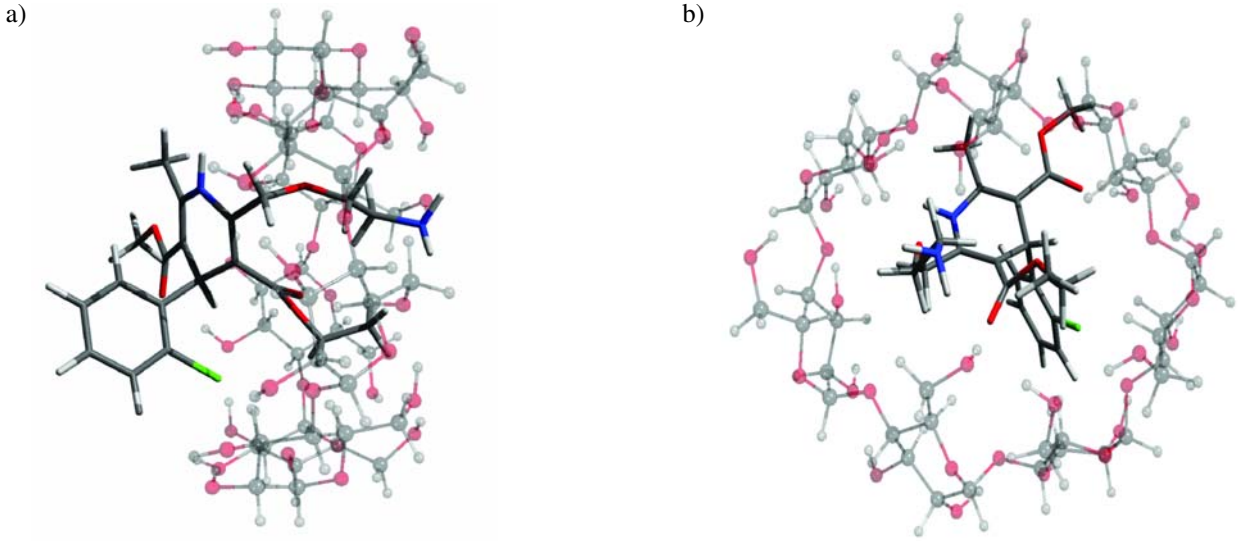

Fig. 8: Molecular modeling of AML–β-CD inclusion compound. (a) Side view, (b) Top view.

4. Conclusions

X-ray diffraction certifies the AML – β-CD inclusion compound formation with a higher amorphous phase content for the freeze-dried product.

FTIR experiments show that the dihydropyridine carboxylate part is included inside CD cavity during the inclusion process, the hydrogen bonds being implied severely in the inclusion compound formation.

DSC measurements shows a strong decreasing of the characteristic endothermal signals in the case of freeze-dried product, probable due to inclusion compound formation by this method.

The increase of the fluorescence intensity with the concentration of the β-CD suggests the formation of the inclusion complex between AML and β-CD. The 1:1 stoichiometry and the association constant (210 M^{-1}) of AML-β-CD inclusion complex were determined by using fluorescence measurements.

Molecular modelling shows that the drug is included with the dihydro-pyridine part inside β-CD cavity.

5. Acknowledgments

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6. References

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Povzetek

Pripravili in okarakterizirali smo spojine, ki smo jih pripravili z vključevanjem zdravilne učinkovine amlodipinbesilata (3-etil 5-metil (4RS)-2-[(2-aminoetoksi)metil]-4-(2-klorofenil)-6-metil-1,4-dihidropiridin-3,5-dikarboksilat bezensulfonat) v β-cyclodelstrin. Spojine smo pripravili na različne načine in jih proučevali z FTIR spectroskopijo, difrakcijo rentgenskih žarkov in diferenčno dinamično kalorimetrijo (DSC). Struktura spojin, dobljena z molekularnim modeliranjem, se dobro ujema s podatki iz FTIR spektroskopije in kaže, da je učinkovina z dihidropiridinskim delom ujeta v notranjost β-cyclodekstrina. Vključevanje amlodipinbesilata v β-cyclodekstrin naj bi povečalo stabilnost in bio-razpoložljivost učinkovine.