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ORIGINAL ARTICLE

A new inclusion complex of amlodipine besylate and soluble β-cyclodextrin polymer: preparation, characterization and dissolution profile

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Abstract Amlodipine besylate (AML) has become the most popular blood pressure medication for hypertensive pets. It belongs to the class I (high solubility and high permeability) according to BCS and is marketed in Europe only as white tablets equivalent to 2.5, 5.0 and 10.0 mg of amlodipine for oral administration. Unfortunately, oral AML dosage for cats and dogs is in the range 0.1–0.2 and 0.625–0.125 mg/kg/die respectively. Moreover, AML shows a slight solubility in water according to Ph. Eur. 7°. According to these considerations, the aim of this work was the complexation between soluble β-cyclodextrin polymer (CD) and AML using the solubilization/freeze-dried method to obtain powders easily dosable and soluble in water for the treatment of hypertension in pets. The complex in solution was evaluated by phase solubility studies that indicated the optimal 2:1 drug/CD ratio to form a stable complex. UV–Vis absorption and circular dichroism showed the formation of a complex with a weak bond such as confirmed by differential scanning calorimetry, infrared spectroscopy and fluorescence microscopy. In vitro dissolution/release tests were performed in water to investigate the influence of formulative parameters on drug dissolution/release properties. The inclusion of AML in CD increased its wettability, dissolution rate and solubility in water. This method could be a suitable approach for the administration of an extemporaneous solution of the antihypertensive drug to guarantee a correct dose to pets increasing the compliance.

Keywords Soluble β-cyclodextrin polymer · Amlodipine besylate · Inclusion complex · Phase solubility · Morphological and physicochemical characterization · In vitro dissolution/release test

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Introduction

Amlodipine besylate (AML) (Fig. 1a) has become the most popular blood pressure medication for hypertensive cats and dogs [1]. It is the besylate salt of amlodipine, a dihydropyridine long-acting calcium channel blocker that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. The consequence relax of arterial muscle allows a decrease of blood pressure [2, 3]. While other calcium channel blockers are rapidly and completely absorbed after ingestion, AML has a much slower absorption rate. Peak plasma concentrations occur at 4–12 h in dogs, and similar rates have been reported in cats [4].

In cats and in dogs, oral AML dosage should be in the range 0.1–0.2 and 0.625–0.125 mg/kg/die respectively. Unfortunately, AML is marketed in Europe as white tablets equivalent to 2.5, 5.0 and 10.0 mg of amlodipine for oral administration, with the consequent need to break the tablet to obtain the correct dose for the treatment of hypertension in pets. Nevertheless, it is sensitive to light [5] and was termed class I (high solubility and high permeability) compound according to BCS. Moreover, AML is slightly soluble in water (Ph. Eur. 7° Ed.) and even if it belongs to soluble and permeable drugs, its oral bioavailability is limited by the dissolution rate in the biological fluids [6],

leading to an irregular absorption from oral solid dosage form.

One promising way to overcome these limits is the improvement of the oral performance of AML using β-cyclodextrin (CD) and its derivatives [7–9] to develop very soluble powders of drug with a consequent enhancement of its dissolution rate.

CDs are well known for their ability to form inclusion complex with a large number of molecules [10–14], among which slightly hydrosoluble pharmaceutical active ingredient. Moreover, these polymers improve stability of drugs to air and light [15, 16]. Among different CDs, β-CD is characterized by a low water solubility (1.8 g/100 g water) as a result of hydrogen bonds between the –OH groups from the neighbouring particles. Recently, increasing attention has been paid to modified β-CDs as inclusion complex due to their high water solubility and high hygroscopicity. Moreover, the large surface, allows the drug incorporation into the modified CD with a consequent increase of water dissolution and bioavailability [8]. Recent studies showed that a derivative of CD, such as hydroxypropyl-β-CD, was not able to improve the dissolution rate of enantiomers of AML when the inclusion complexes were prepared by solvent evaporation method, demonstrated that it was not possible to obtain an inclusion complex [17, 18]. On the other hand, another work reported that the co-precipitation method was effective to achieve inclusion complexes of AML with 2-hydroxypropyl-β-CD and β-CD [7].

In this work we selected soluble β-CD polymer (β-CD crosslinked with epichlorohydrin; β unit >50 %, Product Information Sheet, Cyclolab) (Fig. 1b) among the more adequate coating materials for oral administration due to many advantages such as high water solubility (>25 g/100 mL at 25 °C, Product Information Sheet, Cyclolab), improving of drug chemical stability, increasing drug apparent aqueous solubility, enhancing oral bioavailability of poor or slightly soluble drugs without changing their pharmacokinetic properties [6, 19]. The solubilization/freeze-dried method was used due to the high solubility of the selected CD.

Nevertheless, the efficiency of complexation is often not very high, and therefore, relatively large amounts of CDs must be used to obtain the desired effect [20]. In order to achieve a compromise between AML complexation efficiency, enhancement of the dissolution rate in water, and use of small amounts of CD, the best CD/AML molar ratio was selected by calculating the isotherm solubility, stoichiometric ratio and stability constant.

The influence of different parameters (molar ratio, production method) on drug dissolution/release properties as well as on morphology, stability and thermal behaviour of the produced complex was also studied.

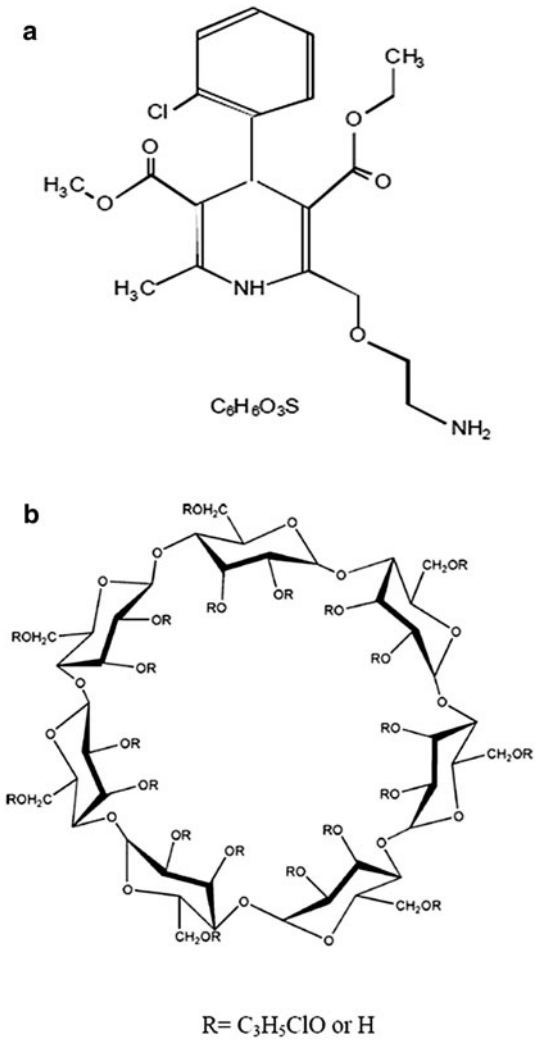

Fig. 1 Chemical structures of AML (**a**, AML) and β-CD crosslinked with epichlorohydrin (**b**, CD)

Materials and methods

Materials

AML (CAS No. 111470-99-6, molecular weight: 567.1 g mol^{-1}), and Ammonium acetate were supplied by Sigma Aldrich (Milan, Italy); soluble β-CD polymer (β-CD crosslinked with epichlorohydrin; CD) was purchased from Cyclolab (CycloLab Cyclodextrin Research and Development Laboratory, Ltd., Hungary, medium molecular weight: 8,500 g mol^{-1}). All other chemicals used were of reagent grade.

Methods

Phase solubility studies

Amlodipine solubility The solubility of AML was previously determined in water (4 g/L) as follows: an excess

amount of AML raw was introduced into glass vials containing 1.5 mL of solvent; the sample was shaken and then stored at room temperature (25 °C). After 3 days, the liquid phase was centrifuged for 5 min at 3,000 rpm, the supernatant was filtered with 0.45 μm filters and analyzed by UV and HPLC.

UV method Calibration curves were previously prepared in distilled water. The proportionality between absorbance and concentration was verified in the range from 2.5 to 10 mg/L ($R^2 > 0.999$).

AML concentration in the supernatant was evaluated by measuring absorbance at λ 237 nm in 1 mm cell (UV/Vis spectrometer Lambda 25, Perkin-Elmer Instruments, MA, USA).

HPLC method AML concentration was also evaluated by an HPLC apparatus (Varian Prostar mod. 230, Varian, Milan, Italy) equipped with a 20 μL Rheodyne 7125 injection valve (Rheodyne, Cotati, Ca, USA) autosampler Varian mod D10 (Varian, Milan, Italy) and a UV–Vis detector set at λ 273 (Ph Eur. 5° Ed.) and Software Galaxie. The chromatographic analysis were performed on a Lichrosphere® 100 C_{18} RP column (particle size 5 μm; 250 × 4 mm ID; Merck, Damstadt, Germany), equipped with a 5 μm Lichrosphere® 100 C_{18} RP guard column (4 × 4 mm ID; Merck, Damstadt, Germany) and eluted isocratically at room temperature. The mobile phase consisted of a 70:30 (v/v) mixture of methanol/water containing 2.3 g of $CH_3COO^-NH_4^+$ (Ph. Eur 5°); the flow rate was set at 0.4 mL/min. *Linearity.* Reference standard solutions were prepared in triplicate at five concentration levels (0.1–0.6 μg/mL). Twenty microliters of each solution were analyzed in triplicate. The standard curve was analyzed using linear least-squares regression equation derived from the peak areas (y = 269.69x + 3.1441, $R^2 = 0.993$) where y is the peak area and x the concentration used). *Specificity*. Peak associated with AML was identified by retention time and confirmed by co-injection, as well as by UV (Perkin-Elmer Lambda 25 spectometer).

Binding constant determination

The equilibrium from AML and CD was

$$\mathrm{nCD} + \mathrm{mS} \overset{\mathrm{K_{m:n}}}{\leftrightarrow} \mathrm{CD_n \cdot S_m} \tag{1}$$

$$K_{m:n} = \frac{[S_m \mathrm{CD}_n]}{[S]^m [\mathrm{CD}]^n} \tag{2}$$

where $K_{m:n}$ is the binding constant [21].

With the molar extinction coefficient of AML, and CD/AML complex, is possible to calculate the $K_{m:n}$ using the Benesi–Hildebrand equation [22] in the following modified form [23, 24]:

$$\frac{[S_t]l}{A - A^0} = \frac{1}{\Delta\varepsilon} + \frac{1}{K_{m:n}[L]\Delta\varepsilon} \tag{3}$$

where $[S_t]$ and [L] represent the concentrations of drug and CD, respectively. A and A^0 are the absorbances of drug in the absence and presence of CD, respectively. $\Delta\varepsilon$ is the difference in molar absorption coefficients between complexed/bound and free drug. l is the optical path length of the solution.

This expression for the formation of a pure 1:2 CD/AML complex and $l = 1$ becomes:

$$\frac{[S_t]^2}{A - A^0} = \frac{1}{\Delta\varepsilon} + \frac{1}{Kb[L]\Delta\varepsilon} \tag{4}$$

Isotherm solubility, stoichiometric ratio and stability constant The solubility phase diagrams of the inclusion complex were determined by Higuchi and Connors method [25]. An excess amount of AML was suspended in 1 mL of water and then different amounts of CD in the molar ratio 1:3, 1:2.5, 1:2, 2:1 (CD/AML) were added. The samples were shaken and then stored at room temperature. After 1 h, the samples were centrifuged for 5 min at 3,000 rpm, the supernatants were filtered with 0.45 μm filters and analyzed in UV apparatus in 1 mm cell at λ 237 nm. The stoichiometric ratio of CD/AML complex was evaluated by Eq. 1

$$\frac{LB - LA}{S_t - S'} \tag{5}$$

where LB − LA was the molar concentration of host to complex the drug; while S_t was the total amount of drug and S′ was the molar concentration of the complexed guest.

From Eq. 2, given that [26]

$$[S] = S_m;\ S_t = S_0 + m[S_m CD_n] \text{ and } [CD]_t = [CD] + n[S_m CD_n] \tag{6}$$

Then the values of $[S_m CD_n]$ and [CD] can be obtained by,

$$[S_m CD_n] = \frac{S_t - S_0}{m} \tag{7}$$

$$[CD] = [CD]_t - n[S_m CD_n] \tag{8}$$

where S_0 is the equilibrium solubility of the drug in absence of CD, S_t is the total concentration of S complexed and uncomplexed and CD_t is the total concentration of CD.

For formation of complexes which are first order respect to CDs, the following equation may be derived:

$$S_t = \frac{mKS_0^m CD_t}{1 + KS_0^m} + S_0 \tag{9}$$

and the general expression for the slope of the straight line of the plot is

$$\text{Slope}: \frac{\left(mK_{m:n}S_0^m\right)}{\left(1+K_{m:n}S_0^m\right)} \quad (10)$$

This expression for the formation of a pure 1:2 CD/AML complex becomes:

$$\text{Slope}: \frac{2K_{1:2}S_0^2}{1+K_{1:2}S_0^2} \quad (11)$$

from which:

$$Kc_{1:2} = \frac{\text{slope}}{S_0^2(2-\text{slope})} \quad (12)$$

where Kc is the stability constant (L mol^{-1}), slope is obtained from the linear relationship between the concentration of AML and CD, and S_0 is the AML solubility (mmol L^{-1}) in absence of CD.

Each experiment was carried out in triplicate (RSD < 3 %).

Complex and physical mixture preparation

Preparation of the solid complex The CD/AML solid complex was prepared with the solubilization/freeze-dried method as follows: 1.7×10^{-6} mol of AML was suspended in 1.5 mL of water and then was added with 8.7×10^{-7} mol of CD. The sample was vortexed for 60 s, stored for 1 h at −4 °C and lyophilized for 24 h to obtain the clathrate CD/AML.

Preparation of the physical mixture Physical mixtures (CD/AML mix) were prepared by simple mixing in mortar CD/AML 1:2 molar ratio.

Complex characterization

Drug content and inclusion efficiency (IE)

The drug content and IE in CD/AML was assessed by both UV and HPLC. *UV method.* Samples (2 mg) of each complex were dissolved in 3 mL of water and vortexed for 60 s. The drug content was determined spectrophotometrically (UV/Vis 1601 Shimadzu, MA, USA) at λ 237 nm (1 mm cell; Spectracomp 602, Advanced Products SRL, Milan, Italy). Each analysis was made in triplicate and the results were expressed as average value. Values were superimposable to those obtained by HPLC analyses. *HPLC Method.* Samples (2 mg) of three complexes were dissolved in 3 mL H_2O, vortexed for 60 s and centrifuged at 3,000 rpm for 5 min. The concentration was determined in the supernatant solutions using the same chromatographic conditions described in the precedent section. Each analysis was performed in triplicate and the results, expressed as average value, are reported in Table 1.

Table 1 Percentage of theoretical drug content (TDC%), actual drug content (ADC%), and inclusion efficiency (IE%) of AML in physical mixture (CD/AML mix) and in CD/AML inclusion complex (CD/AML complex)

Sample	TDC%	ADC%	IE%
AML	100.0	100.1 ± 0.1	100.1 ± 0.1
CD/AML mix	100.0	99.5 ± 0.2	99.5 ± 0.2
CD/AML complex	100.0	100.0 ± 0.1	100.0 ± 0.1

The IE, was calculated from the ratio of actual (ADC) to theoretical drug content (TDC) in freeze-dried complex (Table 1).

Morphology and particle size analyses

Samples were prepared by sprinkling a small amount of dry powders (10 μg) or liquid complex (10 μL) onto a microscope slide and then were observed with a Zeiss Axiophot fluorescence microscope (FM), with 40, 63 and 100 × 1.4 NA plan Apochromat oil immersion objectives (Carl Zeiss Vision, München-Hallbergmoos, Germany) using standard 4′,6-diamidino-2-phenylindole optics that adsorb violet radiation (max 372 nm) and emit a blue fluorescence (max 456 nm).

Absorption spectra

The absorption spectra of CD/AML complex (1:2 molar ratio) and raw materials were taken with a Shimadzu UV–Vis spectrometer model UV-1601. The molar concentrations examined were 5×10^{-4} mol for CD and 1×10^{-3} mol for AML.

Circular dichroism

The circular dichroism spectra were obtained using a Jasco J-600 Spectropolarimeter (Tokyo, Japan). The signal-to-noise ratio was improved by superimposition of three different scans.

Differential scanning calorimetry (DSC)

Raw materials and CD/AML complex (1:2 molar ratio) were analyzed by DSC on an indium calibrated Mettler Toledo DSC 821e (Mettler Toledo, OH, USA). Thermograms were recorded by placing accurately given quantities (1–2 mg weighed with a microbalance MTS Mettler Toledo, OH, USA) of each sample in a 40 μL aluminium pan which was sealed and pierced. The sample underwent two thermal cycles. First, the sample was scanned (10 °C/min) between 25 and 350 °C and then was cooled to 25 °C

(20 °C/min). From heated cycle, melting temperature T_m and heats of fusion (ΔH_m) were measured.

Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectra were obtained using a Jasco FT-300 (Tokyo, Japan) FTIR spectrometer. Samples of complex CD/AML and of raw materials were analyzed as KBr discs in the spectral region 400–4,000 cm^{-1}.

Stability studies

Physicochemical stability

Evaluation of the physicochemical stability of CD/AML complex was performed according to the stress testing and accelerated method reported by the guide lines ICH (International Conference on Harmonization, 2003). Glass vials containing AML or CD/AML complex corresponding to 0.662 mg of AML were stored for 6 h at 50, 60, 70, 80 and 90 ± 2 °C in a thermostatic bath. At given times (0, 1, 2, 3, 4, 5, and 6 h), sample of AML was collected and analyzed by HPLC method. All measurements were performed in triplicate.

In vitro dissolution/release studies

Sample of complex corresponding to about 2.5 mg of AML (sink conditions) was analyzed spectrophotometrically at λ 237 nm in water.

In vitro dissolution/release tests of CD/AML complex were carried out in 10 mL of water (under stirrer (100 rpm) at 37 ± 0.5 °C). The filtrates of samples were analyzed to determine the AML content by the UV–Vis spectrophotometer at 237 nm. All the dissolution/release tests were performed in triplicate; only the mean values are reported in graph (standard deviations <5 %).

AML alone and CD/AML physical mixture (CD/AML mix) were used as control.

Results and discussion

AML was complexed with soluble β-CD polymer to enhance its dissolution rate. The solubilization/freeze-dried method was used due to the high solubility in water of the selected polymer.

Drug content and IE

The actual AML content values determined by both UV and HPLC analyses were perfectly agreed. The percentage of AML in the samples ranged from 99.5 ± 0.2 to 100.1 ± 0.1 ($n = 3$), as reported in Table 1. This indicated that AML was uniformly distributed in all the samples.

Binding constant determination

The binding constants were determined by using two solutions of the same concentration of AML and CD which were mixed in different proportions in order to obtain various solutions with the same total concentration and different ratio between host and guest. The molar absorptivity of the complex was measured on solutions of the inclusions complexes.

Kb calculated from the Eq. 4 was 1.2×10^5 M. This high value reflects the good stability of the obtained compound [27].

Phase solubility studies

AML is a slightly soluble drug (Ph. Eur. 7° Ed.). The solubility of AML in distilled water at room temperature was 4 g/L and was notably affected by the presence of CD.

Phase-solubility analysis of the effect of complexing agents on the compound being solubilized is a traditional approach to determine not only the value of the stability constant but also to give insight into the stoichiometry of the equilibrium [6].

Solubility curves are obtained when the apparent solubility of the substrate increases with ligand concentration throughout the entire concentration range. The phase solubility profile for the complex formation between AML and CD at 25 °C is shown in Fig. 2. A linear increasing in the solubility of AML was observed with the increasing concentrations of CD until 1:2 (CD/AML) molar ratio.

$$CD + 2S \overset{K_{1:2}}{\leftrightarrow} CD \cdot S_2$$

Results show an A_L type of solubility curve that could allow and seems possible a first-order soluble complex at 1:2 (CD/AML) molar ratio (two drug molecules form a complex with one CD molecule).

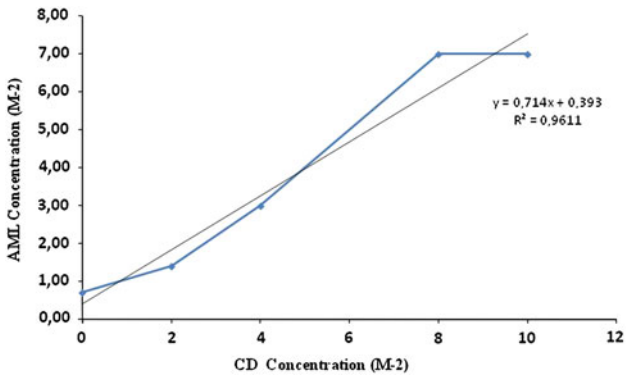

Fig. 2 Solubility phase *diagram* of AML in presence of CD

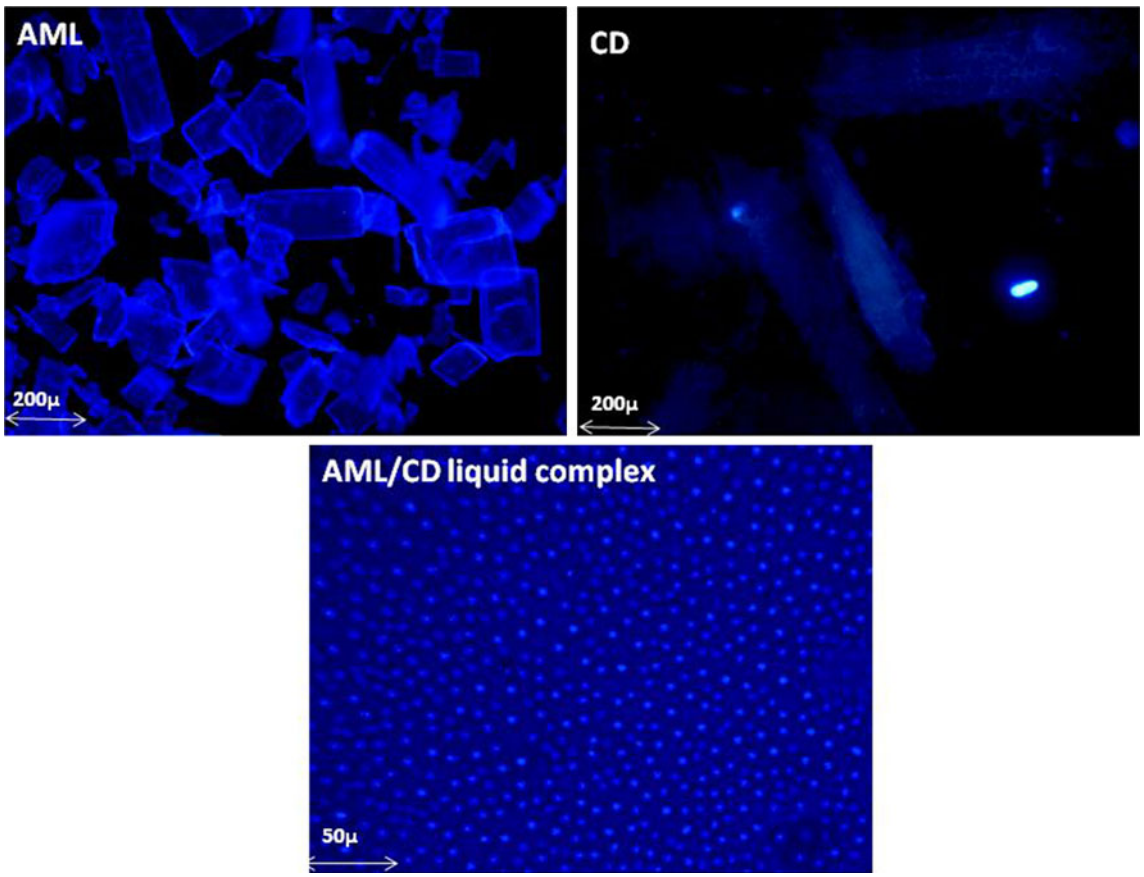

Fig. 3 Fluorescence *microphotographs* of AML alone (AML, **a**, ×10), soluble *β*-CD polymer pure (CD, **b**, ×10) and liquid reconstituted complex (CD/AML–liquid complex, **c**, ×40)

The apparent 1:2 stability constant Kc was calculated from the straight line of the phase solubility diagram to verify the affinity of drug for CD [28].

The Kc value for the CD/AML complex was found to be 1,133.33 M^{-1} (between 100 and 5,000 M^{-1}), which is considered adequate for the improvement of the bioavailability of this slightly soluble drug.

Complexes characterization

To evaluate the inclusion complex formation, morphology and particle size, both analyses in liquid (UV and circular dichroism) and in solid state (DSC and FTIR) were carried out.

Morphology and particle size analyses

AML is commercially available in a crystalline state (intense blue fluorescence, d_{50} 250.0 ± 0.1 μm) characterized by a slight water solubility (4 g/L) (Fig. 3a), while the CD powder show a lamellar structure (slight blue fluorescence, d_{50} 500.0 ± 0.2 μm) (Fig. 3b).

The microphotographs of CD/AML complex were obtained on a droplet (10 μg) of reconstituted lipophilised (CD/AML liquid) in water or dried reconstituted samples (CD/AML dried) (Fig. 3c). The CD/AML dried were obtained drying the droplet of the reconstituted complex on a slide at room temperature in the dark.

The droplet of CD/AML liq shows a spherical structures (d_{50} 3.0 ± 0.15 μm) with an intense fluorescent core. As shown in Fig. 3c, the core of the complex was more fluorescent than the outside layer. These structures seem to be constituted by CD lamellae that are structured to give a hole in which AML appears as an amorphous state. This was probably due to the inclusion of AML into the cavity of the CD [29, 30].

Absorption spectra

The UV spectra of raw materials alone and AML in presence of CD (CD/AML) was reported in Fig. 4.

Amlodipine showed two UV absorption bands: at 214 nm probably due to $\pi \rightarrow \pi^*$ transition of substituted aromatic systems (k band) and at 237 nm, typical absorption band of 1,4-dihydropyridine derivatives (R band) [31].

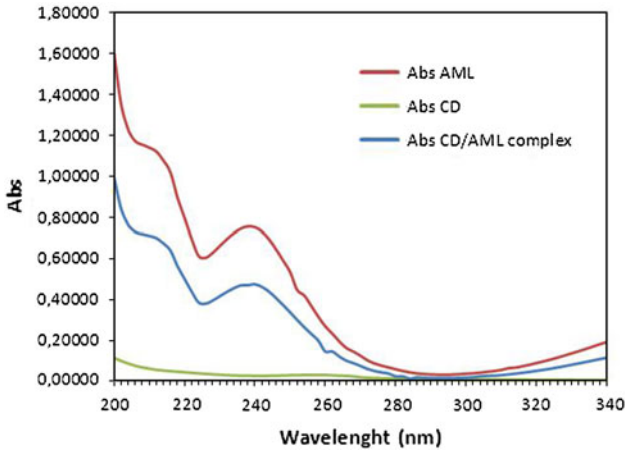

Fig. 4 UV spectra analyses of CD/AML complex in comparison with AML and CD raw materials

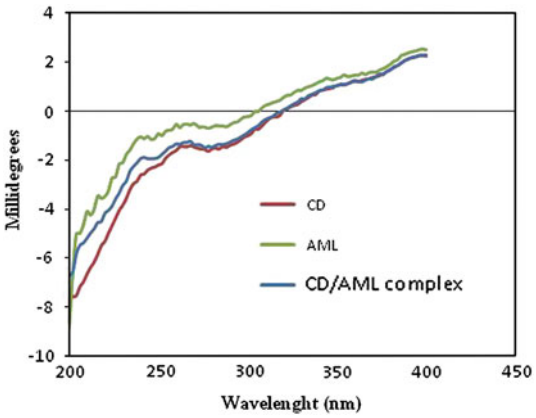

Fig. 5 Circular dichroism *diagrams* of CD/AML complex in comparison with AML and CD raw materials

The presence of CD induced a hypochromic shift of 3 nm (from 237 to 240 nm) and the K band decrease in intensity [32]. These results were probably due to the solubilization of AML in presence of CD and to the depletion of the guest molecule [30]. This confirms the formation of the CD/AML complex with weak bonds.

Circular dichroism

Circular dichroism is a useful method to detect CD inclusion complexes in aqueous solution in presence of achiral and chiral guest molecules [6, 15, 33]. AML, such as other chiral guest molecules, may show changes in circular dichroism (CD) spectra upon the formation of inclusion complex with CD [15, 29]. The circular dichroism spectrum of the AML (2×10^{-6} M)/BCD (1×10^{-6} M) system in aqueous solution is shown in Fig. 5. In the presence of CD, the optical activity was slightly induced in the wavelength region of the drug chromophore with a shift

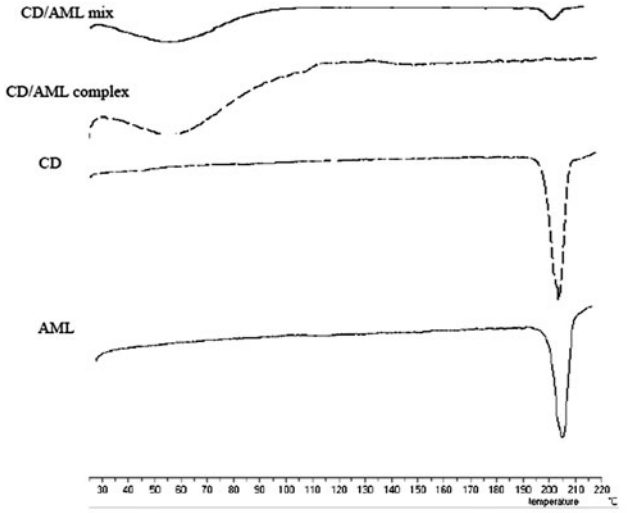

Fig. 6 DSC of AML and CD alone, physical mixture (CD/AML mix) and CD/AML complex

from 237 to 240 nm. Nevertheless it was observed a negative peak at 240 nm without a remarkable extrinsic Cotton effect, but with a slightly hypochromic effect confirmed by the UV analysis. The hypochromic effect and slight in optical activity may be due to the inclusion of the drug in the cavity of CD following complexation. These results were confirmed by DSC and FTIR analyses.

Differential scanning calorimetry

When guest molecules are included in CD cavities or crystal lattice, their melting, boiling and sublimation points shift to different temperatures or disappear [34]. Figure 6 shows the thermal profiles of AML alone, CD alone, CD/AML physical mixture (CD/AML mix) and AML/CD complex. AML and CD as raw materials show a characteristic endothermal peak corresponding to the melting point of the crystalline substances, i.e. 206.3 and 206.4 °C respectively. It is evident the presence of the superimposed melting points in the physical mixture, that disappeared in CD/AML complex thermal profile. These data should confirm the formation of the inclusion complex.

FTIR spectroscopy

The complexation process of AML with the CD has been confirmed by the FTIR spectroscopy. FTIR spectra of CD/AML complex show differences in the peak patterns with respect to raw materials (Fig. 7).

A new peak was expressed at 1,689.6 cm^{-1}. A shift of 1,208.1 and 1,491.9 band at 1,213.6 and 1,512.1 cm^{-1} respectively, might indicate the interaction of the CD through N–H groups with AML considering the type of

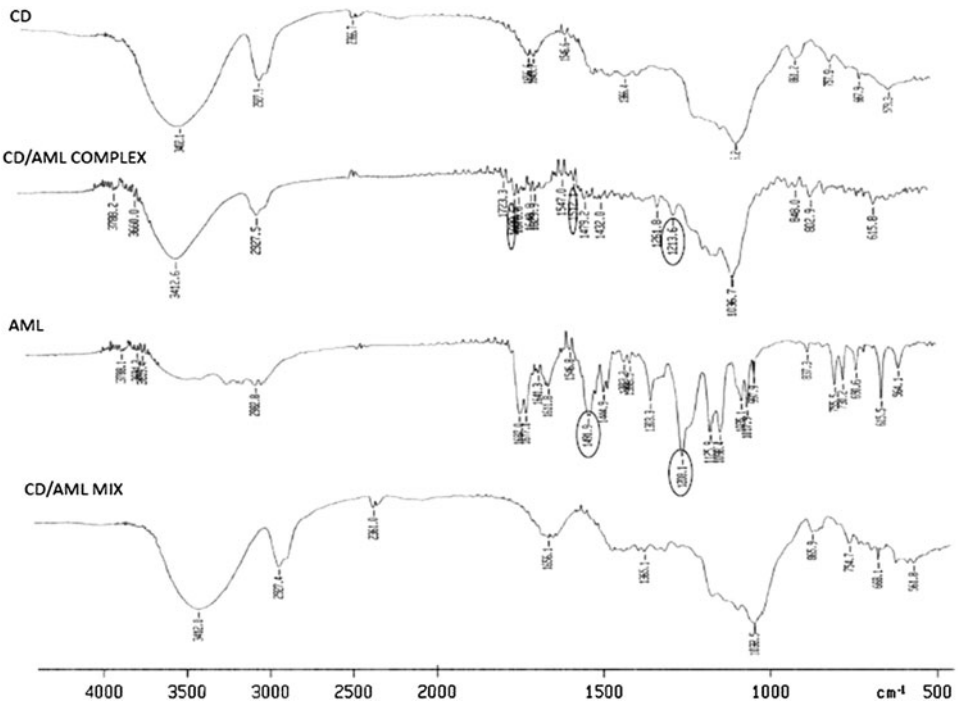

Fig. 7 FTIR of CD/AML complex, in comparison with physical mixture (CD/AML mix), AML and CD raw materials

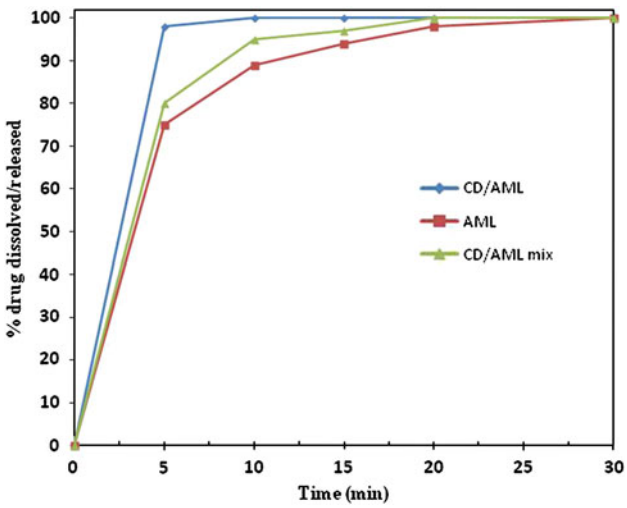

Fig. 8 Dissolution/release profile of CD/AML complex, in comparison with physical mixture (CD/AML mix) and AML raw material dissolution profiles in water

hydrogen bonds, such as reported in literature for AML complexed with other β-CDs [8].

Stability studies

Usually, the drugs in water solution are often instable. For this reason, the physicochemical and functional stability of both free drug and its complex were evaluated under stress conditions. The accelerated stress testing performed for AML and CD/AML provides information concerning shelf-life and efficacy of a product with a specific function. AML or CD/AML complex were analyzed in the range 50–90 °C at different time intervals (0, 1, 2, 3, 4, 5 and 6 h). No degradation products were detected by HPLC analyses, thus confirming the high thermal stability of both the tested samples.

Dissolution/release study

The dissolution/release profiles of AML from AML/CD complex in comparison with dissolution profiles of neat AML and CD/AML physical mixture in water are reported in Fig. 8.

As reported in figure, it is possible to observe that about 97.0 % of AML dissolved/released in 5 min from the CD/AML complex, while about 80.0 % of AML dissolved from the physical mixture CD/AML and only 75.0 % of AML alone dissolved at the same time interval. These results showed that the powder of CD/AML mix was more easily wettable compared to the raw material, and moreover that an important increase in dissolution rate could be achieved with the drug complexation (CD/AML). This behavior may be caused by an increase of AML water interaction due to the soluble β-CD polymer, which is able to improve drug wettability and water solubility.

Conclusion

The present study demonstrated that the aqueous solubility of AML can be increased by its complexation into a CD/

AML binary system. Solubilization/freeze-dried method and the soluble β-CD polymer selected in our work were effective in achieving an inclusion complex of AML, such as confirmed by UV, DSC, and FTIR analyses. The selected 1:2 molar ratio CD/AML was effective in complexing the drug as confirming the high value of Kb that highlights a good interaction between AML and CD. Moreover, as result of inclusion complex, an increase of the wettability, the dissolution rate and the solubility in water of AML was observed. This approach might be suitable to obtain an extemporaneous solution of drug to guarantee the administration of a correct dose to hypertensive pets.

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