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Inclusion Complexes of Felodipine and Amlodipine with Methyl-β-cyclodextrin

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Abstract

The methods of obtaining and physicochemical properties of inclusion complexes of amlodipine (AM) and felodipine (FL) with methyl-β-cyclodextrin (MCD) clathrates have been studied. Solid complexes were obtained by two methods: the kneading one and lyophilization with the drug and MCD at the molar ratio of 1:1. The identity of the obtained clathrates was confirmed by IR, ^{13}C-NMR spectra and DSC measurements. The process of AM and FL complexation with MCD was shown to involve the aromatic ring, the carbonyl groups in the ester bonds and the carbon atoms of the DHP ring linked via the ester bonds. One of the aims of complexation was to improve the drug solubility, so the dissolution rate of the obtained clathrates was tested. As a result of the inclusion complexes formation of AM, obtained both by kneading and lyophilization, the solubility of this therapeutic drug increased 3 times. The inclusion complex formation with FL and MCD brought the most dramatic increase in FL solubility, which increased 16 times.

Introduction

One of the methods used to increase the stability of a pharmaceutical product is the formation of inclusion complexes with the cyclodextrins [1–6]. The native β-cyclodextrin is lowly soluble in water as a result of hydrogen bonds between the –OH groups from the neighbouring particles. β-CD demonstrates particularly low solubility (1.8 g/100 g water). In the recent years in the complexation process the modified cyclodextrins have been more often used, as they are more water soluble, more hygroscopic and more surface active. For that reason the drug incorporated into the modified CD dissolves much better in water and has higher bioavailability [7–12]. The CD derivatives are chemically inhomogeneous mixtures of stereoisomers. For example, the product formed as a result of condensation of β-CD with methyl chloride is statistically defined by molar substitution index. Its value is calculated as the mean number of alkylation substance in moles in relation to one mole of glucopyranose, because each of the 21 –OH groups can react with alkyl chloride.

Introduction of the functional groups into the CD molecule causes displacement or disruption of the intramolecular hydrogen bonds. Depending on the substitution grade, it entails changes in the physicochemical properties of CD, like e.g. solubility or inclusion capabilities of other substances [13–18]. CD's ability to form inclusion complexes was used among others for the rise of bioavailability of the drugs characterized by low solubility in water.

AM and FL just as the other DHP derivatives impede the influx of the Ca^{2+} through the calcium channels type L into the cells. Their main hemodynamic effect is a significant vasodilatation, leading to a decrease in the resistance and increase in the coronary flow, leading to improvement in the blood supply with oxygen [19–25]. Unfortunately, the drugs of this group have certain undesired physicochemical properties, like low water solubility and high photosensitivity [26]. One of the methods to alleviate the effect of these undesired properties is complexation with CDs [27].

Experimental

Materials

Amlodipine bensylate (AM) - Dr. Redoys Laboratories Ltd. - AB 0221095; felodipine (FL) – Cipla Ltd. FX 6050L0TII; methyl-β-cyclodextrin (MCD) – Wacker-Chemie GmbH, mol. weight 1326.8 g mol^{-1}, MS = 1.8. Deuterated dimethylsuphoxide (DMSO-d_6) (99.95%)

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was obtained from Merck KGaA (Darmstadt, Germany).

Preparation of inclusion complexes

The inclusion complexes of FL and AM with MCD were prepared by the kneading and lyophilization methods.

The kneading method

1.62 g MCD was placed into a mortar, moistened with 50% ethanol and kneaded to the paste consistency. Then 0.46 g FL or 0.50 g AM was introduced and kneaded 60 min adding successively 1.6 g 50% ethanol. The obtained samples were dried at 50 °C for 2 days and than over P_2O_5 till constant mass.

The received inclusion complexes were labelled as follows.

K-FLFL with MCD

K-AMAM with MCD.

The lyophilization method

Fifty millilitre ethanolic solution of the FL or AM of the concentration 1×10^{-2} mol $l^{-1}$was placed into the crystallisers. Later 50 ml of a 1×10^{-2} mol l^{-1} water solution of MCD was added and mixed for 30 min with a magnetic stirrer at a constant rate of revolutions. The samples were stored for 24 h at −4 °C and as a result of the 20 h long lyophilization, the clathrates were obtained and labelled as follows.

L-FLFL with MCD

L-AMAM with MCD.

Preparation of physical mixtures

FL or AM and MCD were mixed thoroughly in a molar ratio 1:1 in the dry state. The mixtures were then reduced to 250–315 μm.

Physical mixtures of the FL and AM with the MCD were labelled as follows.

FL-CDFL with MCD

AM-CDAM with MCD.

Identification of the inclusion complexes

The final products were characterised by IR, DSC and ^{13}C-NMR.

Differential Scanning Calorimetry (DSC)

DSC analysis was performed for FL, AM, MCD, their physical mixtures and inclusion complexes. DSC curves were obtained on a Shimadzu DSC-50 instrument equipped with TA-50 WSI. Samples of FL and AM (3 mg) were covered with aluminium pans. The analysis was performed in the nitrogen atmosphere. All samples were run at a scanning rate of 5 °C min^{-1}, from 20 to 450 °C. DSC curves of the pure components and their 1:1 mol mol^{-1} physical mixtures and inclusion complexes are shown in Figure 1.

^{13}C-NMR

^{13}C-NMR spectra for analysis were taken on a Varian Unity 300 spectrometer using the following parameters: ^{13}C frequency 75–43 MHz, frequency range 6000 MHz, memory 65 kB, pulse width 11–3 μs, acquisition time 0.999 s, resolution 0–3 Hz. Spectroscopic measurements of the samples of 0.3 mol l^{-1}concentration were performed in DMSO-d_6. The chemical shifts were determined with respect to the residual signal of the solvent (DMSO-d_6 39,500 ppm) and expressed for TMS, which was the internal standard.

The values of the chemical shifts ($\Delta\ \delta$) at the carbon atoms in the ^{13}C-NMR spectra of the clathrates were determined with respect to those of FL or AM and MCD. The calculated values of the chemical shifts ($\Delta\delta$) of the carbon atoms in the inclusion complexes are shown in Tables 1 and 2.

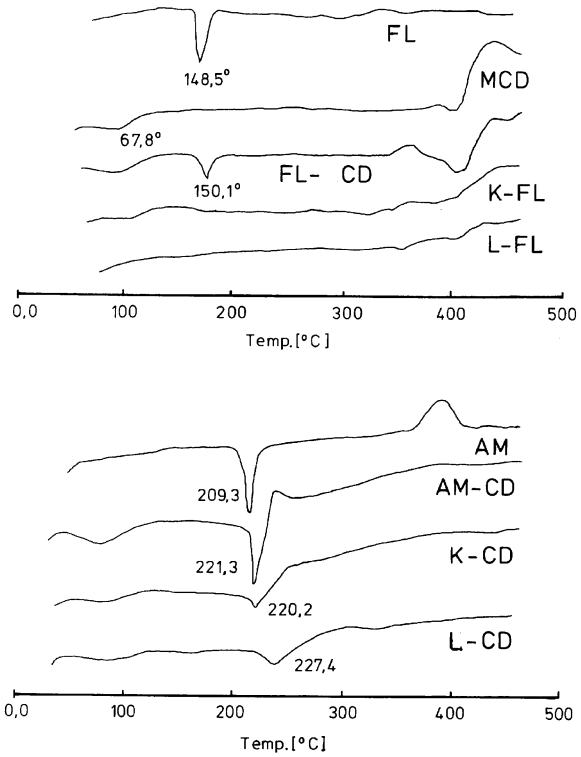
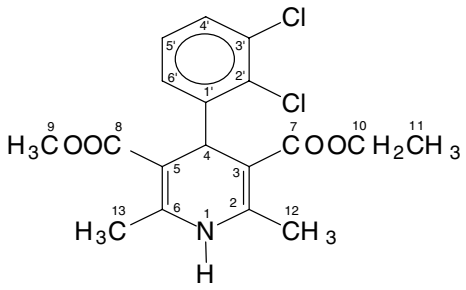


Figure 1. DSC curves of felodipine (FL) and amlodipine (AM), methyl-β-cyclodextrin (MCD), their physical mixtures and the inclusion complexes; MCD – methyl-β-cyclodextrin, FL-CD – physical mixture FL with MCD, AM-CD – physical mixture AM with MCD, K-FL – inclusion complex FL with MCD obtained by kneading, K-AM – inclusion complex AM with MCD obtained by kneading, L-FL – inclusion complex FL with MCD obtained by lyophilization, L-AM – inclusion complex AM with MCD obtained by lyophilization.

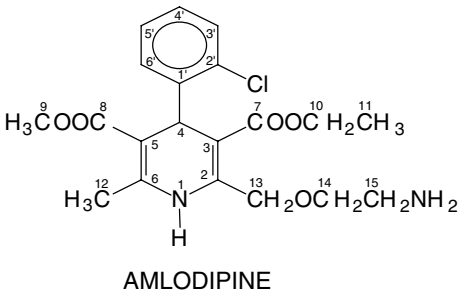
Table 1. The values of the chemical shifts ^{13}C-NMR of the selected absorption peaks of the felodipine (FL), methyl-β-cyclodextrin (MCD) and their clathrates obtained with the method kneading (K-FL) and lyophilization (L-FL)



FELODIPINE

FL[ppm]	MCD[ppm]	Δδ Chemical shifts	
		K-AM	L-AM
$C_7$166.92		0.26	0.24
$C_8$166.39		0.26	0.25
$C_{2'}$148.90		0.22	0.19
$C_{3'}$145.61		0.15	0.23
C_2 131.19		0.18	0.16
C_6 129.43		0.18	0.16
$C_{6'}$ 129.25		0.18	0.14
$C_{5'}$ 127.95		0.19	0.19
$C_{4'}$ 127.87		0.20	0.20
C_3 101.63		−0.24	−0.27
C_5 101.25		0.69	*
C_9 50.47		−0.18	−0.14
	C_I 101.96	−0.20	−0.23
	C_{II} 81.59	0.33	0.31
	C_{III}73.14	−0.23	−0.23
	C_{IV} 72.47	0.17	0.17
	C_V 72.12	0.30	0.33

Table 2. The values of the chemical shifts ^{13}C-NMR of the selected absorption peaks of the amlodipine (AM), methyl-β-cyclodextrin (MCD) and their clathrates obtained with the method kneading (K-AM) and lyophilization (L-AM)



AMLODIPINE

AM[ppm]	MCD[ppm]	Δδ Chemical shifts	
		K-AM	L-AM
$C_8$166.89		0.27	0.26
$C_7$166.00		0.28	0.27
$C_6$147.58		0.50	0.34
$C_{1'}$145.56		0.19	0.26
$C_{2'}$145.11		0.48	0.34
$C_{6'}$ 128.50		0.24	0.24
$C_{3'}$ 127.65		0.12	0.18
$C_{4'}$ 127.26		0.21	0.18
$C_{5'}$ 127.26		0.18	0.20
C_3 102.01		0.13	0.12
C_5 102.86		*	0.10
C_{15} 66.63		0.33	0.10
C_{14} 66.35		0.11	*
	C_{II} 81.60	0.18	0.15
	C_{III} 73.14	0.34	0.31
	C_{IV} 72.47	0.32	0.35
	C_{VI} 60.03	0.36	0.24

IR spectra

The IR spectra recorded on a Brucker Vector Spectrophotometer were taken in potassium bromide disks (2 mg/300 mg KBr) – Figures 2 and 3.

Dissolution rate

A 100 mg sample of FL and 500 mg AM or an equivalent amount of the inclusion complexes or the physical mixtures were placed in the container in order to determine their dissolution rates according to the USP paddle method. The solvent was 500 ml water maintained at 37 ± 0.1 °C. The whole sample was agitated at 100 rpm and after certain time intervals in the range from 5 to 90 min, a 5 ml of the solution was withdrawn and the remaining sample was supplemented with water at 37 °C to the initial volume of 500 ml. After that the removed 5 ml samples were filtered through the membrane filter of the pore diameter 0.65 μm and were assayed spectrophotometrically in the cells of l = 1 cm

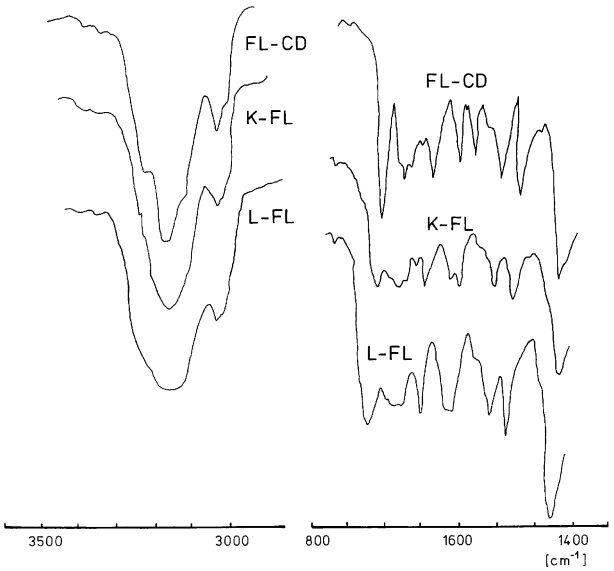


Figure 2. IR spectra of physical mixtures and inclusion complexes of felodipine with methyl-β-cyclodextrin. Symbols like in Figure 1.

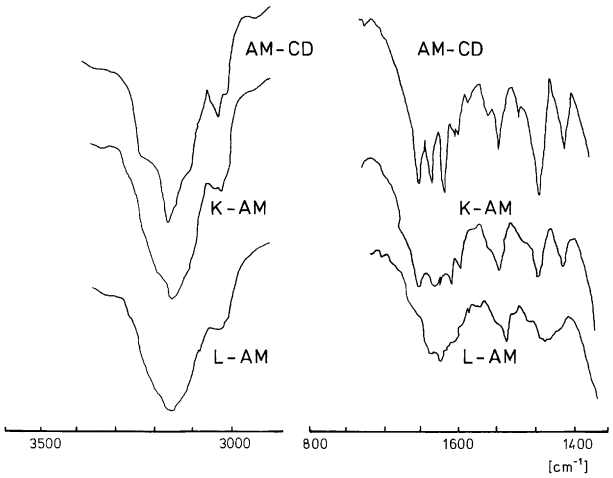


Figure 3. IR spectra of physical mixtures and inclusion complexes of amlodipine with methyl-β-cyclodextrin. Symbols like in Figure 1.

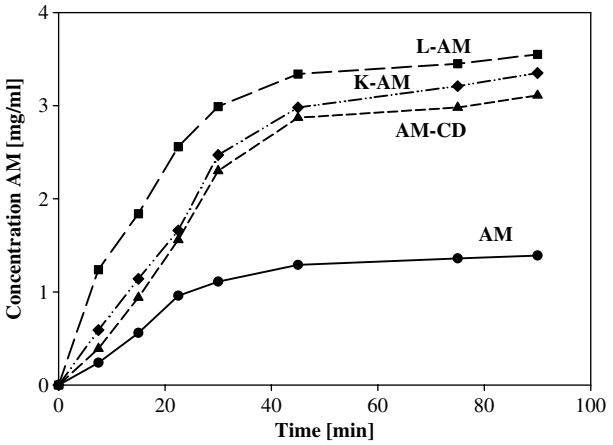


Figure 5. Dissolution rate of amlodipine (AM), physical mixture and the corresponding inclusion complexes with methyl-β-cyclodextrin. Symbols like in Figure 1.

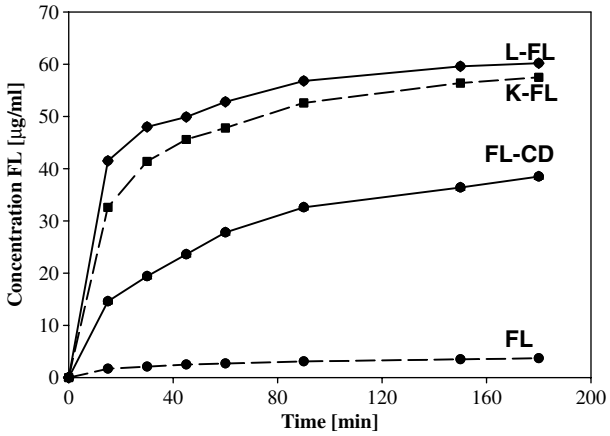


Figure 4. Dissolution rate of felodipine (FL), physical mixture and the corresponding inclusion complexes with methyl-β-cyclodextrin. Symbols like in Figure 1.

in reference to water (λ = 362.0 and 366.8 nm, for the FL and AM, respectively). All samples were analysed in triplicate.

The dissolution rate of the FL and AM, their physical mixtures with MCD and their inclusion complexes are shown in Figures 3 and 4.

Results and discussion

Drugs inclusion complexes with CDs can be obtained by different methods, in this study the kneading method and lyophilization were used, for the therapeutic drug and CDs at the molar ratio of 1:1.

According to literature data, the inclusion complexes of the drugs with CDs are formed in a non-covalent manner, without chemical bonds. Their formation involves mainly hydrophobic interactions and van der Waals bonds, less frequently hydrogen bonds. For this reason the identification of the clathrates is a complex problem. In this study it has been confirmed on the basis of DSC, nuclear magnetic resonance (^{13}C-NMR) and IR spectrophotometry.

The DCS curves obtained for AM and FL and their inclusion complexes are shown in Figure 1. They show a characteristic endotherm, corresponding to the melting point of the crystalline drug i.e. at 148.5 °C (FL) and at 209.3 °C (AM). The thermograms of the inclusion complxes do not show peaks in this region, implying inclusion of the drug into the MCD cavity. The exo- and endotherms on the each thermogram in the temperature range 380–450 °C are interpreted as corresponding to the thermal decomposition of MCD. The not much pronounced broad endothermic peaks at the beginning of the DSC curve have been related to the liberation of water from the MCD groove. As follows from a comparison of the graphs in the Figure 1, the formation of inclusion complexes is indicated by the disappearance or shift of the endothermic peaks corresponding to the drug melting process.

Another method used to identify the clathrates of FL and AM with MCD was ^{13}C-NMR spectroscopy. In order to assign absorption peaks to particular carbon atoms, the DEPT analysis of FL and AM has been carried out, enabling the establishment the atoms position with a proper valency.

The analysis of the ^{13}C-NMR spectra of the FL and AM inclusion complexes has been conducted by calculating the value of the chemical shifts of corresponding carbon atoms. Comparing the results in Table 1. there has been noticed that the considerable values of chemical shifts have been observed in the FL clathrates obtained both by kneading and lyophilization method. The chemical shifts obtained for AM inclusion complexes were also significant and more pronounced for the complexes obtained by the kneading method.

In particular great chemical shifts were observed at the carbon atoms in the aromatic phenyl ring ($C_{1'}$, $C_{2'}$, $C_{6'}$) and those of the carbonyl groups (C_7, C_8). Interesting chemical shifts value was obtained for the aliphatic C carbon atom in the alkylammonium chain (C_{14}, C_{15}).

The complexation process of FL and AM with the MCD has been also confirmed by the IR spectroscopy. The IR spectra of the inclusion complexes of the DHP derivatives studied (Figures 2 and 3) are significantly different from those of the corresponding physical mixtures. In the IR spectra of the inclusion compounds of the two DHP derivatives studied, the bands in the range 3050–3250 cm^{-1}, with a maximum at 3150 cm^{-1}, assigned to the stretching vibrations of the N–H bonds in the dihydropyridine ring are much broader. The differences also appear in the band corresponding to the vibrations of carbonyl groups in the ester bonds. In the spectra of the physical mixtures the band occurs at 1700–1720 cm^{-1}, while in the spectra of the corresponding inclusion complexes it is broadened and shifted towards shorter waves. Some differences are also noted in the range 1500–1600 cm^{-1}, assigned to the vibrations of multiple bonds in the unsaturated DHP ring.

One of the aims of the complexation of FL and AM with the MCD was to increase the drug solubility, therefore, the dissolution rate of all the obtained clathrates and the respective physical mixtures was tested. According to an earlier study, DHP derivatives solubility depends on the kind of the substituent at the phenyl ring [27]. The compounds having the polar group $–NO_2$ are much better soluble than the compounds with alkyl or halogen substituents. However, as shown in Figures 3 and 4, the complexation process changed the solubility of AM and FL solubility in the same way, irrespectively of the method of obtaining the inclusion complex. For example, the solubility of AM in the clathrates obtained by both the kneading and lyophylization methods was 3 times greater than in the crystalline form.

Interestingly, in the clathrate L-AM the solubility of AM decreased. Even more pronounced effect of making inclusion complexes with MCD was observed for FL. In the physical mixture and in the L-FL clathrate, FL dissolved 10 and 16 times better, respectively, than in the crystalline form.

Conclusion

The solubility of felodipine and amlodipine can be significantly improved by converting them into inclusion complexes with methyl-β-cyclodextrin by the kneading and lyophylization methods.

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