VERSITA

Cent. Eur. J. Chem. • 8(4) • 2010 • 834–841
DOI: 10.2478/s11532-010-0061-8

Central European Journal of **Chemistry**

Inclusion complexes of amlodipine besylate and cyclodextrins

Research Article

Agneš Kapor*[1], Vesna Nikolić[2], Ljubiša Nikolić[2], Mihajlo Stanković[2], Milorad Cakić[2], Ljiljana Stanojević[2], Dušica Ilić[2]

[1]*Faculty of Sciences, Department of Physics, University of Novi Sad, 21000 Novi Sad,Serbia*

[2]*Faculty of Technology, University of Nis, 16000 Leskovac, Serbia*

Received 7 December 2009; Accepted 9 April 2010

Abstract: In this paper the procedure for the preparation of inclusion complexes of amlodipine besylate with β-cyclodextrin (β-CD) and 2-hydrohypropyl-β-cyclodextrin (HPβ-CD) and their structural characterization was described. Molecular inclusion complexes of amlodipine besylate are prepared by the coprecipitation method and characterised by the application of spectroscopic methods FTIR, ^{1}H-NMR and XRD. The photosensitivity of amlodipine besylate in the inclusion complexes was also determined with respect to uncomplexed agent. DSC curves indicate the loss of the clear peak due to melting of amlodipine besylate at about 200°C, while on XR diffractograms certain reflections are lost belonging to amlodipine besylate in complexes. This indicates its inclusion in the vacancies of the host. The inclusion of amlodipine besylate with cyclodextrins increases the stability, *i.e.* decreases the photosensitivity of amlodipine besylate.

Keywords: *Amlodipine besylate • Inclusion complexes • Cyclodextrins • Spectroscopic methods • Photosensitivity*

1. Introduction

Amlodipine besylate, 2-[(2-aminoetoxy)-methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine dicarboxylic acid 3-ethyl 5-methyl esterbenzene sulfonate, is the blocker of calcium channels from the group of dihydropyridines [1]. It decreases the number of attacks of angina pectoris, and allows certain physical load by decreasing ischemic changes [2,3]. Amlodipine besylate can be applied in the treatment of hypertension, most often the only successful drug for the regulation of blood pressure with the majority of patients [4,5].

More recent studies show that amlodipine besylate and other blockers of calcium channels inhibit tumor proliferation by inhibiting the incoming flow of calcium ions causing the destruction of internal reservoirs of calcium ions in cancer cells [6,7]. Xhing Li and collaborators have shown in their research that topotekan combined with amlodipine besylate produces a synergetic effect in three types of leukemia cells, *i.e.*, shows expressed cytotoxicity [8].

The main disadvantage of amlodipine besylate, in spite of its good pharmacological characteristics, is its photosensitivity. Like all antihypertensives from 1,4 dihydropyridine groups, amlodipine besylate is degraded under the influence of light, as shown at Fig. 1 [9,10].

One can see that due to influence of light the 1,4 dihydropyridine ring turns into the pyridine ring. The resulting pyridine derivative of amlodipine besylate causes the loss of pharmacological activity and also exhibits some toxicity, and is a potential cause of cancer [11]. In order to protect amlodipine besylate and similar drugs from photodegradation, various protective coatings are used. More recently, attention is being directed towards the production of supramolecular structures which can substantially correct shortcomings of drugs like photosensitivity, bioavailability, volatility and solubility. Many studies were devoted to the formulation of various drugs with cyclodextrins, liposomes,

* *E-mail: akapor@df.uns.ac.rs*

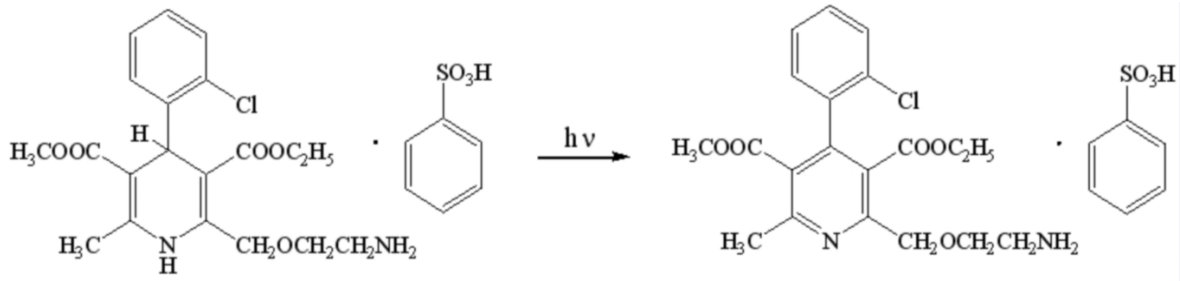

Figure 1. Amlodipine besylate degradation to the pyridine derivative under the influence of light.

microspheres and microcapsules [12-18]. Results indicate that to a high degree, chemical and physical characteristics of the drugs were corrected, enabling their successful application as human therapeutics. For example, Mielcarek *et al.* [19] improved the low solubility of amlodipine and thus reduced the bioavailability by forming a complex with methyl-β-cyclodextrin.

2. Experimental Procedure

2.1. Materials

Amlodipine besylate, 2-[(2-aminoetoxy)-methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine dicarboxylic acid 3-ethyl 5-methyl esterbenzene sulfonate, racemate, nonhydrated, purity 98% was purchased from Ipca Laboratories Limited, India. Complexing agents β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin of purity 98% and 97.5% were obtained from Merck, Darmstadt and Sigma-Aldrich, Wisconsin, respectively. Water used in the study was distilled twice.

2.2. Spectral measurements

FTIR spectra were recorded in a KBr pellet (0.6 mg sample, 140 mg KBr) at spectrophotometer Bomem Hartmann & Braun MB-series in the wave number range from 4000 to 400 cm^{-1}. This same method was used for following the photosensitivity of amlodipine besylate in the pure and complexed state. Namely, once the prepared KBr pellets with samples are exposed to daylight for some period of time the recording of the spectra is performed and repeated.

^{1}H-NMR spectra of samples are recorded at Bruker AC 250 E NMR spectrometer at 250 MHz, in glass cuvete of 5 mm diameter at room temperature by pulse method with multiple pulse repetition. The solvent was deuterated chlorophorm, $CDCl_3$.

X-ray powder diffraction was performed at Philips X'Pert powder diffractometer under the following conditions: samples were irradiated by monochromatic CuK_α radiation and measured under the angle 2θ between 5 and 40° with the step 0.05° and recording time τ=5 s. Voltage and current were 40 kV, 20 mA, respectively.

DSC curves were recorded on DuPont DSC differential scanning calorimeter with a scaning rate of 10°C min^{-1} in the temperature range 20-300°C, with 5 mg sample in aluminum closed vessels in a nitrogen atmosphere.

2.3. Preparation of inclusion complexes by coprecipitation method

Amlodipine besylate (567 mg, 1 mmol) and β-cyclodextrin (1135 mg, 1 mmol) or 2-hydroxypropyl-β-cyclodextrin (1540 mg, 1 mmol) are suspended in 150 cm^3 of distilled water. Obtained mixtures are mixed at room temperature for 24 h, evaporated at vacuum evaporator at 50°C till the volume of about 20 cm^3, then dried at the exicator above concentrated sulphuric acid at the temperature of 25°C till dry. The whole proces is performed in darkness in order to protect amlodipine besylate from photodegradation. The molar ratio in formed complexes of amlodipine besylate and complexing agents (β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin) is 1:1.

2.4. Preparation of the physical mixture

Physical mixtures were prepared by simple mixing of amlodipine besylate and complexing agents, β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin in the molar ratio 1:1, host molecule : guest molecule.

3. Results and Discussion

Fig. 2 shows FTIR spectra of amlodipine besylate (A), β-cyclodextrin (B), inclusion complex of β-cyclodextrin : amlodipine besylate (C), 2-hydroxypropyl β-cyclodextrin (D) and inclusion complex of 2-hydroxypropyl β-cyclodextrin : amlodipine besylate (E). In IR spectrum of amlodipine besylate in the range from 3500 to 3300 cm^{-1}, a band is present at 3410 cm^{-1} as the result of N-H valence vibrations, while deformation vibrations of N-H lead to the band at 1493 cm^{-1}. Valence vibrations

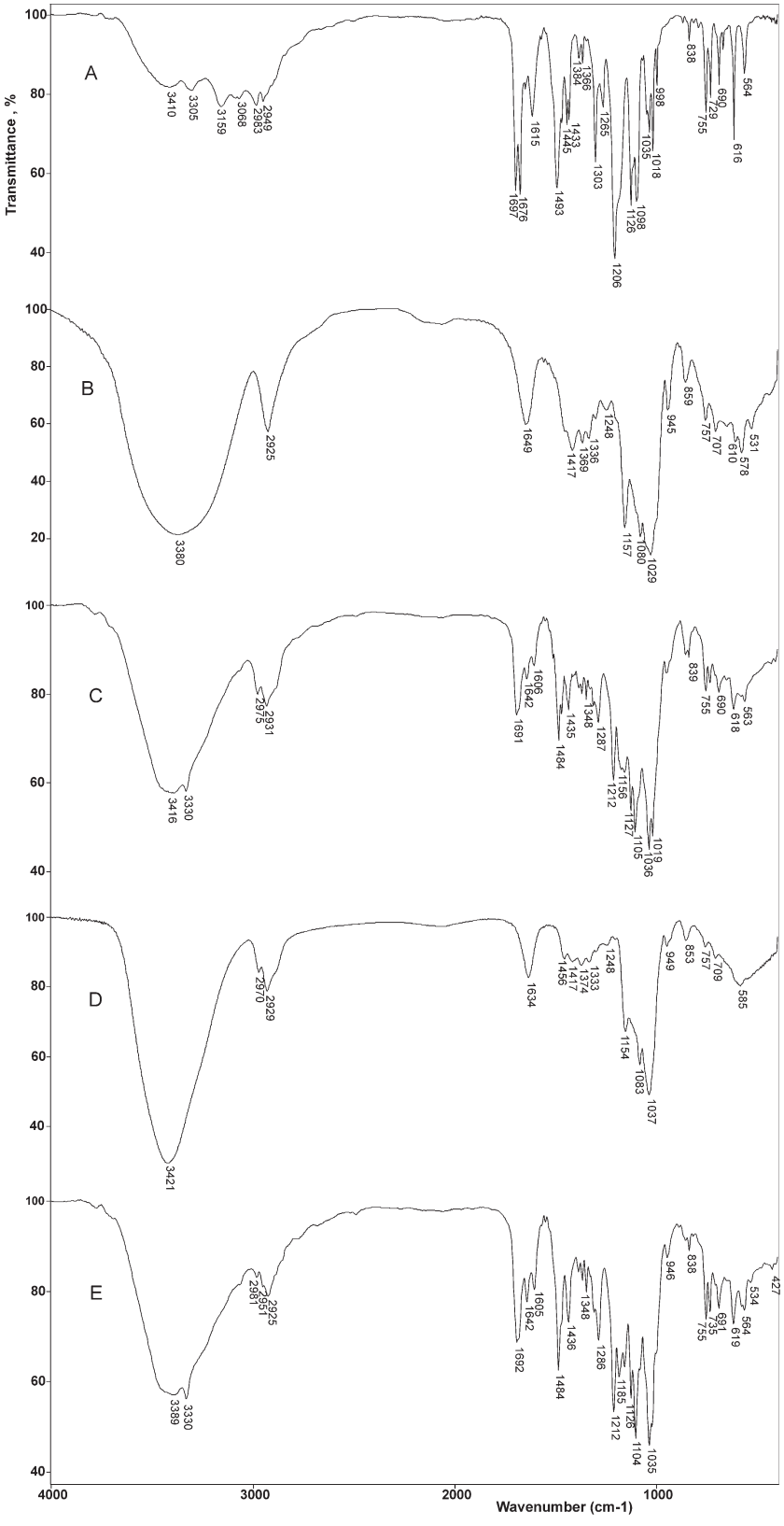

Figure 2. FTIR spectra of amlodipine besylate (A), β-cycldextrin (B), inclusion complex of β-cyclodextrin : amlodipine besylate (C), 2-hydroxypropyl β-cyclodextrin (D) and inclusion complex 2-hydroxypropyl β-cyclodextrin : amlodipine besylate (E)

of aliphatic C-H groups in the spectrum of amlodipine besylate produce the band at 2983 cm^{-1}. Intensive bands at 1697 cm^{-1} and 1676 cm^{-1} are characteristic for the valence vibrations of C=O and C=C groups, respectively. Two bands at 1303 cm^{-1} and 1098 cm^{-1} are the result of C-O valence vibrations. C-N valence vibrations result in the appearance of a rather intensive band at 1206 cm^{-1}. Aromatic parts of the structure of amlodipine besylate is characterised by the bands appearing at 1615 cm^{-1} and 755 cm^{-1} which result from C=C valence and C-H deformation vibrations. The band at 729 cm^{-1} is the result of C-S valence vibrations.

In FTIR spectra of inclusion complexes of amlodipine besylate with β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin there is no record of the bands whose position, shape and intensity are characteristic for amlodipine besylate. This fact indicates that the molecule of amlodipine besylate is screened in the vacancies of the host molecules (β-cyclodextrin and 2-hydroxypropyl β-cyclodextrin). Also, if one compares IR spectra of β-cyclodextrin and 2-hydroxypropyl β-cyclodextrin with corresponding IR spectra of the complexes, one can notice in the complexes the appearance of complex bands in the range from 1600 cm^{-1} up to 1700 cm^{-1} with an expressed peak at 1691 cm^{-1} in the complex with β-cyclodextrin and at 1692 cm^{-1} in the complex with 2-hydroxypropyl β-cyclodextrin. Both complexes possess bands at 1484 cm^{-1} and 1212 cm^{-1} not present in the IR spectra of the host molecules, while in the IR spectrum of guest molecule these bands appear at 1493 cm^{-1} and 1206 cm^{-1}. Displacement of these bands for 9 and 6 units might indicate the interaction of the guest molecule through N-H groups with host molecules considering the type of hydrogen bonds.

Complexes of amlodipine besylate with cyclodextrins, as well as individual components were submitted to ^1H NMR analysis. Fig. 3 shows the structures of amlodipine besylate and glucoside units of β-cyclodextrin and 2-hydroxypropyl β-cyclodextrin with marked C-atoms necessary for clear identification of chemical shifts of the H atoms .

Numerical values of δ-shift of H atoms in the molecule of amlodipine besylate are given in Table 1, while Table 2 shows the numerical values of chemical shifts and changes of chemical shifts of protons in β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin before and after the complexation with amlodipine besylate.

Based on the given $\Delta\delta$ values one can see that the largest shifts appear in the complex of β-cyclodextrin : amlodipine besylate for protons occuring at the C_3 and C_1 atom, while in the complex of 2-hydroxypropyl-β-cyclodextrin : amlodipine besylate, largest shifts occur for protons at C_1 and C_2-C_8 atoms of the corresponding host molecules. The above results can also indicate that just these hydrogen atoms participated most in the interaction with the guest molecule, amlodipine besylate.

Figs. 4a and 4b show the X-ray powder diffractograms (XRD) of amlodipine besylate, complexes with β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin, complexing agents and corresponding physical mixtures [20].

Comparative analysis of these diffractograms leads to the conclusion that the diffractograms of the complexes are almost identical with the diffractograms of the corresponding host molecules, β-cyclodextrin and 2-hydroxypropyl β-cyclodextrin, which supports the assumption that the amlodipine besylate molecule is built in into the vacancies of the given cyclodextrins, which completely screen it from the XRD. On the other hand, one notices a large crystallinity of amlodipine besylate in the form of commercial sample (of purity 98%) as supported by rather distinct peaks in the diffractogram of amlodipine besylate. In the

Table 1. Chemical shifts (δ) in ^1H-NMR spectrum of amlodipine besylate

Proton	4	7	8	9	10	12	13	3' to 6'	NH	2" to 6"
δ, ppm	5.35	2.11	3.56	4.65	1.18	3.65	3.15	7.05 to 7.25	8.05	7.45 to 7.85

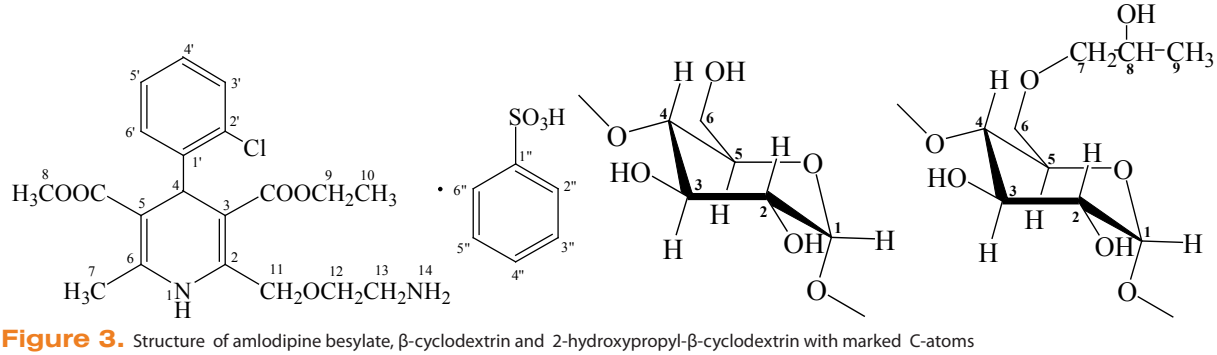

Figure 3. Structure of amlodipine besylate, β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin with marked C-atoms

Table 2. Chemical shifts (δ) and changes of chemical shifts of the proton (Δδ) in ^{1}H-NMR spectra of β—cyclodextrin, complex of amlodipine besylate with β—cyclodextrin and 2-hydroxypropyl—β—cyclodextrin and complex of amlodipine besylate with 2-hydroxypropyl-β-cyclodextrin

C-atom	δ, ppm		Δδ, ppm	C-atom	δ, ppm		Δδ, ppm
	β-cyclodextrin	Complex			2-hydroxypropyl-β-cyclodextrin	Complex	
1	5.12 s	5.03 s	-0.09	1	5.14 d	5.21 d	+0.07
2 and 4	3.65 m	3.60 m	-0.05	2 to 8	3.73 m	3.78 m	+0.05
3	4.03 t	3.89 t	-0.14	9	1.11 d	1.12 d	+0.01
5 and 6	3.92 m	3.85 m	-0.07				

s - singlet, d - doublet, t - triplet, m - multiplet.

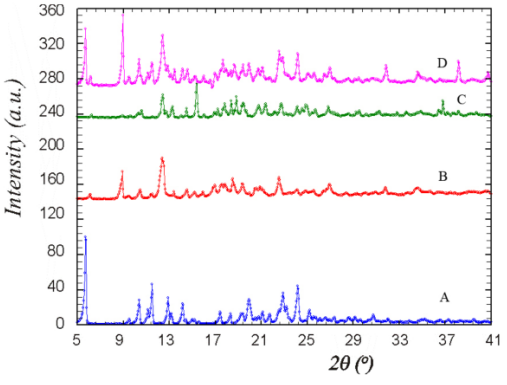

Figure 4a. X-ray diffractograms of amlodipine besylate (A), β-cyclodextrin (B), inclusion complex of amlodipine besylate and β-cyclodextrin (C) and physical mixture of amlodipine besylate and β-cyclodextrin (D).

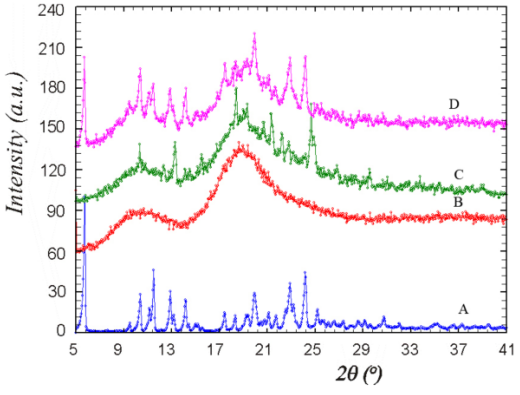

Figure 4b. X-ray diffractograms of amlodipine besylate (A), 2-hydroxypropyl-β-cyclodextrin (B), inclusion complex of amlodipine besylate and 2-hydroxypropyl-β-cyclodextrin (C) and physical mixture of amlodipine besylate and 2-hydroxypropyl-β-cyclodextrin (D).

diffractogram of 2-hydroxypropyl β-cyclodextrin there exists a broad diffraction peak in the range about 2θ=10.516° and 2θ=18.882° which is not structured and indicates the disorder of the crystal structure at long range. Diffractogram of physical mixtures show that it is really a mixture of amlodipine besylate with β-cyclodextrin or 2-hydroxypropyl-β-cyclodextrin as confirmed by the presence of the reflections of both components in corresponding mixtures, which are not present in complexes. X-ray diffraction indicates that the formation of a complex of amlodipine besylate is realized as an inclusion of the molecule into the crystal lattices of the matrices, in this case β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin.

DSC curves of amlodipine besylate (1), inclusion complex of amlodipine besylate and β-cyclodextrin (3), inclusion complex of amlodipine besylate and 2-hydroxypropyl-β-cyclodextrin (2), β-cyclodextrin (4) and 2-hydroxypropyl-β-cyclodextrin (5) are presented at Fig. 5.

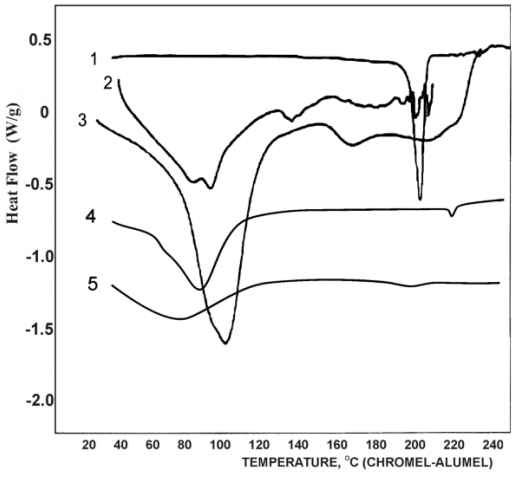

Figure 5. DSC curve of amlodipine besylate (1), inclusion complex of amlodipine besylate and β-cyclodextrin (3), inclusion complex of amlodipine besylate and 2-hydroxypropyl-β-cyclodextrin (2), β-cyclodextrin (4) and 2-hydroxypropyl-β-cyclodextrin (5).

DSC curves of inclusion complexes (2 and 3) show the temperature changes which differ both from the components of the hosts (4 and 5) and components of the guest (1). Amlodipine besylate shows a strongly expressed endothermal peak in a narrow range around 200°C which actually represents its melting point. In both complexes, this peak is rather weak and complex indicating an interaction of a guest molecule and the host components, so its changes accompanied by the thermal effects in complexes occur in a different regime.

Amlodipine besylate as well as other representatives of 1,4-dihydropyridines are unstable compounds, and under the influence of light easily oxidise to aromatic pyridine derivatives [10,21,22]. In this work, the photosensitivity of amlodipine besylate was followed in the solid state both in the noncomplexed as well as the complexed state. The suitable method was chosen to be FTIR, and samples in KBr pellets were exposed continually to daylight during various time intervals (0-8 days) and their FTIR spectra were recorded. After irradiating the amlodipine besylate, the 1,4-dihydropyridine ring becomes pyridine ring, *i.e.*, two protons are lost in the molecule (Fig. 1). This implies that the changes in FTIR spectra should be looked for in the bands originating from the vibrations of these groups (N-H and Ar-H). Figs. 6a and 6b show FTIR spectra of amlodipine besylate in a freshly prepared KBr pellet, and after irradiation with light during 8 days.

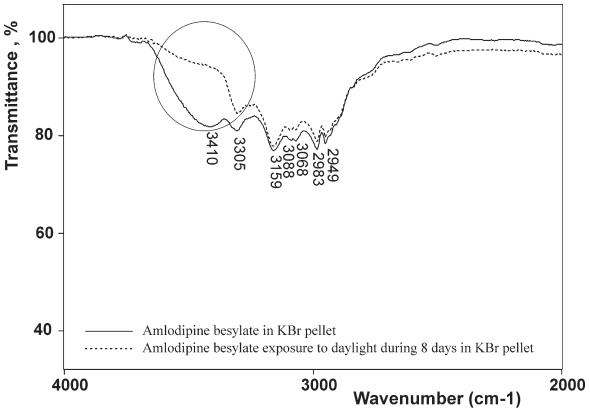

Figure 6a. FTIR spectrum of amlodipine besylate without and with irradiation with daylight during 8 days (range 2000 - 4000 cm^{-1})

The analysis of these two spectra shows that after 8 days a complete loss of the band arising from $\nu_{(N-H)}$ vibrations of the secoundary NH group of 1,4-dihydropyridine ring of amlodipine besylate appearing at 3410 cm^{-1} in the spectrum of non-irradiated amlodipine besylate occured. This points to the dehydrogenation process and the transition of the 1,4-dihydropyride ring into the pyridine ring, as confirmed by the changes in the bands in the range 600-800 cm^{-1}, as well as the bands in the range 1206-1670 cm^{-1} which are more intense, and which are important for $\delta_{(C-H)}$ deformation and $\nu_{(C=C)}$ and $\nu_{(C-N)}$ valence vibrations in the pyridine ring.

FTIR spectra of inclusion complexes of amlodipine besylate with cyclodextrins before and after the exposure to the daylight for given time intervals are presented in Fig. 7.

One cannot notice any significant changes in the number, position or intensity of the bands in both inclusion complexes with respect to the initial spectra, which means that for the observed time period amlodipine besylate in the inclusion complexes did not undergo photodegradation. Namely, by molecular incapsulation of amlodipine besylate into cyclodextrins vacancies, its increased stability to daylight is achieved.

4. Conclusion

Amlodipine besylate can be complexed to produce inclusion complexes with cyclodextrins, giving supramolecular systems which could be of interest for the production of pharmaceutical formulations. Such

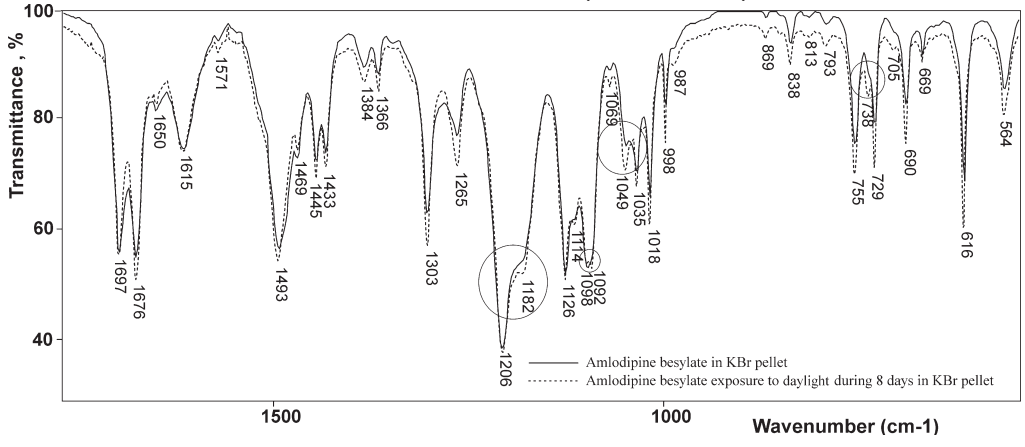

Figure 6b. FTIR spectrum of amlodipine besylate without and with irradiation with daylight during 8 days (range 500 - 1800 cm^{-1})

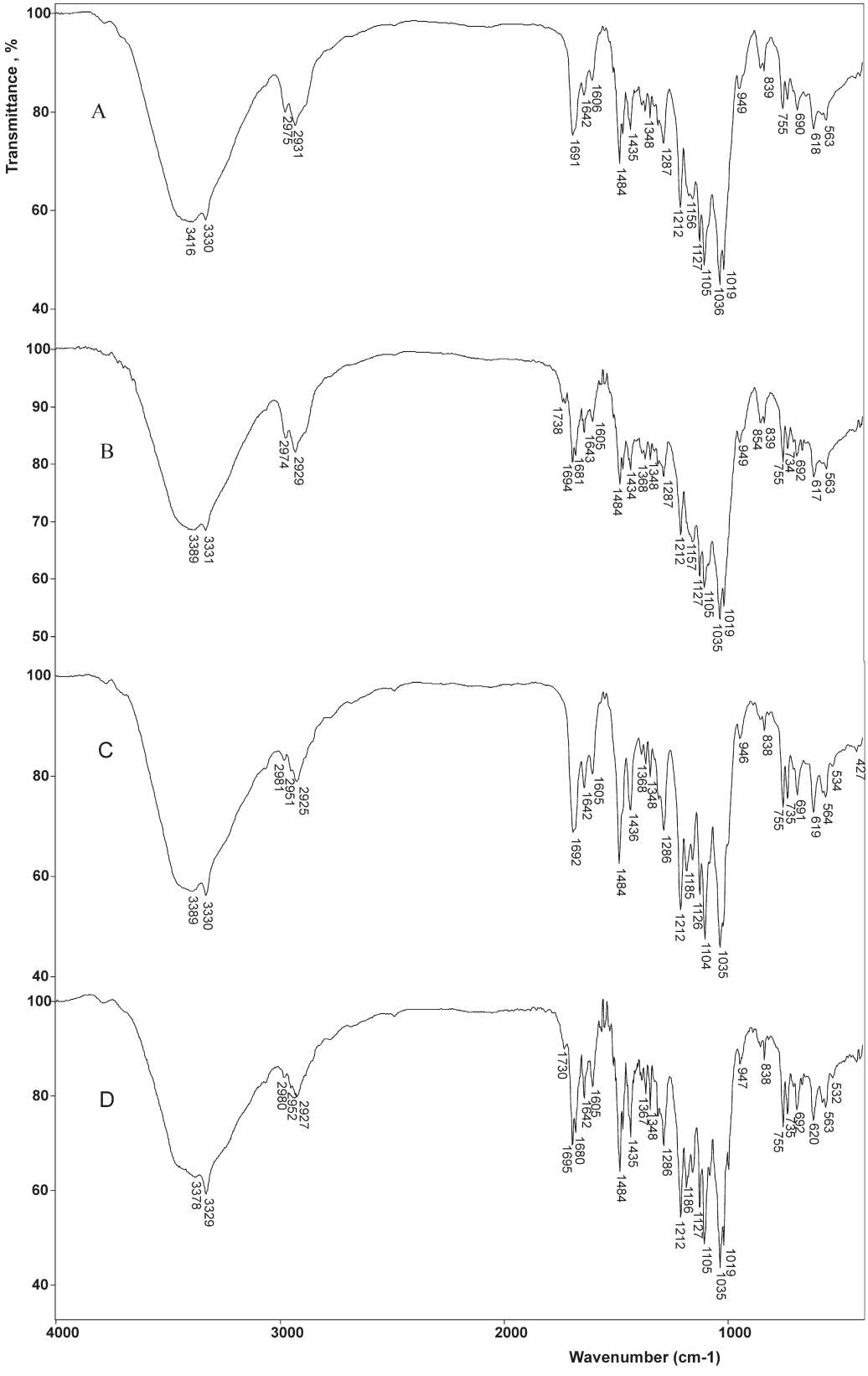

Figure 7. FTIR spectra of complex of amlodipine besylate with β-cyclodextrin non-irradiated (A) and irradiated by visible light during 8 days (B), complex of amlodipine besylate with 2-hydroxypropyl-β-cyclodextrin non-irradiated (C) and irradiated by visible light during 8 days (D)

inclusion complexes correct physical and chemical characteristics of the guest molecule, *i.e.*, amlodipine besylate. Complexes prepared for this study have shown a significantly larger degree of protection from the continual exposition to daylight with a degradation level much lower than in the solid amlodipine besylate. Photosensitivity of amodipine besylate and inclusion complexes with cyclodextrins was followed by simple FTIR spectrometry using KBr pellets.

Acknowledgements

This paper was supported by Ministry of Science and Technological Development – Republic of Serbia, project TR-19048 and OI-142037.

References

[1] J. Stressnig, M. Grabner, J. Mitterdorfer, S. Hering, M. Sinnegger, H. Glossmann, Trends Pharmacol. Sci. 19, 108 (1998)
[2] D.R. Ambernethy, Am. Heart J. 118, 1100 (1989)
[3] P.A. Meredith, H.L. Elliott, Clin. Pharmacokinet. 22, 22 (1992)
[4] E.M. Sorkin, S.P. Clissold, Drugs 33, 296 (1987)
[5] D. Murdoch, R.C. Heel, Drugs 41, 478 (1991)
[6] J. Yoshida, T. Ishibashi, M. Nishio, Eur. J. Pharmacol. 472, 23 (2003)
[7] J. Yoshida, T. Ishibashi, M. Nishio, Eur. J. Pharmacol. 492, 103 (2004)
[8] X. Li, G.R. Ruan, W.L. Lu, H.Y. Hong, G.W. Liang, Y.T. Zhang, Y. Liu, C. long, X. Ma, L. Yuan, J.C. Wang, X. Zhang, Q. Zhang, Journal of Controlled Release 112, 186 (2006)
[9] G. Ragno, A. Garofalo, C. Vetuschi, J. Pharm. Biomed. Anal. 27, 19 (2002)
[10] G. Ragno, E. Cione, A. Garofalo, G. Genchi, G. Ioele, A. Risoli, A. Spagnoletta, Int. J. Pharm. 265, 125 (2003)
[11] A. Michelitsch, J. Reiner, M. Schubert-Zsilavecz, W. Likussar, Pharmazie 50, 548 (1995)
[12] V. Nikolić, Lj. Nikolić, M. Stanković, A. Kapor, M. Popsavin, D. Cvetković, J. Serb. Chem. Soc. 72, 737 (2007)
[13] V. Nikolić, M. Stanković, A. Kapor, Lj. Nikolić, D. Cvetković, J. Stamenković, Pharmazie 59, 845 (2004)
[14] T. Cserhati, E. Forgacs, J. Szejtli, Int. J. Pharm. 141, 1 (1996)
[15] K. Csabai, M. Vikmon, J. Szejtli, E. Redenti, G. Poli, P. Vrntura, J. Inclusion Phenom. Macromol. Chem. 31, 169 (1998)
[16] R.H. Bisby, C. Mead, C.G. Morgan, Photochem. Photobiol. 71, 57 (2000)
[17] P. Sansdrap, A.J. Moë, Int. J. Pharm. 98, 157 (1993)
[18] N. Yüksel, T. Tincer, T. Baykara, Int. J. Pharm. 140, 145 (1996)
[19] J. Mielcarek, A. Czernielewska, B. Czarczynska, J. Incl. Phenom. Macro. 54, 17 (2006)
[20] A. Kapor, S. Skuban, V. Nikolić, Ž. Cvejić, S. Rakić, J. Res. Phys. Vol. 32, No.1, 25 (2008)
[21] V. Marinković, D. Agbaba, K. Karljiković-Rajić, J. Comor, D. Živanov-Stakić, Il Farmaco 55, 128 (2000)
[22] J. Mielcarek, J. Pharm. Biomed. Anal. 15, 681 (1997)