J. Serb. Chem. Soc. 72 (8–9) 737–746 (2007)
JSCS–3607
UDC 539.219.1+535.42+54–77:547.458.68
Original scientific paper

A molecular inclusion complex of atenolol with 2-hydroxypropyl-β-cyclodextrin; the production and characterization thereof

VESNA NIKOLIĆ[1*#], LJUBIŠA NIKOLIĆ[1#], MIHAJLO STANKOVIĆ[1#], AGNEŠ KAPOR[2], MIRJANA POPSAVIN[3#] and DRAGAN CVETKOVIĆ[1]

[1]Faculty of Technology, Bulevar oslobođenja 124, 16000 Leskovac, [2]Faculty of Science, Department of Physics, Trg Dositeja Obradovića 4, 21000 Novi Sad and [3]Faculty of Science, Department of Chemistry, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia

(Received 28 August 2006, revised 21 March 2007)

Abstract: The molecular inclusion complex of atenolol with 2-hydroxypropyl-β-cyclodextrin was synthesized using the coprecipitation method. The complex obtained was characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR spectroscopy, as well as by DSC and X-ray diffraction analysis. The DSC analysis confirmed the existence of the complex with the endothermic atenolol melting peak at about 155 ºC disappearing. The X-ray diffraction patterns of the complex and 2-hydroxypropyl-β-cyclodextrin were very similar, thus confirming the complete inclusion of the atenolol molecule within the cavity of the 2-hydroxypropyl-β-cyclodextrin. The peaks originating from atenolol were completely absent in the diffractogram of the complex. ^{1}H-NMR and ^{13}C-NMR spectra showed certain changes in the chemical shifts of protons and C atoms from atenolol and 2-hydroxypropyl-β-cyclodextrin, indicating that a complex had been formed and also which protons participated in the hydrogen bonds which formed the complex. The atenolol solubility in water was improved (254 mg complex cm^{-3}, *i.e.,* 37.5 mg atenolol cm^{-3}), and in pH 3 HCl solution (251 mg complex cm^{-3}, *i.e.,* 37 mg atenolol cm^{-3}) when compared to pure atenolol, and even when compared to the atenolol complex with β-cyclodextrin. The increased solubility ensures greater bioavailability of the active component and, due to the low solubility, significantly corrects for the lack of the basic active substance and, simultaneously, increases its overall therapeutic effect, combined with reduced side effects.

Keywords: atenolol, 2-hydroxypropyl-β-cyclodextrin, inclusion complex, FT-IR, DSC, NMR, X-ray.

INTRODUCTION

Cyclodextrins and their derivatives build canal inclusion compounds, so-called "molecular inclusion compounds". In these compounds, a single molecule can receive a molecule of another compond into the cavity of its large ring (canal ca-

* Corresponding author. E-mail: nvesna@yahoo.com
# Serbian Chemical Society member.
doi: 10.2298/JSC0709737N

vity diameter 0.5–0.9 nm). In crystalline state, some spirally wound molecules of cyclodextrin are placed one above the other, thus forming longer canal where molecules of various organic substances can be located, representing so-called molecular encapsulation.[1–9] There are a number of examples of molecular inclusion complexes of cyclodextrins and their derivatives, especially with respect to various types of drugs.

Ammar gave a description of the formation of an ampicillin and β-cyclodextrin complex, which eliminates one of the drawbacks of ampicillin, *i.e.*, its tendency to polymerize when alone in an aqueous solution.[10] With most drugs, their bioavailability, *i.e.*, the absorption of the remedial substance is low due to their poor solubility and, therefore, their application is limited. This is the case with some antibiotics, such as polymixin and tetracycline hydrochloride.[11,12] By forming a complex of naproxen (a steroid anti-inflammatory agent with an analgesic effect) and β-cyclodextrin, the absorption of the former is increased and, thus, its bioavailability.[13] Heptacain, a local anesthetic, has better activity when in a complex with β-cyclodextrin because of its improved physico–chemical properties, *i.e.*, solubility, dissolution rate and membrane permeability.[14] The chemical and photochemical stabilities of retinol, meclozin hydrochloride and nicerdipin are improved after formation of inclusion complexes with cyclodextrins.[11,15,16] In order to form an inclusion complex, the diameter of the guest molecule must correspond to the diameter of the host molecule cavity.[11] Drugs from the β-blocker group are characterized by poor solubility in aqueous and gastric fluids and, therefore, the low solubility rate and the variability of their bioavailability directly affect the efficiency of the drug.[17] Consequently, in order to increase the solubility and solubility rate, atenolol was complexed into an inclusion complex with β-cyclodextrin, whereby a better, but still not completely satisfactory, solubility of the complex was achieved.[18] For the same reason, celiprolol, a well known β-blocker, was complexed with β-cyclodextrin and a correction of the solubility of this drug was achieved.[17] An improved solubility of felodipine was achieved by complexing with β-cyclodextrin.[19] Prazosin hydrochloride, an α_1-adrenoceptor antagonist used in clinical practice for the treatment of hypertension, vascular sclerosis and cardiac arrest, also has the problem of poor solubility in water, which is very important for its activity. By complexing this drug with β-cyclodextrin and hydroxypropyl-β-cyclodextrin, these complexes obtained solubility superior to those of the non-complexed drug.[20]

In a patent application, a method for the separation of racemic atenolol by a solid–liquid separation method is given. Moreover, another patent gives a description of the slow release of some drugs from the corresponding forms, including atenolol, carvedilol and hydrochlorothiazide.[21,22] A further patent gives a descryption of the increased bioavailability of atenolol in connection with cholic acid, but there is no data on inclusion type complexes with β-cyclodextrin derivatives

with the purpose of increasing the solubility of this drug.[23] 2-Hydroxypropyl-β-cyclodextrin was chosen for complexing atenolol because it has a better solubility in water at room temperature than β-cyclodextrin.[3]

EXPERIMENTAL

Reagents

Atenolol (*RS*)-2-{-4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl}acetamide is a racemic mixture of (*R*)-(+) and (*S*)-(–) stereoisomers of 99.43 % purity, purchased from Ipca Laboratories Limited, India, while 2-hydroxypropyl-β-cyclodextrin (average molecular weight 1540 g mol^{-1}) was obtained from Sigma–Aldrich, Wisconsin.

Preparation of the inclusion complex by co-precipitation

Atenolol (266 mg) and 2-hydroxypropyl-β-cyclodextrin (1540 mg) were mixed and dissolved in 150 cm^3 of the water. The solution was stirred at room temperature for 24 h, evaporated using rotary evaporator at 50 ºC to a volume of approximately 20 cm^3, and then dried in a desiccator above concentrated sulfuric acid at 25 °C.

Preparation of a physical mixture

A homogenous physical mixture was prepared by mixing atenolol and 2-hydroxypropyl-β-cyclodextrin in a 1:1 molar ratio in a mortar.

X-Ray crystallography

X-Ray diffraction analysis was performed on a Phillips X'Pert powder diffractometer under the following conditions: the samples were exposed to monochromatic CuK_α radiation and analyzed in the 2θ range between 5 and 65º with a step of 0.05º and the recording time $\tau = 5$ s. The voltage and the strength of the electric current were 40 kV and 20 mA, respectively.

^{1}H-NMR and ^{13}C-NMR spectroscopy

The ^{1}H-NMR and ^{13}C-NMR spectra of the samples were recorded in a 5 mm diameter glass cuvette at room temperature on a Bruker AC 250 E NMR spectrometer, operating at frequencies of 250 and 62.5 MHz, respectively, by the pulse method and with multiple pulse repetitions. D_2O was used as the solvent.

Differential scanning calorimetry (DSC)

DSC Curves of the samples were recorded on a DuPont DSC differential scanning calorimeter in the temperature range 30–280 ºC at a scanning rate of 10 ºC min^{-1}. The measurements were performed on 5 mg of the sample in closed aluminum containers under a nitrogen atmosphere.

Fourier transformation infrared (FTIR) spectroscopy

The FTIR spectra of the samples were recorded in KBr pellets (0.6 mg sample, 140 mg KBr) in the wavenumber range 4000–400 cm^{-1}, on a Bomem Hartmann & Braun MB-series FTIR spectrophotometer.

RESULTS AND DISCUSSION

X-Ray crystallography

The diffractograms of atenolol (A), 2-hydroxypropyl-β-cyclodextrin (B), the molecular inclusion complex of 2-hydroxypropyl-β-cyclodextrin and atenolol (C), and the physical mixture (D) are shown in Fig. 1. A comparative analysis of these diffractograms shows that the diffractogram of the complex (C) is almost identical to that of 2-hydroxypropyl-β-cyclodextrin (B), confirming the hypothe-

sis that the atenolol molecule was included into the cavity of 2-hydroxypropyl-β-cyclodextrin, which shielded it completely from the X-rays. On the other hand, the commercially available, high purity (99.46 %) atenolol sample had a high degree of crystallinity, as evidenced by the very articulated peaks in its diffractogram. The diffractogram of 2-hydroxypropyl-β-cyclodextrin indicates the existence of two wide diffraction peaks in the range of about $2\theta = 10.516°$ to $2\theta = 18.882°$, which are not structured and show the existence of a disorganized crystal structure at great distances. The diffractogram of the physical mixture (D) confirms by the presence of the peaks of both components of the mixture that this really was a mixture of atenolol and 2-hydroxypropyl-β-cyclodextrin; the peaks arising from atenolol were absent in the diffractogram of the complex. The X-ray diffraction pattern of the complex confirms that atenolol is complexed as a molecular inclusion into the crystalline grid of the matrix, 2-hydroxypropyl-β-cyclodextrin.

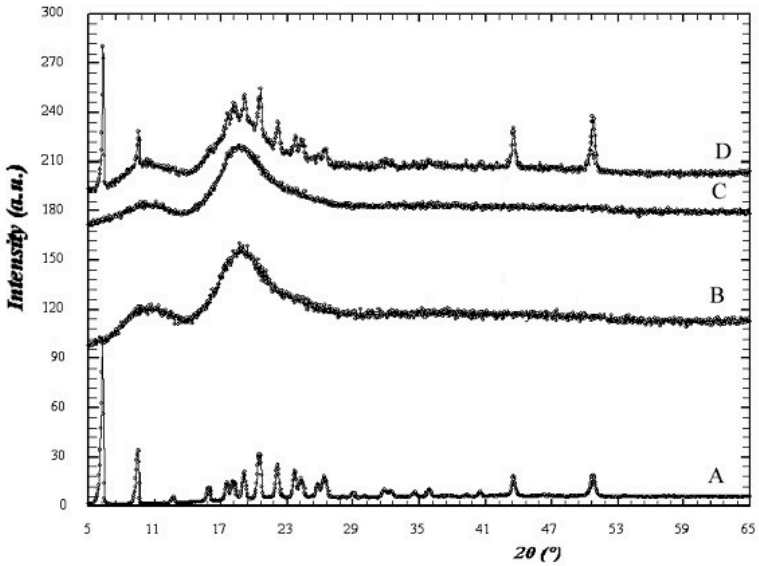

Fig. 1. X-ray diffractograms of atenolol (A), 2-hydroxypropyl-β-cyclodextrin (B), the inclusion complex of atenolol and 2-hydroxypropyl-β-cyclodextrin (C) and a mixture of atenolol and 2-hydroxypropyl-β-cyclodextrin (D).

NMR analysis

The ^{1}H-NMR analysis shows the occurrence of the molecular encapsulation of atenolol into the hydrophobic cavity of 2-hydroxypropyl-β-cyclodextrin because the spectra of the basic components are different from those of the molecular inclusion complex. The labeling of the C atoms in atenolol and 2-hydroxypropyl-β-cyclodextrin where the protons are situated is shown in Figs. 2 and 3 and the chemical shifts, as well as the changes in proton chemical shifts in the complex with respect to the pure components, are shown in Table I.

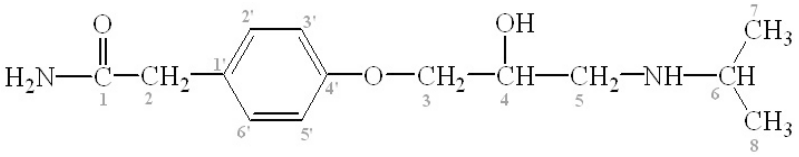

Fig. 2. Labeling of the C atoms in an atenolol molecule.

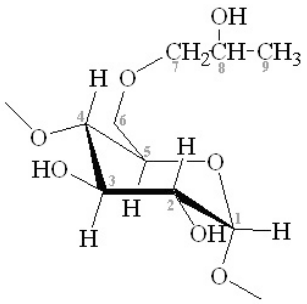

Fig. 3. Labeling of the C-atoms in the hydroxypropylglucose unit of a 2-hydroxypropyl-β-cyclodextrin molecule.

TABLE I. Chemical shifts (δ) and changes of proton chemical shifts ($\Delta\delta$) in the ^{1}H-NMR spectra of atenolol, 2-hydroxypropyl-β-cyclodextrin, and the complex of atenolol with 2-hydroxypropyl-β-cyclodextrin

C atom	δ / ppm		$\Delta\delta$ / ppm
	Atenolol	Complex	
2	3.500 *s*	3.852 *s*	+0.352
3 and 4	4.000 *m*	4.200 *m*	+0.200
5 and 6	2.750 *m*	2.963 *m*	+0.213
7 and 8	1.017 *d*	1.179 *d*	+0.162
2' and 6'	6.958 *d*	7.037 *d*	+0.079
3' and 5'	7.208 *d*	7.296 *d*	+0.088
	2-Hydroxypropyl-β-cyclodextrin	Complex	
1	5.137 *d*	5.200 *d*	+0.063
2 to 8	3.731 *m*	3.852 *m*	+0.121
9	1.108 *d*	1.179 *d*	+0.071

On the basis of these results it can be concluded that the protons from atenolol on the 2, 3, 4, 5, and 6 C atom had the greatest shifts ($\Delta\delta$ from 0.352 to 0.200) in the complex, which means that they participated to a great extent in the interaction with 2-hydroxypropyl-β-cyclodextrin. The other protons from atenolol also showed shifts, but they were slightly less in magnitude. The greatest proton shifts in 2-hydroxypropyl-β-cyclodextrin in the complex relative to the uncomplexed compound occurred at the H atoms bonded to carbons C_2 to C_8, while the shifts of those bonded to C_1 and C_9 were slight.

This analysis indicates an inclusion type of complexing between atenolol and 2-hydroxypropyl-β-cyclodextrin, in which hydrogen bonds, apart from physical factors, play an important role in the guest–host interactions.

The analysis of the ^{13}C-NMR results, also indicating the inclusion of the guest into the host cavity, show that the greatest chemical shifts after complexing occurred at the C_1, C_4, C_5, C_7, and C_8 atoms in atenolol, while in 2-hydroxypropyl-β-cyclodextrin, the greatest shifts were found at the C_1, C_4, and C_5 atoms. The differences in chemical shifts for each C atom in atenolol and in 2-hydroxypropyl-β-cyclodextrin with respect to the molecular inclusion complex are shown in Table II.

TABLE II. Chemical shifts and variations of the chemical shifts of the C-atoms in ^{13}C-NMR spectra of atenolol, 2-hydroxypropyl-β-cyclodextrin and the complex of atenolol with 2-hydroxypropyl--β-cyclodextrin

C-atom	δ / ppm		$\Delta\delta$ / ppm	δ / ppm		$\Delta\delta$ / ppm
	Atenolol	Complex		2-Hydroxypropyl-β--cyclodextrin	Complex	
1	180.818	179.912	–0.906	102.933	103.361	+0.428
2	43.585	43.897	+0.312	74.506	74.383	–0.123
3	73.312	73.405	+0.093	75.629	75.806	+0.177
4	71.342	70.660	–0.682	83.212	83.622	+0.410
5	51.011	51.526	+0.515	74.787	75.058	+0.271
6	50.922	51.030	+0.108	63.113	62.994	–0.119
7 and 8	23.844	23.420	–0.424	–	–	–
1'	130.521	130.762	+0.241	–	–	–
2' and 6'	117.883	117.651	–0.232	–	–	–
3' and 5'	133.285	133.079	–0.206	–	–	–
4'	160.143	160.308	+0.165	–	–	–
7	–	–	–	79.497	80.861	+1.364
8	–	–	–	69.219	69.224	+0.005
9	–	–	–	20.919	20.941	+0.022

DSC analysis

The DSC curves of atenolol (A), 2-hydroxypropyl-β-cyclodextrin (B), a mixture of atenolol and 2-hydroxypropyl-β-cyclodextrin (C), and the complex of atenolol and 2-hydroxypropyl-β-cyclodextrin (D) are shown in Fig. 4. A comparative analysis of the DSC curves also confirms the inclusion of the guest molecule into the cavity of the host. The DSC curve of the inclusion complex has no endothermic melting peak of racemic atenolol at about 155 ºC, which is, on the other hand, present in the curve of atenolol and of the mixture of atenolol and 2-hydroxypropyl-β-cyclodextrin.

Infrared spectroscopy

By comparing IR spectrum of the inclusion complex of 2-hydroxypropyl-β-cyclodextrin and atenolol to those of the pure substances and the physical mixture, it can be concluded that there are no variations in the spectrum of the physical

mixture with respect to the spectra of the pure components, but that there are variations in the IR spectrum of the complex (Fig. 5). Namely, pure atenolol has a strong band at 1638 cm^{-1}, originating from C=O valence vibrations (amide band I), which, in the inclusion complex, has a significantly lower intensity and occurs at 1668 cm^{-1}, which results from this vibration being covered due to the inclusion of atenolol into the cavity of 2-hydroxypropyl-β-cyclodextrin. For the same reason, the band at 3180 cm^{-1}, originating from the NH valence vibrations, present in the spectrum of atenolol is absent in spectrum of the complex, as is the amide band III, originating from the C–N valence vibration coupled with NH bending vibrations, which is present in the spectrum of atenolol at 1417 cm^{-1}. Also, the band at 1516 cm^{-1}, originating from C=C valence vibrations of the aromatic part of the atenolol system, does not appear in the IR spectrum of the complex. This analysis is in accordance with the results of the previous methods used to analyze the complex and it confirms the realization of the inclusion of the atenolol molecule into the hydrophobic cavity of the host molecule, 2-hydroxypropyl-β-cyclodextrin, *i.e.*, the formation of the molecular inclusion complex.

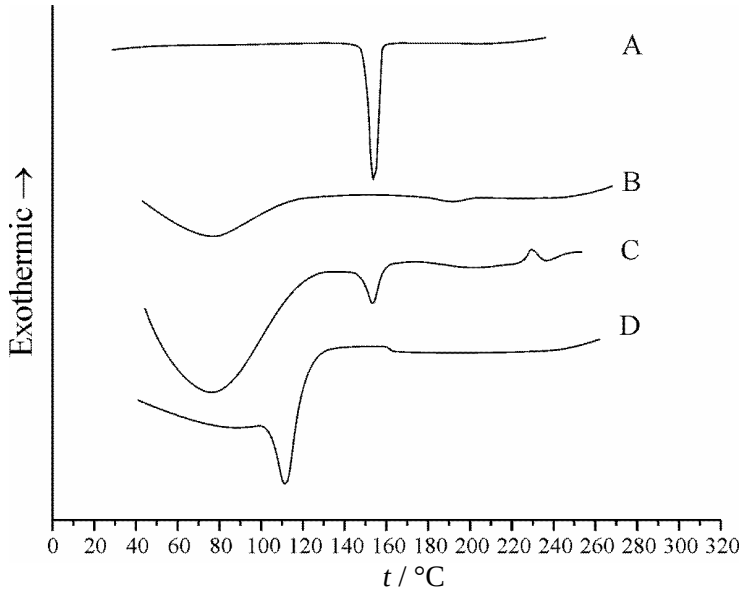

Fig. 4. DSC curves of atenolol (A), 2-hydroxypropyl-β-cyclodextrin (B), a mixture of atenolol and 2-hydroxypropyl-β-cyclodextrin (C), and the inclusion complex of atenolol with 2-hydroxypropyl-β-cyclodextrin (D).

Dissolution study

The room temperature solubility of pure atenolol in water is 0.3 mg cm^{-3}.[24] On complexing it with 2-hydroxypropyl-β-cyclodextrin, its solubility is increased to 254 mg complex cm^{-3}, *i.e.*, to 37.5 mg atenolol cm^{-3}, in water and 251 mg com-

plex cm^{-3}, *i.e.*, 37 mg atenolol cm^{-3} in a pH 3 solution of HCl. In this way, the bioavailability of the drug to the organism can be increased and the effect of the therapy can be achieved by administration a lower quantity of the substance.

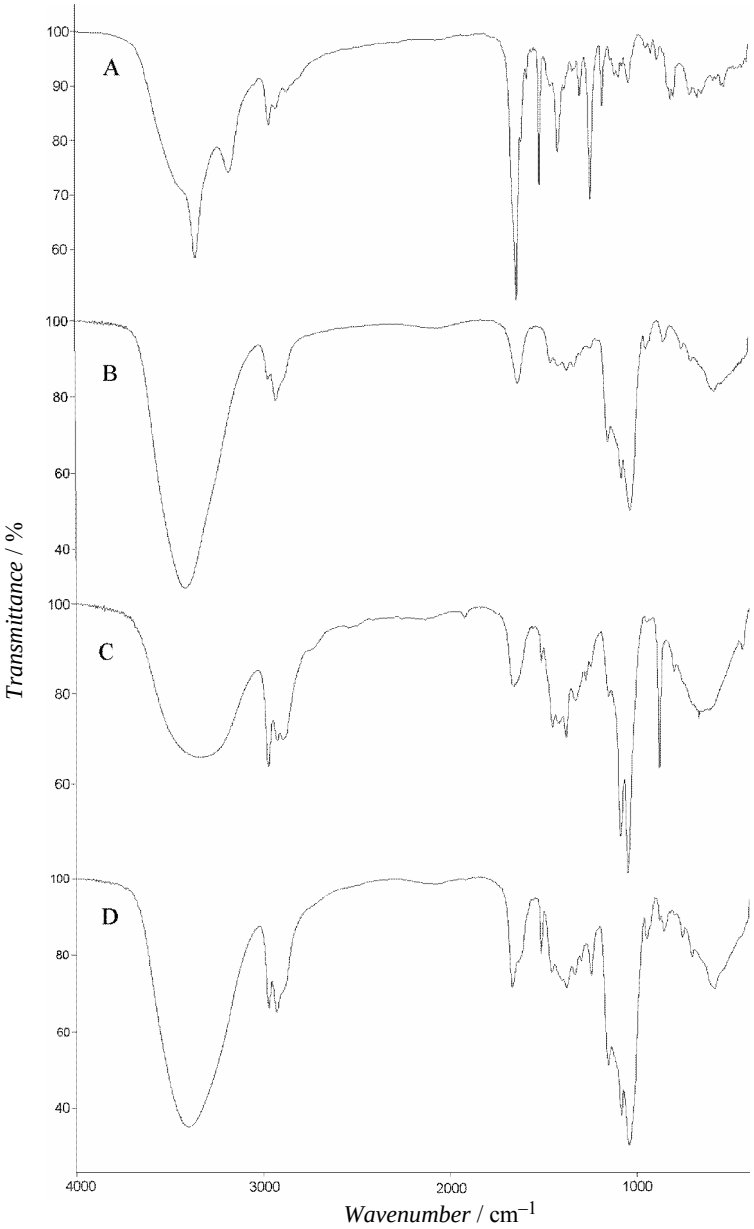

Fig. 5. FT-IR Spectra of atenolol (A), 2-hydroxypropyl-β-cyclodextrin (B), a mixture of atenolol and 2-hydroxypropyl-β-cyclodextrin (C), and the inclusion complex of atenolol and 2-hydroxypropyl-β-cyclodextrin (D).

CONCLUSIONS

From the point of view of pharmacologically active formulations with basic components of varied physical properties, inclusion complexes are of interest. In such supramolecular structures the active substance, in this case atenolol, which will have excellent solubility in this form, can be incorporated in an exact quantity, thus ensuring better bioavailability and functional value in medicine.

Compared to free atenolol of hard consistency and with poor solubility in water and, consequently, low bioavailability, the molecular inclusion complexes of atenolol with cyclodextrin derivatives are also solid, powdery substances containing the active substance in a form giving them manifold solubility, and consequently, better bioavailability and efficiency.

Acknowledgements: This paper was supported by the Ministry of Science of the Republic of Serbia through the project TR–6708B.

ИЗВОД

МОЛЕКУЛСКИ ИНКЛУЗОНИ КОМПЛЕКС АТЕНОЛОЛА СА 2-ХИДРОКСИПРОПИЛ-β--ЦИКЛОДЕКСТРИНОМ; ПОСТУПАК ДОБИЈАЊА И КАРАКТЕРИЗАЦИЈА

ВЕСНА НИКОЛИЋ[1], ЉУБИША НИКОЛИЋ[1], МИХАЈЛО СТАНКОВИЋ[1], АГНЕШ КАПОР[2], МИРЈАНА ПОПСАВИН[3] и ДРАГАН ЦВЕТКОВИЋ[1]

[1]Технолошки факултет, Булевар Ослобођења 124, Лесковац, [2]Природно–математички факултет, Департман за физику, Трг Доситеја Обрадовића 4, Нови Сад и [3]Природно–математички факултет, Департман за хемију, Трг Доситеја Обрадовића 3, Нови Сад

Молекулски инклузиони комплекс атенолола са 2-хидроксипропил-β-циклодекстрином синтетисан је методом копреципитације. Добијени комплекс је окарактерисан методама FT-IR, DSC, ^{1}H-NMR, ^{13}C-NMR и дифракцијом X-зрака. DSC анализа је потврдила постојање комплекса у коме нестаје ендотермни пик од топљења атенолола на око 155 °C. Дифрактограми комплекса и 2-хидроксипропил-β-циклодекстрина су веома слични чиме је потврђена потпуна заклоњеност молекула атенолола унутар шупљине 2-хидроксипропил-β-циклодекстрина. Пикови који потичу од атенолола на дифрактограму комплекса у потпуности нестају. ^{1}H-NMR и ^{13}C-NMR спектри су показали извесне промене у хемијским померањима протона и C атома из атенолола и 2-хидроксипропил-β-циклодекстрина, што такође указује на стварање комплекса, као и на то који протони учествују у стварању водоничних веза којима је формиран комплекс. Постигнута је побољшана растворљивост комплексираног атенолола у води (254 mg комплекса cm^{-3}, тј. 37,5 mg атенолола cm^{-3}) и у раствору HCl, pH 3 (251 mg комплекса cm^{-3}, тј. 37 mg атенолола cm^{-3}) у односу на чист атенолол, па чак и у односу на атенолол који је комплексиран β-циклодекстрином. Повећана растворљивост обезбеђује и већу биорасположивост активне компоненте и значајно коригује недостатак основне активне супстанце услед слабе растворљивости повећавајући њен укупни терапеутски учинак са смањеним споредним појавама.

(Примљено 28. августа 2006, ревидирано 21. марта 2007)

REFERENCES

1. A. R. Hedges, *Chem. Rev.* **98** (1998) 2035
2. V. T. Karathanos, I. Mourtzinos, K. Yannakopoulou, N. K. Andrikopoulos, *Food Chem.* **101** (2007) 652
3. H. J. Schneider, F. Hacket, V. Rudiger, *Chem. Rev.* **98** (1998) 1755

4. J. Szejtli, *Chem. Rev.* **98** (1998) 1743
5. M. Morgan Conn, J. Rebek, Jr., *Chem. Rev.* **97** (1997) 1647
6. P. Wallimann, T. Marti, A. Furer, F. Diederich, *Chem. Rev.* **97** (1997) 1567
7. K. Uekama, F. Hirayama, T. Irie, *Chem. Rev.* **98** (1998) 2045
8. F. Hapiot, S. Tilloy, E. Monflier, *Chem. Rev.* **106** (2006) 767
9. A. R. Khan, P. Forgo, K. J. Stine, V. T. D'Souza, *Chem. Rev.* **98** (1998) 1977
10. H. O. Ammar, S. A. El–Nahhas, M. M. Ghorab, *Pharmazie* **51** (1996) 568
11. A. Angelova, C. R. Lefebvre, A. Baszkin, *J. Colloid Interface Sci.* **212** (1999) 275
12. S. T. Stankov, N. Lambov, E. Minkov, *Pharmazie* **47** (1992) 125
13. N. Celebi, M. Iscanoglu, T. Degim, *Pharmazie* **46** (1991) 863
14. K. Kralova, J. Cizmarik, J. Szejtli, *Pharmazie* **47** (1992) 460
15. M. Berićević, R. Senjković, *Pharmazie* **47** (1992) 202
16. J. Mielcarek, *Pharmazie* **51** (1996) 477
17. R. Ficarra, P. Ficarra, M. R. Di Bella, D. Raneri, S. Tommasini, M. L. Calabro, M. C. Gamberini, C. Rustichelli, *J. Pharm. Biomed. Anal.* **23** (2000) 33
18. R. Ficarra, P. Ficarra, M. R. Di Bella, D. Raneri, S. Tommasini, M. L. Calabro, A. Villari, S. Coppolino, *J. Pharm. Biomed. Anal.* **23** (2000) 231
19. J. Mielcarek, *J. Inclusion Phenom. Mol. Recognit. Chem*. **30** (1998) 243
20. L. Liu, S. Zhu, *J. Pharm. Biomed. Anal.* **40** (2006) 122
21. T. Yoshikazu, S. Nobuaki, K. Kazuhiro, EP 0 605 384 A1 (1994)
22. Z. Shuyi, W. Jin, WO 2005/030201 (2003)
23. P. James, C. Andrew, M. Dean, L. Kimberley, WO 2001/076531 (2001)
24. M. Moneghini, A. Carcano, G. Zingone, B. Perissutti, *Intern. J. Pharmaceutics* **175** (1998) 177.