VERSITA

Cent. Eur. J. Chem. • 8(4) • 2010 • 744–749
DOI: 10.2478/s11532-010-0043-x

Central European Journal of **Chemistry**

The protection of Nifedipin from photodegradation due to complex formation with β-cyclodextrin

Research Article

Vesna Nikolić*[1], Dušica Ilić[1], Ljubiša Nikolić[1], Mihajlo Stanković[1], Milorad Cakić[1], Ljiljana Stanojević[1], Agneš Kapor[2], Mirjana Popsavin[3]

[1]*Faculty of Technology, University of Nish, 16000 Leskovac, Serbia*

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

[3]*Faculty of Sciences, Department of Chemistry, University of Novi Sad, 21000 Novi Sad, Serbia*

Received 19 November 2009; Accepted 26 February 2010

Abstract: The inclusion complex β-cyclodextrin:nifedipin was prepared in solid state by coprecipitation with 1:1 mol ratio. The structure of the obtained complex and nifedipin was characterized by use of X-ray diffraction (XR), infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), and differential scanning calorimetry (DSC) methods. The photodegradation of nifedipin and the β-cyclodextrin:nifedipin inclusion complex in solid state was monitored under natural daylight by infrared spectroscopy, whereby the free nifedipin degraded four to five times faster than the complexed nifedipin. The photodegradation products of both free and complexed nifedipin, formed during irradiation at 350 nm (with corresponding energy flux of 18 W m^{-2}) were monitored by liquid chromatography during various time intervals. The speed of formation of nitroso- and nitro-phenyl derivatives by nifedipin irradiation was significantly higher than those of complexed nifedipin irradiation, which indicates its increased photostability in the inclusion complex. The effect on this property is significant because it contributes both to the improvement of the therapeutic effect of nifedipin and to the safer application thereof.

Keywords: *Nifedipin • Inclusion complex • β-cyclodextrin • Photodegradation • Liquid chromatography*

1. Introduction

Nifedipin [1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine carboxylate] is a derivative of 1,4-dihydropyridine. It belongs to a group of active substances with therapeutic use as calcium channel blockers and coronary vasodilators [1-5]. o-Nitro derivatives of 1,4-dihydropyridine are very unstable compounds, which readily oxidize under the influence of light to the nitroso and nitro derivatives shown in Fig. 1 [6-8].

Oxidation of nifedipin brings about the loss of its pharmacological activity, to the point that nitroso derivatives even show some toxicity. The limiting factors for the use of nifedipin for clinical purposes are its high photosensitivity and poor solubility [2].

Building inclusion complexes with β-cyclodextrins can increase nifedipin's stability 5- to 10-fold [9-11]. Among the natural cyclodextrins, α-, β- and γ-cyclodextrins – consisting of 6, 7 and 8 D-glucopyranose units, respectively – are the most frequently used for these purposes. The glucosidal units are linked by α-1,4 glycoside links to form the cyclic structures shown in Fig. 2 [12,13].

X-ray analysis proved that the glucosidal residues in cyclodextrins are constrained to the more thermodynamically stable chair conformation because all the substituents are in equatorial positions. As a consequence of this conformation, all secondary

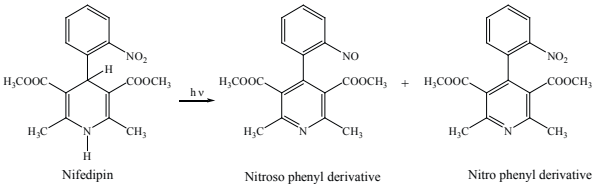

Figure 1. Nifedipin degradation under the influence of light to nitroso derivatives [4-(2-nitrosophenyl)-2,6-dimethyl-3,5-dimethoxy carbonyl pyridine] and nitro derivative [4-(2-nitrophenyl)-2,6-dimethyl-3,5-dimethoxy carbonyl pyridine].

* *E-mail: nvesna@yahoo.com*

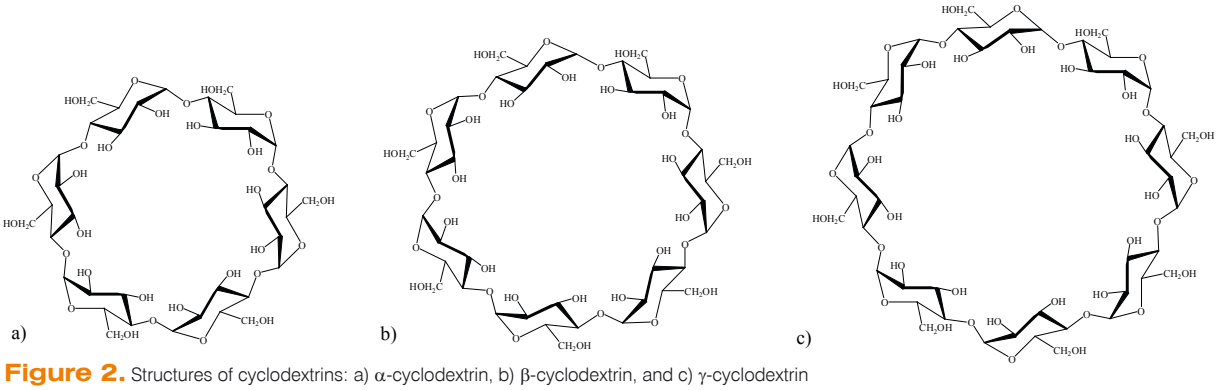

Figure 2. Structures of cyclodextrins: a) α-cyclodextrin, b) β-cyclodextrin, and c) γ-cyclodextrin

hydroxyl groups (OH-2 and OH-3) are located on one side of the cylindrical molecule, while all primary hydroxyl groups (OH-6) are on the opposite side. As such, the wider side of the cavity is surrounded by secondary hydroxyl groups, *i.e.,* the outer side of cyclodextrin is hydrophilic and, therefore, soluble in aqueous media. Conversely, the inner cavity of cyclodextrin is much more hydrophobic in character because the free electron pairs of the glycoside bridge oxygens are within the cavity [14,15]. Various organic molecules can be encapsulated in the cyclodextrin cavity, forming so-called inclusion complexes. Formation of inclusion complexes alters physical properties of the included components, such as solubility, photosensitivity, stability, volatility, flavor and others [16-22]. Alterations to these characteristics of the active component contribute to its safer and more efficient application, especially for the production of pharmaceutical formulations.

In this work, the inclusion complex of nifedipin with β-cyclodextrin was prepared in order to increase its solubility and protect it from daylight. Nifedipin and the inclusion complex β-cyclodextrin:nifedipin were structurally characterized by use of various methods: XR, ^{1}H-NMR, FTIR and DSC. Photostability of nifedipin and of the β-cyclodextrin:nifedipin inclusion complex in solid state exposed to daylight was assessed by FTIR spectrometry. Finally, aqueous solutions of nifedipin and of β-cyclodextrin:nifedipin inclusion complex were subjected to UV-radiation and the degradation products were analyzed by high-pressure liquid chromatograpy (HPLC).

2. Experimental Procedure

2.1 Reagents

Nifedipin,[1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine carboxylate], 99.67% pure, was purchased from Ipca Laboratories Limited, India, and β-cyclodextrin from Merck, 98%, Darmstadt. Other solvents and reagents used were p.a. and HPLC purity.

2.2 Preparation of inclusion complex by co-precipitation

Nifedipin (1 mmol, 346 mg) and β-cyclodextrin (1 mmol, 1135 mg) were mixed and dissolved in 150 cm^3 of water. The light-protected solution was mixed at room temperature for 24 hours, evaporated in a vacuum evaporator at 50°C to approx. 20 cm^3 volume, and then dried in a desiccator above concentrated sulfuric acid at 25°C. After drying, a yellow cristalline complex was obtained and used for further investigations in this work as such.

2.3 Preparation of physical mixture

Physical mixtrures were prepared by simply blending nifedipin and β-cyclodextrin with 1:1 molar ratio in a mortar until uniformity.

2.4 X-ray crystallography

X-ray diffraction was performed on a Phillips X'Pert powder diffractometer under the following conditions: samples were exposed to monochrome CuK_α radiation and analyzed under angle 2θ between 5 and 55° with 0.05° increments and recording time, τ=5 sec. The voltage and the strength of the electric current were 40 kV and 20 mA, respectively.

2.5 ^{1}H NMR spectrometry

^{1}H-NMR spectra of the samples were measured on a Bruker AC 250 E NMR spectrometer with operating frequencies of 250 MHz, in a 5 mm diameter glass cuvette at room temperature, by pulse method with multiple pulse repetitions. D_2O was used as the solvent for nifedipin:β-cyclodextrin complex and β-cyclodextrin, while $CDCl_3$ was used for nifedipin.

2.6 Differential scanning calorimetry (DSC)

DSC curves of the samples were recorded on a DuPont DSC differential scanning calorimeter with the scanning rate of 10°C min^{-1} and temperature range 20-320°C. Thermal properties were studied by heating about 5 mg of the sample in closed aluminum containers in nitrogen atmosphere.

2.7 Fourier transformation infrared spectrometry (FTIR)

KBr pellets prepared from the samples and protected from moisture were exposed to daylight for several days and FTIR spactra were recorded periodically to monitor the degradation of complexed and non-complexed nifedipin to degradation products under the influence of daylight.

2.8 HPLC analysis

Liquid chromatography was used to analyze the water solutions of nifedipin and nifedipin:β-cyclodextrin complex before and after irradiation with UV-light (λ=350 nm). Measurements were made on HPLC Agilent 1100 Series apparatus under the following conditions: column Zorbax Eclipse XDB-C18 4.6x150 mm, 5 μm; mobile phase acetonitrile/water 85/15 v/v; flow rate 0.5 cm^3 min^{-1}; column temperature 25°C; injected volume 20 μL. Detector DAD at 205 nm.

2.9 UV-irradiation

Continuous irradiation of samples were performed in a cylindrical photochemical reactor "Rayonet" with 14 symmetrically placed lamps with emission maxima at 350 nm (UV-A). Samples were irradiated in quartz cuvettes (1×1×4.5 cm) placed on a rotating circular holder, in the middle of the cylinder, 10 cm from the walls. The total measured energy flux at the samples was 18 W m^{-2} at 350 nm.

3. Results and Discussion

The diffractograms of nifedipin, β-cyclodextrin, β-cyclodextrin:nifedipin inclusion complex, and a physical mixture of nifedipin and β-cyclodextrin are shown in Fig. 3. There is a great difference between the diffractograms of the inclusion complex (diffractogram 3 in Fig. 3) and that of the physical mixture of nifedipin and β-cyclodextrin (diffractogram 4 in Fig. 3). The diffractogram of the physical mixture resembles the cumulative diffractograms of nifedipin and β-cyclodextrin.

On the other hand, there is a great similarity between the diffractograms of β-cyclodextrin (diffractogram 2 in Fig. 3) and the inclusion complex of nifedipin and β-cyclodextrin (diffractofram 3 in Fig. 3). These represent clear evidence that the inclusion of nifedipin within the cavities of β-cyclodextrin has been achieved in the complex, so that the reflection planes originating from nifedipin are not visible in the diffractogram, while they are present in the diffractogram of the physical mixture.

In Fig. 4, protons of the nifedipin molecule (a) and the glucosidal unit of β-cyclodextrin (b) are enumerated, and the results of ^{1}H-NMR testing of nifedipin, β-cyclodextrin and β-cyclodextrin:nifedipin complex are given in Table 1.

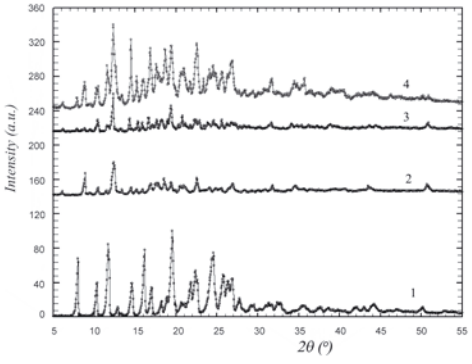

Figure 3. X-ray diffractograms of (1) nifedipin, (2) β-cyclodextrin, inclusion complex of nifedipin and (3) β-cyclodextrin, and (4) physical mixture of nifedipin and β-cyclodextrin

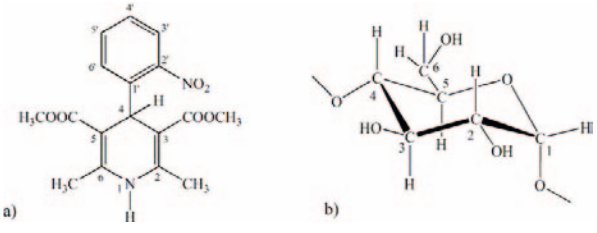

Figure 4. (a) Nifedipin molecule and (b) glucopyranose unit with indicated protons which give signals in 1H-NMR spectra

Table 1. Chemical shifts (δ) and changes of proton chemical shifts (Δδ) in 1H-NMR spectra of nifedipin, β-cyclodextrin, and the complex of nifedipin with β-cyclodextrin

C-atom, nifedipin	δ, ppm	C-atom	δ, ppm		Δδ, ppm
			β-cyclodextrin	Complex	
2 and 6 (6H,2xCH3)	2.28 s				
3 and 5 (6H, 2xOCH3)	3.54 s	1	5.12 s	5.01 s	-0.110
4 (1H)	5.68 s	2 and 4	3.65 m	3.595 m	-0.055
1(1H, NH)	5.92 s	3	4.03 t	3.822 t	-0.208
3'-6' (4H, Ar)	7.2-7.8 m	5 and 6	3.92 m	3.79 m	-0.130

The greatest $\Delta\delta$ shifts after nifedipin complexation with β-cyclodextrin were recorded at protons of the C_3, C_5 and C_6 atoms of the glucopyranose unit of β-cyclodextrin, indicating that these protons were those most involved in the interaction with the nifedipin molecule during the inclusion.

The FTIR spectra of nifedipin (A), β-cyclodextrin (B), and the inclusion complex of nifedipin and β-cyclodextrin (C) are given in Fig. 5. The FTIR spectra of β-cyclodextrin and the inclusion complex β-cyclodextrin:nifedipin are almost identical with respect to the position and intensity of the bands. The FTIR spectrum of nifedipin shows absorption bands originating from ν_{as}(N=O) at 1530 cm^{-1}, ν_s(N=O) at 1350 cm^{-1}, ν(C=O) at 1680 cm^{-1}, ν(C-O) at 1129 cm^{-1}, ν(N-H) at 3332 cm^{-1} that are directly related to its structure. In the FTIR spectrum of the β-cyclodextrin:nifedipin complex the nifedipin absorption bands are not present with the same intensity and shape as in the FTIR spectrum of nifedipin, which indicates covering of nifedipin within the β-cyclodextrin cavities, *i.e.*, the forming of the inclusion-type supramolecular structure.

DSC curves of nifedipin (A), β-cyclodextrin (B), complex of β-cyclodextrin:nifedipin (C) and the physical mixture of nifedipin and β-cyclodextrin (D) are shown in Fig. 6. DSC curves show the nifedipin melting peak at about 172°C. The peak is lower in intensity for the complex than for the physical mixture, which is the result of interaction during complex formation.

Shown in Fig. 7 is the chromatogram of nifedipin and its nitroso derivative formed by the irradiation of nifedipin by light at 350 nm wavelength for various time intervals.

Nifedipin in the solution (Fig. 7 peak 1) was largely converted into the nitroso derivative (Fig. 7 peak 2) even during the first 5 minutes of light exposure, and with further exposure the remaining quantities were also converted into the nitroso derivative. When the nifedipin complex solution was subjected to exposure under the same condition, the transformation of nifedipin into the nitroso derivative was found to be considerably slower (Fig. 8 peaks 1 through 7 and 8). This indicates an increased protection of the complexed nifedipin compared to free nifedipin.

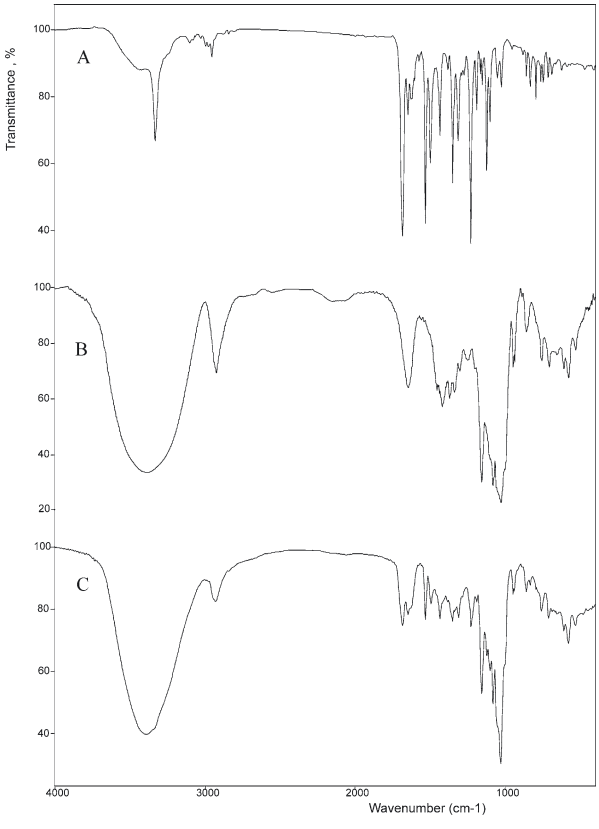

Figure 5. FTIR spectra of (A) nifedipin, (B) β-cyclodextrin and (C) the inclusion complex of nifedipin and β-cyclodextrin.

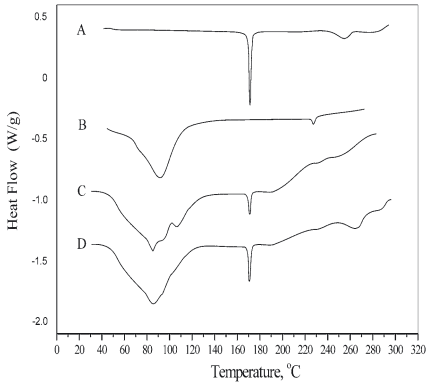

Figure 6. DSC curves of (A) nifedipin, (B) β-cyclodextrin, the inclusion complex of nifedipin and (C) β-cyclodextrin and (D) the physical mixtrure of nifedipin and β-cyclodextrin

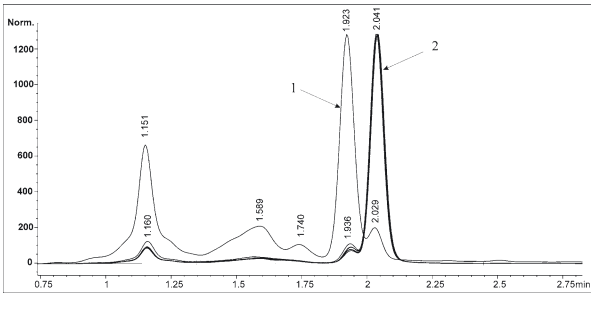

Figure 7. HPLC chromatogram of nifedipin: (1) nitro-derivative of nifedipin without irradiation, (2) nitroso-derivative of nifedipin obtained by irradiation at 350 nm for 5, 10, 15, 20, 25 and 30 min.

FTIR was used to monitor the effect of daylight on the stability of solid forms of nifedipin and complexes of nifedipin with β-cyclodextrin. Prepared KBr pellets with nifedipin and with β-cyclodextrin:nifedipin complex were exposed to daylight for several days (0 to 15 days) and FTIR spectra were recorded periodically. Figs. 9 and 10 display the spectra for pure nifedipin and nifedipin complex with β-cyclodextrin, respectively.

The bands that are indicative and can be used to monitor nifedipin degradation to nitroso derivatives during exposure to daylight are found at wave numbers 1681, 1731, and 3332, and they originate from the corresponding vibrations of the nifedipin carbonyl, the nitroso derivative carbonyl, and the nifedipin amine, respectively. With the increase of nifedipin exposure to daylight, the formation of the nitroso derivatives also increases. This conclusion is based on the absorption bands from C=O groups in nifedipin and the nitroso derivative, which occur at different frequencies, namely, 1681 and 1731 cm^{-1}, respectively. This phenomenon can be explained by the existence of an acid proton at the fourth carbon atom of the nifedipin molecule that may hydrogen bond with two oxygen atoms of the ester group, causing the C=O band to appear at a lower frequency (1681 cm^{-1}). In the nitroso derivative of nifedipin there is no acid proton, so the C=O group frequency has the usual value, *i.e.,* it appears at 1731 cm^{-1}. The irradiation of nifedipin also brings about the decrease of two intensive bands at 1530 and 1350 cm^{-1}, corresponding to the asymmetric and symmetric vibrations of the nitro group of nifedipin. The appearance of a medium intensity band (1558 cm^{-1}) during photodegradation of nifedipin corresponds to the vibrations of N=O group in the nitroso derivative of nifedipin. The incomplete stability of nifedipin in the complex is indicated by the decreased intensity of the band at 3392 cm^{-1}, resulting from the vibrations of OH groups of β-cyclodextrin and NH groups of nifedipin. Since nifedipin derivatives generated by exposure to daylight lack the NH, the decreased total absorption in the band around 3392 cm^{-1} may be attributed to the loss of NH absorption.

The stability of nifedipin in the complex is higher compared to pure nifedipin, based on the decrease in intensity of the aforementioned bands in the complex. The complete loss of the band at 1681 cm^{-1} for complexed nifedipin was achieved after 10 days of exposure to daylight, and there were no changes in peak intensities at 3391 and 1733 cm^{-1} under continued exposure. This indicates an increased photostability of nifedipin in the inclusion complex compared to both solid nifedipin and the solutions of nifedipin and β-cyclodextrin:nifedipin complex.

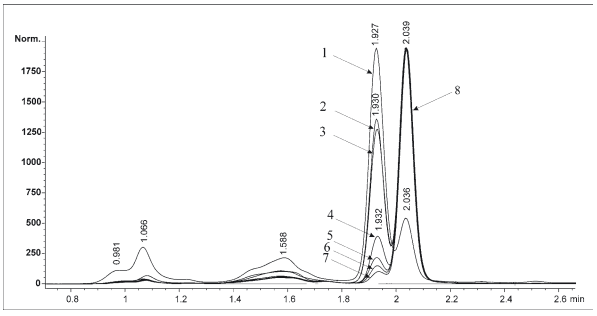

Figure 8. HPLC chromatogram of nifedipin and β-cyclodextrin complex: nitro-derivative of (1) nifedipin complex without irradiation, (2) products after complex irradiation at 350 nm for 5 min; (3) 10 min; (4) 15 min; (5) 20 min; (6) 25 min; (7) 30 min and (8) nitroso-derivative of nifedipin from complex.

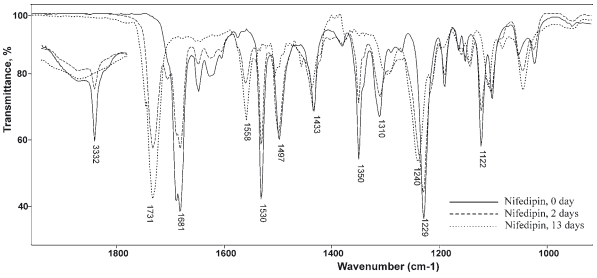

Figure 9. FTIR spectra of nifedipin exposed to daylight for several days

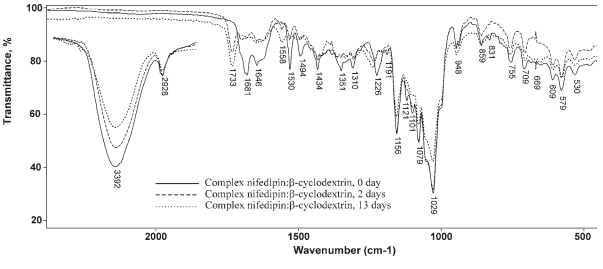

Figure 10. FTIR spectra of nifedipin complex with β-cyclodextrin exposed to daylight for several days

4. Conclusion

Nifedipin:β-cyclodextrin is an inclusion-type complex. Structural characterization of the prepared inclusion complex was carried out by use of various methods. Nifedipin photosensitivity in the inclusion complex is decreased more in solid state than in the solution. Pure nifedipin shows significantly higher photosensitivity compared to the inclusion complex, both in solution and in solid state. In this work, it was shown that the photosensitivity of solid state nifedipin, both pure and in complex, may be monitored by a fast and simple FTIR method.

Acknowledgements

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

References

[1] M. Shamsipur, B. Hemmateenejad, M. Akhond, K. Javidnia, R. Miri, J. Pharm. Biomed. Anal. 31, 1013 (2003)
[2] M.A. Bayomi, K.A. Abanumay, A.A. Al-Angary, Int. J. Pharm. 243, 107 (2002)
[3] D.L. Savigni, D. Wege, G.S. Cliff, M.L.H. Meesters, E.H. Morgan, Biochem. Pharmacol. 65, 1215 (2003)
[4] V.B. Patravale, V.B. Nair, S.P. Gore, J. Pharm. Biomed. Anal. 23, 623 (2000)
[5] R. Teraoka, M. Otsuka, Y. Matsuda, Int. J. Pharm. 184, 35 (1999)
[6] I.A. Majeed, W.J. Murray, D.W. Newton, S. Othman, W.A. Alturk, J. Pharm. Pharmacol. 39, 1044 (1987)
[7] Y. Mastuda, R. Teraoka, I. Sugimoto, Int. J. Pharm. 54, 211 (1989)
[8] I. Matsuura, M. Imaizumi, M. Sugiyama, Chem. Pharm. Bull. 38, 1692 (1990)
[9] N. Škalko, M. Brandl, M. Bećirević-Laćan, J. Filipović-Grčić, I. Jalšenjak, Eur. J. Pharm. Sci. 4, 359 (1996)
[10] J. Mielcarek, Pharmazie 51, 477 (1996)
[11] K.L. Andrew Chan, S.G. Kazarian, Mol. Pharm. 1, 331 (2004)
[12] J. Szejtli, Chem. Rev. 98, 1743 (1998)
[13] P. Giastas, K. Yannakopoulou, I.M. Mavridis, Acta Cryst. B59, 287 (2003)
[14] M.M. Pop et al., Acta Cryst. B58, 1036 (2002)
[15] H.J. Schneider, F. Hacket, V. Rodiger, Chem. Rev. 98, 1755 (1998)
[16] V.T. D'Souza, K.B. Lipkowitz, Chem. Rev. 98, 1749 (1998)
[17] T. Loftsson, J. Buldvinsdottr, A.M. Sigurdardottir, Int. J. Pharm. 98, 225 (1993)
[18] R.A. Rajewski, V.J. Stella, J. Pharm. Sci. 85, 1142 (1996)
[19] V. Nikolić, M. Stanković, A. Kapor, Lj. Nikolić, D. Cvetković, J. Stamenković, Pharmazie 59, 845 (2004)
[20] V. Nikolić, Lj. Nikolić, M. Stanković, A. Kapor, M. Popsavin, D. Cvetković, J. Serb. Chem. Soc. 72, 737 (2007)
[21] R. Ficarra et al., J. Pharm. Biomed. Anal. 23, 33 (2000)
[22] R. Ficarra et al. J. Pharm. Biomed. Anal. 23, 232 (2000)