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The effects of β-cyclodextrin and pH on bifonazole hydrosolubility

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Abstract: The equlibria of bifonazole in saturated water solution within the pH range 4 to 10 [I = 0.1 mol/dm^3 (NaCl); 25 ºC] were studied spectrophotometrically. Based on the equlibrium constants determined in a heterogeneous system, the acidity constant (pK_a = 5.72) and solubility in water ($S = 10^{-5.79} + 10^{-(pH + 0.07)}$ were calculated. The influence of β-cyclodextrin on bifonazole solubility was examined in the presence of 10^{-3} and 10^{-4} mol/dm^3 β-cyclodextrin. At pH values over 8, bifonazole solubility was two- and 13-fold increased in the presence of 10^{-4} and 10^{-3} mol/dm^3 β-cyclodextrin, respectively, compared to its solubility in water. The ratio of bifonazole solubility in the presence of β-cyclodextrin and that in water decreased in parallel to the increase of acidity.

Keywords: bifonazole, acidity constant, solubility, β-cyclodextrin.

INTRODUCTION

Bifonazole, 1-[(1,1'-biphenyl)-4-ylphenylmethyl]-1H-imidazole, belongs to a group of antimycotics, imidazole derivatives with a broad spectrum of activity. It is applied for skin and nail infections with the fungus *Malassezia furfur* and *Candida spp.*.[1] It also expresses *in vitro* antibacterial action against some gram-positive cocci. Chemically, bifonazole is a base sparingly soluble in water in its molecular form (intrinsic solubility).

The solubility of sparingly soluble organic molecules was shown to be increased in the presence of cyclodextrins, which are non-reducing, cyclic oligosaccharides consisting of six or more α-(1,4)-linked D-glucopyrannose units.[2] Due to their unique structure, as the host molecules, they can form inclusion complexes with a variety of hydrophobic molecules which are either completely or partially included in the internal cavity of cyclodextrin.

Morin *et al.*[3] observed an increase in bifonazole solubility in water (in neutral medium) in the presence of β-cyclodextrin (β-CD) affording two particular struc-

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tures: β-CD-bifonazole and $(\beta\text{-CD})_i$-bifonazole (where $2 < i < 3$). The first structure is an inclusion compound and the second one may be a simple binary compound without bifonazole inclusion in the cyclodextrin internal cavity.

Since bifonazole chemically represents a protolyte, its solubility is influenced not only by β-CD but also by the pH of the medium. The aim of the present study was to examine the effects of acidity on bifonazole solubility in water in the absence and in the presence of β-CD. In addition, the acidity constant of bifonazole, missing so far in the available literature, was determined.

The results of studies on both the solubility and the distribution of the cationic and molecular forms of bifonazole in dependence on pH, could be of great significance not only for the examination of the penetration and adsorption of antimycotic hydrophobic imidazole derivatives through human nail matrices, but also for the choice of the optimal conditions for the analyses of the corresponding pharmaceutical formulations.

EXPERIMENTAL

Apparatus and reagents

Spectrophotometric measurements were performed on a GBC Cintra 20 spectrophotometer (GVC Scientific Equipment Pty Ltd., Dandenog, Australia) using 1.0 cm quartz cuvettes. For pH measurements, a pH meter PHM-84 with a combined electrode GK 2401B (Radiometer, Denmark) was used. The electrode was calibrated using standard buffer solution of pH 4.01, 7.00 and 9.18 (Radiometer). The conversion of the recorded pH values [25± 0.1 ºC; $I = 0.1$ mol/dm^3 (NaCl)] into pc_H values was performed by the following relation[4]: $pc_H = -\log[H_3O^+]$=pH–A, where A represents a correction factor the value of which (0.12) was obtained by titration of the standard HCl solution with the standard NaOH solution at 25 ± 0.1 ºC and ionic strength of 0.1 mol/dm^3 (NaCl). Bifonazole (Medilom & Co., Antwerpen, Belgium) of pharmaceutical purity grade (99.98 %) was kindly provided by Srbolek (Belgrade, Yugoslavia) and β-cyclodextrin was a Sigma product. Other reagents were of analytical grade (Merck). Standardization of the HCl and NaOH solutions was performed potentiometrically.

Procedures

Determination of bifonazole solubility in water. Saturated aqueous solutions within the pH range from 4 to 10 were obtained by the partial precipitation of free bifonazole base from bifonazole·HCl (approximately 10^{-3} mol/dm^3 in 0.1 mol/dm^3 NaCl) by additing different aliquots of NaOH solution. The samples were thermostated (25 ± 0.1 ºC) with intensive stirring until complete equilibration (2 h). The insoluble part was removed by filtration. The aliquots of the resulting solutions were diluted, aciditied to pH 2 (0.01 mol/dm^3 HCl) and the concentration of bifonazole (protonated form) was determined spectrophotometrically at 257 nm (the absorption maximum). The reliability of Beer's Law had been verified previously in the bifonazole concentration range 5×10^{-6} – 5×10^{-5} mol/dm^3. Standard solutions for the calibration curve were prepared in 0.01 mol/dm^3 HCl solution. The absorbance of these solutions were measured using 0.01 mol/dm^3 HCl as the blank.

Determination of bifonazole solubility in the presence of β-CD. Saturated aqueous solutions of bifonazole in the presence of β-CD (10^{-4} and 10^{-3} mol/dm^3), at an ionic strength of 0.1 mol/dm^3 (NaCl) within the pH range from 4 to 10 were prepared by the general procedure described for the determination of bifonazole solubility in water. The samples were thermostated (25 ± 0.1 ºC) with strong stirring until complete equilibration (5 h). The filtrate aliquots were diluted, HCl added (to a

concentration 0.01 mol/dm^3), and β-CD (to a concentration of 10^{-4} mol/dm^3, *i.e.*, 10^{-3} mol/dm^3). Measurements of the absorbance were made at 257 nm against the corresponding blank. The reliability of Beer's Law was checked in the bifonazole concentration range 5×10^{-6} – 5×10^{-5} mol/dm^3. Standard solutions for the calibration curves were prepared in 0.01 mol/dm^3 HCl by addition of β-CD to a concentrations of 10^{-4}, *i.e.*, 10^{-3} mol/dm^3.

RESULTS AND DISCUSSION

Solubility of bifonazole in water

Bifonazole in its molecular form is sparingly soluble in water. Due to the protonation of the imidazole nitrogen atom in its molecule, the following equilibria are estabilished between the solid phase (B_s) and the solution in saturated aqueous solution:

$$B_s \rightleftharpoons B \qquad K_{s0} = [B] \tag{1}$$

$$B_s + H_3O^+ \rightleftharpoons HB^+ + H_2O \qquad K_{s1} = \frac{[HB^+]}{[H_3O^+]} \tag{2}$$

Between the acidity constant and the equilibrium constants in the heterogeneous system, the following relationship exists:

$$K_a = \frac{K_{s0}}{K_{s1}} \tag{3}$$

Equilibrium constants in the heterogeneous bifonazole system were determined by the solubility method.[4] Bifonazole solubility (S) in aqueous solution can be presented by the following expression:

$$S = [B] + [HB^+] = K_{s0} + K_{s1}\,[H_3O^+] \tag{4}$$

On the basis of the spectrophotometrically determined solubility of bifonazole in acidic media and the dependence given by Eq. (4), the constants K_{s0} and K_{s1} of bifonazole were calculated by linear regression analysis from the intercept and the slope of the plot of total solubility of bifonazole *vs* $[H_3O^+]$ (Fig. 1a).

In alkaline solution, the concentrations of HB^+ could be neglected, so, Eq. (4) can be transformed into the following form:

$$S = [B] = K_{s0} \tag{4a}$$

from which it can be seen that the solubility of bifanozole in alkaline media is constant and equal to the solubility of its molecular form. Determinations of the constant K_{s0} were performed at different pH values within the range from 8 to 10 (Fig. 1b).

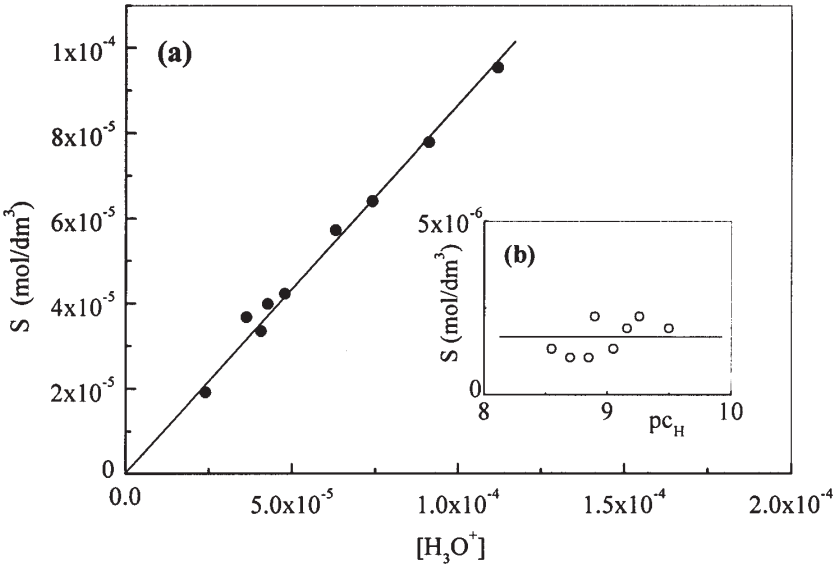

Fig. 1. Total solubility of bifonazole (*S*) in water as a function of H_3O^+ concentration. (a) Acidic solution; (b) alkaline solution.

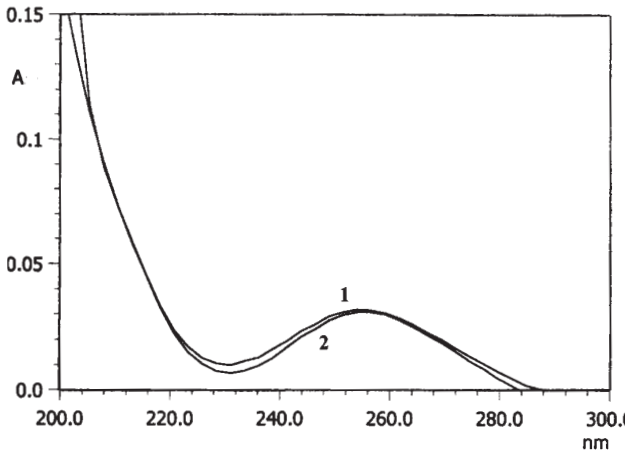

Fig. 2. Absorption spectra of bifonazole (1.5×10^{-6} mol/dm^3). a) Molecular form (pH 10); 2) protonated form (pH 2).

From the equilibrium constants determined in the heterogeneous system, the acidity constant was calculated from Eq. (3). It was not possible to determine the acidity constant of bifonazoile directly employing classical methods, such as potentiometric and specrophotometric methods, because of the extremely low solubility of the molecular form of bifonazole ($K_{s0} = 1.64 \times10^{-6}$ mol/dm^3) and the almost complete overlapping of the absorption spectra of the molecular ($\lambda_{max} = 253$ nm) and protonated forms ($\lambda_{max} = 257$ nm) of this compound (Fig. 2).

The determined and calculated equilibriuim constants of bifonazole are listed in Table I. It can be seen that the constant K_{s0} could be determined more precisely

TABLE I. The equilibrium concentration constants of bifonazole in a heterogeneous aqueous system. I = 0.1 mol/dm^3 (NaCl); t = 25 ºC

Constant	Value*	Equation applied
pK_{s0}	5.5 ± 0.3 5.79 ± 0.05	(4) (4a)
pK_{s1}	0.07 ± 0.02	(4)
pK_a	5.72 ± 0.05	(3)

*Mean ± standard deviation (n = 9)

applying Eq. (4a). Hence, this value was used further to calculate the bifonazole acidity constant. Since the solubility of bifonazole in water is a linear function of the H_3O^+ concentration [Eq. (4)], based on the obtained values of the equilibrium constnats K_{s0} and K_{s1}, it was possible to calculate the total bifonazole solubility in water at different pc_H values by applying the following equation derived from Eq. (4):

$$S = 10^{-5.79} + 10^{-(pc_H + 0.07)} \quad (5)$$

Solubility of bifonazole in the presence β-CD

The absorption spectra of bifonazole obtained in the absence and in the presence of β-CD demonstrate the interaction of bifonazole with β-CD, as shown in Fig. 3. It can be seen that the intensity of the bifonazole absorption maximum is decreased and its position is slightly increased (from 257 to 257.5 nm), in the presence of β-CD.

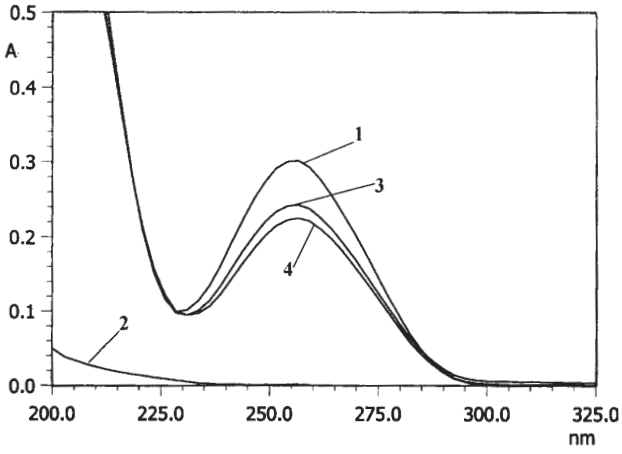

Fig. 3. Absorption spectra of: 1) bifonazole (pH 2); 2) β-CD; 3) bifonazole in the presence of β-CD (pH 10); 4) bifonazole in the presence of β-CD (pH 2). Concentrations: bifonazole 1.5×10^{-5} mol/dm^3, β-CD 10^{-3} mol/dm^3.

The solubility of bifonazole in the presence of β-CD was determined spectrophotometrically. As presented in Table II, the solubility of bifonazole within the pH range from 8 to 10, was 2- and 13-fold increased in the presence of 10^{-4} and 10^{-3}

TABLE II. Solubility of bifonazole in water ($S_{Bf}^{H_2O}$) and in the presence of β-cyclodextrin ($S_{Bf}^{\beta\text{-CD}}$) at varying pH values. I = 0.1 mol/dm^3 (NaCl); t = 25 ºC; pc_H = pH – 0.12

pH	$S_{Bf}^{\beta\text{-CD}}$/mol dm^{-3}	$S_{Bf}^{H_2O}$ */mol dm^{-3}	$S_{Bf}^{\beta\text{-CD}} : S_{Bf}^{H_2O}$
10^{-4} mol/dm^3 β-CD			
8–10	3.19×10^{-6}	1.64×10^{-6} **	1.95
5.35	1.23×10^{-5}	6.63×10^{-6}	1.86
5.04	2.05×10^{-5}	1.19×10^{-5}	1.72
4.64	3.86×10^{-5}	2.73×10^{-5}	1.41
4.33	7.17×10^{-5}	5.41×10^{-5}	1.33
4.05	1.09×10^{-4}	1.02×10^{-4}	1.07
3.81	1.73×10^{-4}	1.75×10^{-4}	0.99
10^{-3} mol/dm^3 β-CD			
8–10	2.13×10^{-5}	1.64×10^{-6} **	13.01
5.63	4.59×10^{-5}	4.25×10^{-6}	10.80
5.26	6.37×10^{-5}	7.79×10^{-6}	8.18
4.48	2.18×10^{-4}	3.88×10^{-5}	5.62
4.22	3.14×10^{-4}	6.92×10^{-5}	4.53
4.00	5.11×10^{-4}	1.14×10^{-4}	4.48
3.83	6.55×10^{-4}	1.68×10^{-4}	3.90

*Calculated according to Eg. (5); **Solubility of the molecular form (K_{s0})

mol/dm^3 β-CD, respectively, compared to its solubility in water, *i.e.*, in the absence of β-CD. At pH values under 8, the solubility of bifonazole is influenced by the acidity of the medium (Fig. 4). It can be seen from Fig. 4 that the solubility of bifonazole in the presence of β-CD is not a linear function of the H_3O^+ concentation. This deviation from linearity can be explained in terms of a decreased concentration ratio of β-CD and bifonazole. Namely, increasing the adicity of the solution leads to an increase in the concentration of the protonated bifonazole form and, as a consequence, the solubility of bifonazole is enhanced, while the concentration of β-CD is constant. The solubility of bifonazole in water calculated by applying Eq. (5) is presented in Table II, together with experimentally determined solubility in the presence of β-CD at selected pH values. These data demonstrate that the ratio of the solubility of bifonazole in the presence of β-CD and its solubility in water decrease parallel to the decrease of pH of the solution. When the pH was changed from 8 to 4, this ratio decreased from 13 to 4.5 and from 2 to 1 in the presence of 10^{-3} and 10^{-4} mol/dm^3 β-CD, respectively. At pH values under 4, the solubility of bifonazole in the presence

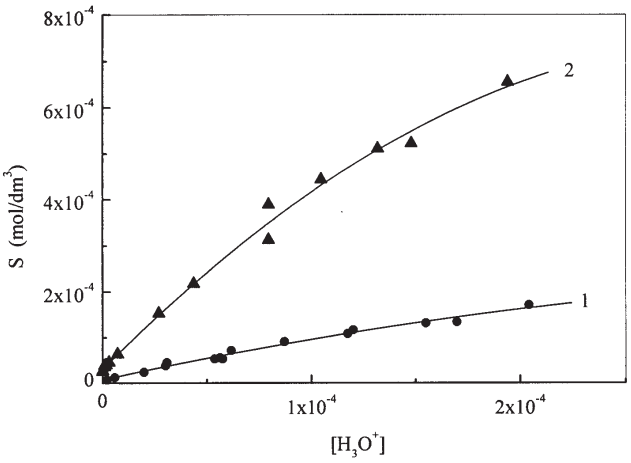

Fig. 4. Total solubility of bifonazole (*S*) in the presence of β-CD as a function of H_3O^+ concentration. Concentration of β-CD: 1) 10^{-4} mol/dm^3, 2) 10^{-3} mol/dm^3.

of 10^{-4} mol/dm^3 β-CD was equal to that in water.

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И З В О Д

УТИЦАЈ β-ЦИКЛОДЕКСТРИНА И pH НА РАСТВОРЉИВОСТ БИФОНАЗОЛА У ВОДИ

ГОРДАНА ПОПОВИЋ и МИРА ЧАКАР

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Спектрофотометријски су проучаване равнотеже у засићеном воденом раствору бифоназола у pH интервалу 4–10. На основу равнотежних константи одређених у хетерогеном систему израчуната је киселинска константа ($pK_a = 5{,}72$) и растворљивост бифоназола у функцији киселости раствора ($S = 10^{-5{,}79} + 10^{-(pc_H+0{,}07)}$). Утицај β-циклодекстрина (β-CD) на растворљивост испитиван је у присуству 10^{-3} и 10^{-4} mol/dm^3 β-CD. Утврђено је да при pH вредностима већим од 8 долази до повећања растворљивости 2 пута (у присуству 10^{-4} mol/dm^3 β-CD) и 13 пута (у присуству 10^{-3} mol/dm^3 β-CD) у односу на растворљивост бифоназола у води (у одсуству β-CD). Однос растворљивости бифоназола у присуству β-CD и растворљивости у води смањује се са повећањем киселости раствора.

(Примљено 1. августа, ревидирано 4. новембра 2003)

REFERENCES

1. M. Pedersen, M. Edelsten, V. Nielsen, A. Scarpellini, S. Skytte, C. Slot, *Intern. J. Pharmaceutics* **90** (1993) 247
2. *Cyclodextrins and their Inclusion Complexes*, J. Szejtli, Ed., Akademiai Kiadó, Budapest, 1982
3. N. Morin, G. Crini, C. Cosentino, J. Millet, J. Verbrel, J. C. Rouland, *J. Chem. Soc. – Perkin Trans.* **2** (1999) 2647
4. L. Pfendt, D. Sladić, T. Janjić, G. Popović, *Analyst* **115** (1990) 383.