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Study on the inclusion complexes of bromazepam with β- and β-hydroxypropyl-cyclodextrins☆

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Abstract

Solubility enhancement of the water insoluble bromazepam was studied during the formation of its inclusion complexes with β-cyclodextrin (β-CD) and β-hydroxypropyl-cyclodextrin (β-HP-CD). The phase solubility technique established by Higuchi and Connors and UV-spectrophotometric methods (zero- and second-order derivative approaches) were used to measure the changes introduced in this chemical system. The amount of time, which was necessary to reach equilibrium between inclusion complexes and their free components, was estimated and found equal to 24 h. The study was carried out at (i) pH 7.0 and 25 °C and (ii) pH 7.4 and 37 °C. The solubility of bromazepam increased linearly as a function of concentration for both β-and β-hydroxypropyl-cyclodextrins. Thus, the phase solubility diagrams were classified as of A_L type in all cases. Under the above-mentioned conditions, the formation constants of the inclusion complexes were calculated and their stoichiometry was evaluated, found in the range of 69–85 M^{-1} and 1:1, respectively.

Keywords: Inclusion complexes of bromazepam; β- and β-Hydroxypropyl cyclodextrins; Solubility enhancement of bromazepam; Phase solubility diagrams; UV-spectrophotometric methods; Formation constants and stoichiometry

1. Introduction

Bromazepam, 7-bromo-5-(2-pyridyl)-2,3-dihydro-1H-benzo[e]1,4-diazepin-2-one [1] is a member of the 1,4-benzodiazepine series. These drugs are widely used as minor tranquilizers, anticonvulsants, sedatives, muscle relaxants and sleep inducers in psychotherapy. Yet, they are almost insoluble in aqueous solutions. However, many biopharmaceutical studies on benzodiazepines showed that their rapid plasma appearance is therapeutically essential, particularly in the treatment of acute convulsive attacks. In this respect, a fast-dissolving form of them with high aqueous solubility is desirable for rapid absorption in oral benzodiazepine therapy. Also, their parenteral and intravenous use necessitated the invention of

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ways to increase their solubility. Complexation of such drugs with cyclodexrtins has become one of these ways, recently [2].

Cyclodextrins [3–7], have recently been widely used in the pharmaceutical formulation of various drugs. Their unique structure enables formation of host–guest complexes by accommodating a wide variety of drug molecules inside their hydrophobic cavity. The binding forces within these inclusion complexes may involve hydrophobic, van der Waals, hydrogen bonding, or dipole interactions [8]. β-Cyclodextrin (β-CD) has been used to improve the dissolution characteristics of sparingly soluble drugs [9–12]. Complexation of pharmaceuticals with β-CD causes enhancement of their solubility and bioavailability as well as stabilization against oxidation, decomposition, hydrolysis etc. [13,14].

In the literature, formation of inclusion complexes of bromazepam has been studied with (a) β-CD in aqueous solutions [15], (b) α-, β-, γ-, DM-β- and TM-β-CDs in aqueous solutions (pH 9.0 and 30 °C), in solid mixtures [16,17] and (c) α-, β- and γ-cyclodextrins in aqueous solutions ($\theta = 25$ °C), [18].

The aim of this research work was the comparative study on the probable solubility enhancement of bromazepam caused by its complexation with β-CD and β-hydroxypropyl-cyclodextrin (β-HP-CD). The latter is the most accepted representative of hydroxyalkylated derivatives as hydrophilic drug carrier, because of its amorphousness, high water solubility and solubilizing power, low cost and toxicity. Hydroxyalkylated cyclodextrin derivatives have been proved to be very useful in intravenous and other parenteral preparations, because of their low hemolytic activity and irritation compared with that of β-CD and its alkylated forms [19–23].

Our studies took place at pH 7.0 and 25 °C imitating shelf-storage conditions, as well as at pH 7.4 and 37 °C imitating human conditions during intravenous use. In addition to the comparison between the unmodified form of β-CD and the modified one of β-HP-CD, the effect of the extent of substitution of β-HP-CD was also examined.

2. Experimental

2.1. Equipment

A Hitachi (Tokyo, Japan) double-beam UV–vis spectrophotometer (Model U-2000) was used for zero- and second-order derivative spectrophotometric measurements.

A Julabo SW1-V shaking water-bath (Julabo Labortechnik GmbH, Germany) was used with a shaking speed of 160 vibrations per min.

2.2. Reagents

All reagents used were of analytical-reagent grade and distilled, de-ionized water was consumed.

Bromazepam ($M_r = 316.6$) was of pharmaceutical purity grade, generous donation of Roche Hellas A.E. pharmaceutical company; β-CD ($\bar{M}_r = 1134.9$) was purchased by Serva Co. and β-HP-CD with two different degrees of substitution ($\bar{M}_r = 1500$ and 1380) was purchased by Aldrich Co.

Phosphate buffer solutions of pH 7.0 and 7.4 containing 0.1 M NaH_2PO_4, were prepared and used throughout this study.

2.3. Measurement procedure

2.3.1. Optimization of experimental parameters

It took place for both β-CD and β-HP-CD in two sets of experimental conditions (pH 7.0, $\theta =$ 25 °C and pH 7.4, $\theta = 37$ °C). The prepared solutions were shaken for a certain amount of time and filtered through filter papers (Whatman® 5, UK) to remove the undissolved quantity of bromazepam. Solutions after filtration were diluted 25 times with the corresponding buffer solution and their zero- and second-order derivative absorbance spectra were taken. The appropriate buffer solution was used as the proper blank in every case.

2.3.2. Solubility studies

Solubility measurements were based on the phase solubility technique established by Higuchi and Connors [24] and carried out under the opti-

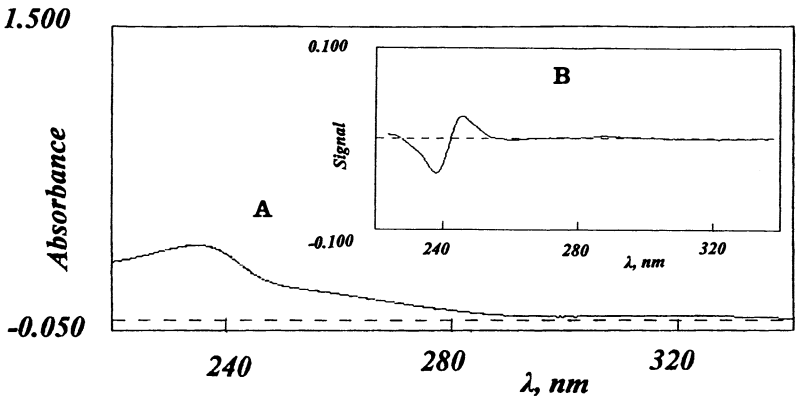

Fig. 1. Absorbance spectra of zero-order (A) and second-order (B) derivatives of 1×10^{-5} M bromazepam in buffer aqueous solution of 0.1 M NaH_2PO_4 (pH 7.0).

mized experimental conditions found during the above study. Constant quantity (0.0032 g) of solid bromazepam was placed in a series of 25-ml Erlenmeyer flasks that contained increasing amounts of cyclodextrins (0.13, 0.26, 0.39, 0.52, 0.65, 0.78, 0.91, 1.04 and 1.17×10^{-2} M). The final volume in each flask was constant and equal to 10 ml. After shaking under the appropriate conditions, solutions were processed as described above.

3. Results and discussion

3.1. Spectral characteristics

The zero-order spectrum of bromazepam is shown in Fig. 1. Due to its shape, absorbance measurements of bromazepam were quite depending on the background signal. This fact led us to the examination of its second-order derivative spectrum (Fig. 1), where the influence of the background on the analyte signal was diminished.

Absorbance spectra of bromazepam in aqueous solutions in the presence or absence of β-CD and β-HP-CD are shown in Fig. 2. Their maxima appeared at the same wavelength and the only difference between them was the magnitude of the absorbance signal, which was related to the concentration of the dissolved bromazepam. The change in the absorbance signal of bromazepam in the presence of the used β-CDs was probably due to the formation of inclusion complexes with them. What it was actually seen was not the complex itself but the quantity of the dissolved bromazepam. This fact was verified by a series of experiments, performed at pH 7.0, where the dissolved bromazepam was kept constant while the concentration of β-CDs increased. The results of this study are tabulated in Table 1. It was noticed that the signal of the second-derivative spectra remained statistically constant. However, the absorbance values at 235 nm increased as the concentration of cyclodextrin increased, because, cyclodextrins showed a small absorbance in the UV range when their concentration became sig-

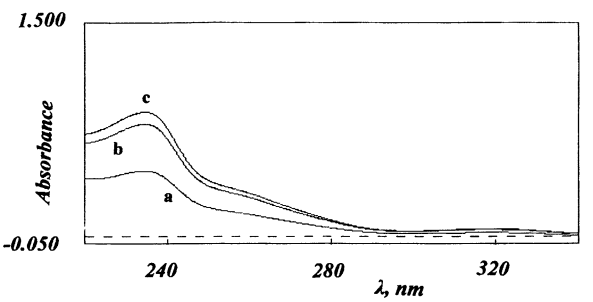

Fig. 2. Zero-order absorbance spectra of bromazepam in, (a) buffer solution of NaH_2PO_4 0.1 M (pH 7.0); (b) β-hydroxypropylcyclodextrin (pH 7.0) and (c) β-CD (pH 7.0). The above solutions were prepared by weighing 0.0032 g of bromazepam, diluting to 10.0 ml with buffer solution or cyclodextrin solution 1.3×10^{-2} M, buffered at pH 7.0, shaking for 24 h, filtration through filter papers and final dilution 1:25 with buffer solution.

Table 1
Signal measurements of a series of solutions prepared at pH 7.0 and shaken for 24 h

Concentration of bromazepam (M)	β-CD			β-HP-CD		
	Concentration (M)	$A^a \pm s^b$	$d_2{}^a \pm s^b$	Concentration (M)	$A^a \pm s^b$	$d_2{}^a \pm s^b$
1.0×10^{-5}	–	0.382 ± 0.002	0.062 ± 0.001	–	0.382 ± 0.002	0.062 ± 0.001
	1.3×10^{-3}	0.388 ± 0.009	0.062 ± 0.001	1.3×10^{-3}	0.387 ± 0.007	0.063 ± 0.002
	2.6×10^{-3}	0.409 ± 0.015	0.064 ± 0.003	2.6×10^{-3}	0.378 ± 0.004	0.060 ± 0.001
	5.2×10^{-3}	0.405 ± 0.012	0.058 ± 0.003	5.2×10^{-3}	0.377 ± 0.006	0.058 ± 0.003
	7.8×10^{-3}	0.404 ± 0.010	0.059 ± 0.002	7.8×10^{-3}	0.392 ± 0.010	0.059 ± 0.002
	1.0×10^{-2}	0.433 ± 0.020	0.058 ± 0.003	1.0×10^{-2}	0.450 ± 0.052	0.064 ± 0.003

The solutions contained a constant dissolved amount of bromazepam and increasing amounts of β-CD and β-HP-CD.
[a] A is the measured absorbance of the zero-order spectra at 235 nm and distance d_2 (235–245 nm) is the peak-to-trough amplitude of the second-order derivative spectra of bromazepam.
[b] s is the standard error of the measured signal. The experimental measurements have been performed in triplicate.

nificant. To compensate for such background absorbance and avoid any other experimental complication, the second-order derivative signals were used in this work. Other workers [22], reported that the absorbance measurements were carried out against a suitable blank of β-HP-CD with the aim to cancel any absorbance exhibited by cyclodextrin molecules. For the purpose of comparison, measurements of the absorbance at the wavelength of 235 nm in the zero-order spectrum of bromazepam were also performed.

3.2. Linearity and reproducibility of calibration curves

Calibration curves of bromazepam in buffer solutions of pH 7.0 and 7.4 were constructed using absorbance measurements at 235 nm from the zero-order spectra and the signal d_2, peak-to-trough amplitude (235–245 nm), from its second-derivative spectra. A least-squares regression analysis was carried out and the results are presented in Table 2. The obtained values indicated good reproducibility in all modes.

Measuring each standard solution three times, a relative standard deviation (s_r) of less than 1% for zero-order spectra and less than 3% for the second-order derivative spectra was calculated. This agrees with the fact that the S/N ratio decreases as long as higher derivative orders are used.

Limits of detection (LOD) and quantitation (LOQ) were defined as the concentrations that give signals equal to $b \pm 3.3s_b$ and $b \pm 10s_b$, respectively, where b is the signal of the blank and s_b is its standard deviation. LOD were calculated at both pH values and found approximately equal to 1×10^{-7} and 6×10^{-7} M for zero-order and second-order derivative approaches, respectively, while LOQ were equal to 3×10^{-7} and 2×10^{-6} M, respectively.

3.3. Optimization of the experimental parameters

Before studying the probable solubility enhancement of bromazepam, caused by its complexation with β-CD and β-HP-CD, optimization of the experimental parameters that affected it, was performed. It was essential, first to find the amount of time needed for this complexation reaction to reach equilibrium, second, to examine whether the form of cyclodextrin (solid or in solution) affected the grade and the rate of its complexation with bromazepam and third, to verify that the amount of solid bromazepam used, was sufficient to maintain saturated solutions in bromazepam for all concentrations of cyclodextrin. The last remark was a requirement for the applicability of the phase solubility technique of Higuchi–Connors in the proposed study. This approach demanded saturated solutions of the drug.

Indicative results of optimization of the shaking time of buffered solutions at pH 7.0 (25 °C), saturated in bromazepam, and containing 1.3×10^{-2} M β-CD or β-HP-CD were tabulated in Table 3. This concentration was the highest of cyclodextrins used in this work. It seemed that equilibrium of the complexation reaction under study, was reached in 24 h for both cyclodextrins. A very slight drift to higher values was rather caused by some evaporation of the solvent after certain days and not by slow equilibration of the reaction. This was easier to conclude looking at the second-order derivative data. Similar results were obtained at pH 7.4 and 37 °C.

In the literature [15–18], the amounts of bromazepam added in each vial were 0.0500 g and β-CD concentration was up to 2.0×10^{-2} M. The shaking time used was 3–7 days, without reporting any optimization.

In our work, the short shaking time was not only saving of time but also a means of avoiding any degradation of bromazepam during the experiments. Moreover, the smaller amounts of cyclodextrins used, lowered the cost of the phase solubility technique of Higuchi–Connors. In addition to these, the very small amount of bromazepam added in each vial (0.0032 g) allowed the completion of this study since access to pharmaceutical purity grade drug was very difficult.

Optimization of the complexation rate, related to the choice of the form (solid or in solution) of β-CDs, led to the following observations. In the case of β-CD, which dissolved very slowly in aqueous solution, extra amount of time was necessary for its dissolution, in addition to the time needed for the equilibration of reactants and product. However, in the case of β-HP-CD, which was very easily dissolved in water, there was no difference in shaking time whether it was in solid or in solution form. In both cases, β-CDs were added in solution form to obtain better precision during these experiments, minimizing the weighing errors.

Proper quantities of bromazepam should be used during the Higuchi–Connors experiments. Since saturated solutions of drug were required and known that bromazepam solubility in aqueous solutions was in the order of 10^{-4} M, an amount of 0.0032 g of bromazepam (10 times more than the quantity that it could be dissolved) was added to 10 ml of buffer solution. In order to assure that the solutions would be saturated after the addition of β-CDs, during optimization of experimental conditions, some vials contained

Table 2
Analytical parameters of calibration curves of bromazepam in buffer solutions of pH 7.0 and 7.4, using zero- and second-order derivative spectral data

Concentration range of bromazepam (M)	pH	Mode[a]	Selected wavelength or distance (nm)	Regression equation[b]			
				Intercept $a \pm s$[c]	Slope $b \pm s$[c]	$r(n)$[d]	s_r (%)[e]
$0.2–2 \times 10^{-5}$	7.0	A	235	-0.0004 ± 0.004	34484 ± 344	0.9999	<1
		d_2	235–245	-0.0006 ± 0.0004	5718 ± 36	0.99994	<3
	7.4	A	235	0.006 ± 0.003	37068 ± 310	0.9999	<1
		d_2	235–245	-0.0003 ± 0.0002	6060 ± 14	0.999991	<3

[a] A is the measured absorbance of the zero-order spectra and distance d_2 is the peak-to-trough amplitude of the second-order derivative spectra of bromazepam.

[b] A or $d_2 = a + b \cdot c$, where c is the bromazepam concentration in M.

[c] s is the standard error of slope and intercept.

[d] $r(n)$ is the correlation coefficient and $n = 5$ is the number of points in each calibration curve; each point is the mean of three experimental measurements.

[e] s_r is the relative standard deviation of the standard solutions used for the construction of calibration curves and prepared three times each.

Table 3
Optimization of the shaking time, of bromazepam saturated solutions, with or without β-CD and β-HP-CD

Bromazepam inclusion complex with	Mode[a]	Time (h)								
		3	6	15	24	48	72	96	120	144
–	A	0.420	0.430	0.453	0.458	0.458	0.448	0.448	0.448	0.447
	$(\pm s)$[b]	($\pm$0.018)	($\pm$0.008)	($\pm$0.017)	($\pm$0.002)	($\pm$0.010)	($\pm$0.013)	($\pm$0.006)	($\pm$0.016)	($\pm$0.009)
	d_2	0.070	0.074	0.078	0.076	0.076	0.071	0.075	0.076	0.076
	$(\pm s)$[b]	($\pm$0.003)	($\pm$0.001)	($\pm$0.003)	($\pm$0.001)	($\pm$0.002)	($\pm$0.003)	($\pm$0.001)	($\pm$0.001)	($\pm$0.002)
β-CD	A	0.813	0.821	0.863	0.873	0.897	0.882	0.892	0.892	0.896
	$(\pm s)$[b]	($\pm$0.014)	($\pm$0.012)	($\pm$0.012)	($\pm$0.015)	($\pm$0.026)	($\pm$0.006)	($\pm$0.007)	($\pm$0.004)	($\pm$0.011)
	d_2	0.135	0.138	0.146	0.148	0.149	0.151	0.149	0.152	0.150
	$(\pm s)$[b]	($\pm$0.003)	($\pm$0.006)	($\pm$0.001)	($\pm$0.004)	($\pm$0.004)	($\pm$0.001)	($\pm$0.003)	($\pm$0.002)	($\pm$0.002)
β-HP-CD	A	0.711	0.725	0.767	0.801	0.809	0.808	0.797	0.821	0.820
	$(\pm s)$[b]	($\pm$0.019)	($\pm$0.012)	($\pm$0.009)	($\pm$0.012)	($\pm$0.020)	($\pm$0.015)	($\pm$0.001)	($\pm$0.014)	($\pm$0.016)
	d_2	0.117	0.119	0.129	0.134	0.134	0.140	0.134	0.138	0.136
	$(\pm s)$[b]	($\pm$0.004)	($\pm$0.001)	($\pm$0.001)	($\pm$0.004)	($\pm$0.003)	($\pm$0.003)	($\pm$0.001)	($\pm$0.001)	($\pm$0.004)

[a] A is the measured absorbance of the zero-order spectra and distance d_2 is the peak-to-trough amplitude of the second-order derivative spectra of bromazepam.
[b] s is the standard error of the A and d_2 measurements; each solution was prepared and measured three times.

Table 4
Effect of the concentration ratios of bromazepam to β-HP-CD, on the degree of complexation

Bromazepam (g)	β-HP-CD concentration (M)	$A^a \pm s^b$	$d_2{}^a \pm s^b$
0.0032	–	0.0449 ± 0.002	0.076 ± 0.001
	1.3×10^{-4}	0.464 ± 0.006	0.079 ± 0.001
	1.3×10^{-3}	0.494 ± 0.004	0.084 ± 0.001
	1.3×10^{-2}	0.837 ± 0.020	0.141 ± 0.004

The experiments were performed at pH 7.0, were done in triplicate and the shaking time was 72 h. The final volume of each sample was constant and equal to 10 ml.

[a] A is the measured absorbance of the zero-order spectra and distance d_2 is the peak-to-trough amplitude of the second-order derivative spectra of bromazepam.

[b] s is the standard error of the measured signals.

double quantities of bromazepam. As it was expected, no change was noticed in the signal measurements after 24 h for both β-CD and β-HP-CD used. This meant that the quantity of 0.0032 g of bromazepam added, in every vial, was sufficient for the solubility studies to be carried out.

Another topic that was examined was the effect of the concentration ratio, of bromazepam to cyclodextrin, on the degree of complexation. As it was shown in Table 4, as this ratio increased, the amount of dissolved bromazepam, in other words the degree of complexation, increased as well.

3.4. Solubility studies

In our work, solubility enhancement of bromazepam was noticed in the presence of β-HP-CD, which became more remarkable when β-CD was used (Fig. 2). Solubility enhancement may be due to the complexation reaction of this drug with cyclodextrin molecules. In our case, the complex itself was not absorbing; only the increase of dissolved bromazepam was seen, as it was reported in the Section 3.1.

Then, solubility studies were performed by the phase solubility technique established by Higuchi–Connors [24]. Phase solubility diagrams of bromazepam were drawn in the presence of β-CD, β-HP-CD ($\bar{M}_r = 1500$) and β-HP-CD ($\bar{M}_r = 1380$), at pH 7.0, $\theta = 25$ °C and pH 7.4 and $\theta = 37$ °C.

The last two substances differ in the average molecular substitution (MS); β-HP-CD with $\bar{M}_r = 1380$ has a MS = 0.6, while β-HP-CD with $\bar{M}_r = 1500$ has a MS = 0.8. All phase solubility diagrams obtained were linear, and they could be classified as an A_L type. Their analytical parameters were tabulated in Table 5. A typical phase solubility diagram of bromazepam in the presence of β-HP-CD at pH 7.0 and $\theta = 25$ °C was presented in Fig. 3.

According to Higuchi–Connors, A_L type complexes can be accepted as complexes containing one cyclodextrin molecule. Moreover, straight lines for systems classified as A_L, Higuchi type curves, clearly indicate the formation of a stoichiometric 1:1 complex in solution. In our study, all linear curves received, showed slopes < 1 (Table 5), which means that all complexes formed had a stoichiometry 1:1. Formation constances, K, of such complexes can be calculated from the slope b and the intercept a of the linear phase solubility diagrams, according to the equation [24]:

$$K = \frac{b}{a(1-b)}$$

Calculated formation constants of the inclusion complexes of bromazepam, studied in this work, are shown in Table 6. All experiments were performed in triplicate and the relative standard deviation (s_r) of the formation constants calculated was less than 5%.

Table 5
Analytical parameters of the phase solubility diagrams drawn by the method Higuchi and Connors [24]

Conditions	Bromazepam inclusion complex with	Mode[a]	Selected wavelength or distance (nm)	Regression equation[b]		
				Intercept $a \pm s^c \times 10^4$	Slope $b \pm s^c \times 10^4$	$r(n)^d$
pH 7.0 ($\theta = 25$ °C)	β-CD	A	235	3.08 ± 0.02	250 ± 3	0.9995
		d_2	235–245	3.07 ± 0.04	254 ± 6	0.998
	β-HP-CD ($\bar{M}_r = 1500$)	A	235	2.98 ± 0.03	213 ± 4	0.999
		d_2	235–245	3.04 ± 0.06	224 ± 9	0.993
	β-HP-CD ($\bar{M}_r = 1380$)	A	235	3.03 ± 0.03	206 ± 4	0.998
		d_2	235–245	3.02 ± 0.04	206 ± 6	0.997
pH 7.4 ($\theta = 37$ °C)	β-CD	A	235	3.44 ± 0.04	273 ± 6	0.998
		d_2	235–245	3.48 ± 0.06	296 ± 8	0.997
	β-HP-CD ($\bar{M}_r = 1500$)	A	235	3.48 ± 0.05	254 ± 8	0.996
		d_2	235–245	3.45 ± 0.06	267 ± 8	0.996

[a] A is the measured absorbance of the zero-order spectra and distance d_2 is the peak-to-trough amplitude of the second-order derivative spectra of bromazepam.
[b] A or $d_2 = a + b \times c$, where c is the bromazepam concentration in M.
[c] s is the standard error of slope and intercept.
[d] $r(n)$ is the correlation coefficient and $n = 10$ is the number of points in each calibration curve; each point is the mean of three experimental measurements.

4. Conclusions

In the present study, enhancement of the solubility of bromazepam was noticed in the presence of β-CD and β-HP-CD, under the experimental conditions used. This enhancement was probably due to the formation of inclusion complexes between these molecules with stoichiometry 1:1, as was indicated from Higuchi–Connors phase diagrams. However, the values of formation constants calculated were very low in the range of 69–85 M^{-1}. Such values were reported from other authors in similar studies on bromazepam with β-CD, under slight different experimental conditions [15,16,18].

In our work, all formation constants calculated were very similar and there was no significant difference observed using bromazepam with β-HP-CD (that had never used before) instead of β-CD. Also, there was no effect, of the degree of substitution of β-HP-cyclodextrins used (β-HP-CD of $\bar{M}_r = 1500$ and 1380), on the extent of complexation studied.

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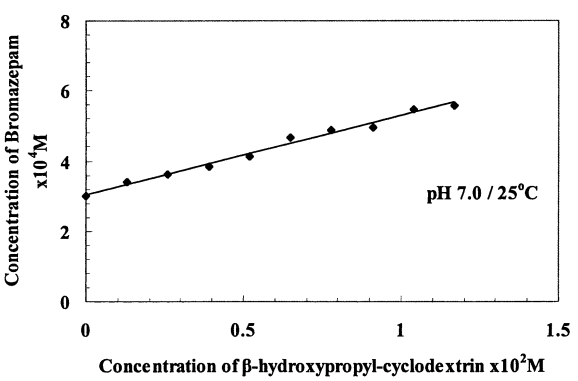

Fig. 3. A typical phase solubility diagram of an inclusion complex of bromazepam with β-HP-CD at pH 7.0 and $\theta =$ 25 °C.

Table 6
Formation constants of inclusion complexes of bromazepam with β-CD, β-HP-CD ($\bar{M}_r = 1500$), and β-HP-CD ($\bar{M}_r = 1380$)

Conditions	Bromazepam inclusion complex with	Mode[a]	Selected wavelength or distance (nm)	Formation constant, K (M^{-1})
pH 7.0 ($\theta = 25$ °C)	β-CD	A	235	83 ± 1
		d_2	235–245	85 ± 3
	β-HP-CD ($\bar{M}_r = 1500$)	A	235	73 ± 2
		d_2	235–245	75 ± 3
	β-HP-CD ($\bar{M}_r = 1380$)	A	235	69 ± 1
		d_2	235–245	70 ± 1
pH 7.4 ($\theta = 37$ °C)	β-CD	A	235	81 ± 1
		d_2	235–245	84 ± 4
	β-HP-CD ($\bar{M}_r = 1500$)	A	235	72 ± 3
		d_2	235–245	76 ± 4

[a] A is the measured absorbance of the zero-order spectra and distance d_2 is the peak-to-trough amplitude of the second-order derivative spectra of bromazepam.

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