American Journal of Analytical Chemistry, **2012, 3, 811-819**
http://dx.doi.org/10.4236/ajac.2012.312107 Published Online December 2012 (http://www.SciRP.org/journal/ajac)

Effect of Cyclodextrin Complexation on the Aqueous Solubility of Diazepam and Nitrazepam: Phase-Solubility Analysis, Thermodynamic Properties

Jasmina Hadžiabdić, Alisa Elezović, Ognjenka Rahić, Indira Mujezin
Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Sarajevo, Sarajevo, Bosnia and Herzegovina
Email: hadziabdicjasmina@yahoo.com

Received October 12, 2012; revised November 22, 2012; accepted November 30, 2012

ABSTRACT

The solubility enhancement of diazepam and nitrazepam in water was analyzed depending on temperature and amount of α-cyclodextrin (α-CD), β-cyclodextrin (β-CD) and 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD). The interactions of drug-cyclodextrin in solution were investigated by the phase-solubility analysis. Diazepam (nitrazepam) content in aqueous complexation medium was analyzed UV spectrophotometrically. Classical solubility data were used to derive apparent stability constants ($K_{1:1}$) which were used to derive thermodynamic parameters for the diazepam (nitrazepam)-cyclodextrin complexes. Since all phase solubility plots were of A_{L-} types, and calculated *Slopes* after linear regression analysis were found to be less than 1, it could be assumed that stoichiometry of the formed binary systems was 1:1. According to the calculated $K_{1:1}$ values, the stability of the complexes of diazepam and nitrazepam with α-CD, β-CD and 2-HP-β-CD varies as follows: 2-HP-β-CD > β-CD > α-CD. The α-CD has higher affinity for dissolving nitrazepam compared to diazepam. While all parameters lead to an improvement in solubility, the largest effect was obtained for guest-host complexation with 2-HP-β-CD. The solubility of diazepam and nitrazepam in water increased 93.02 times and 64.23 times, respectively, in the presence of 40% (w/w) 2-HP-β-CD, at 25˚C. Solubility data for diazepam and nitrazepam in aqueous 2-HP-β-CD were used to derive thermodynamic parameters, ΔG° at 298 K = −14.43 kJ·mol^{-1}, ΔH° = 0.79 kJ·mol^{-1}, ΔS° at 298 K = 51.17 J mol^{-1}·K^{-1} and ΔG° at 298 K = −13.43 kJ·mol^{-1}, ΔH° = 2.38 kJ·mol^{-1}, ΔS° at 298 K = 53.01 J·mol^{-1}·K^{-1}, respectively. Formation of inclusion complexes substantially increases the water solubility of diazepam and nitrazepam. Diazepam and nitrazepam dissolution thermodynamics in aqueous 2-HP-β-CD were characterized by spontaneous and endothermic dissolution and hydrophobic interactions.

Keywords: Diazepam; Nitrazepam; Phase-Solubility Analysis; Thermodynamic; Parameters

1. Introduction

Diazepam and nitrazepam are benzodiazepine drugs (**Figure 1**). They are commonly used for treating anxiety, insomnia and muscle spasms [1]. The solubility was found to be 1.6228 × 10^{-4} mol·L^{-1} for diazepam, while the solubility of nitrazepam was 1.1554 × 10^{-4} mol·L^{-1}, at 25˚C [2]. In liquid medical forms, diazepam and nitrazepam are available as oral solutions (0.4 mg or 1 mg of diazepam/mL solution and 1 mg nitrazepam/mL solution), as an oral suspension (0.5 mg or 1 mg of nitrazepam/mL suspension) and as a solution for injection (5 mg diazepam/mL solution for injection).

Cyclodextrins (CDs) are non-toxic cyclic oligosaccharides containing at least 6 D-(+)-glucopyranose units attached by α-(1,4) glucoside bonds (**Figure 2**). As a result of their molecular structure, with hydrophilic exterior surface and hydrophobic cavity interior, cyclodextrins possess a unique ability to form inclusion complexes with many drugs [6,7].

In aqueous solutions, cyclodextrins form complexes with many drugs through a process in which water molecules located in the central cavity are replaced by either the whole drug molecule, or more frequently, by some lipophilic portion of the drug structure (**Figure 3**). Since no covalent bonds are formed or broken during the drug-cyclodextrin complex formation, the complexes are in dynamic equilibrium with free drug and cyclodextrin molecules [3-5].

The objectives of the present work were to prepare aqueous solutions of diazepam and nitrazepam with α-cyclodextrin (-CD), β-cyclodextrin (β-CD) and 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD) and to investigate the possibility of improving the solubility of poorly water soluble diazepam and nitrazepam by their comple- xation with selected cyclodextrins. The effects of α-CD, β-CD and 2-HP-β-CD on equilibrium solubility were assessed

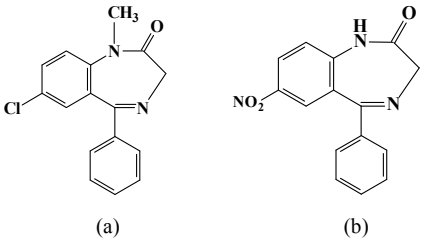

Figure 1. Structural formulas of (a) diazepam and (b) nitrazepam.

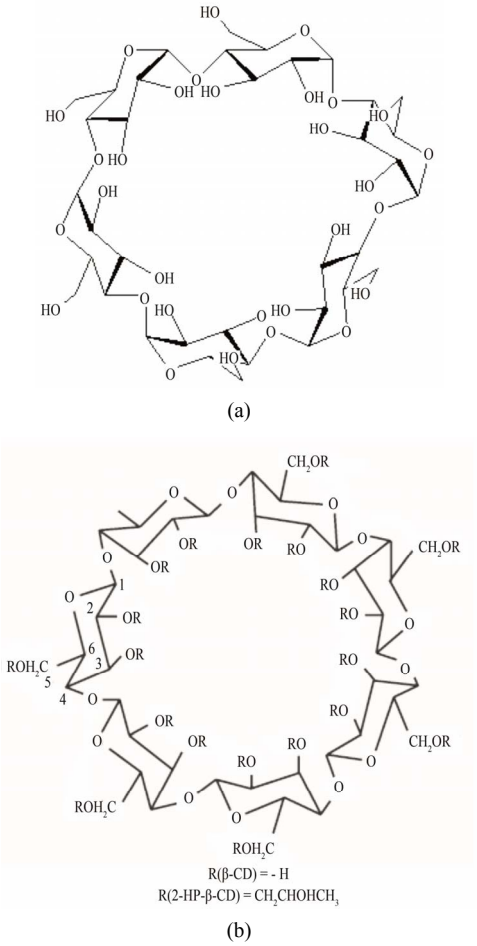

Figure 2. The chemical structures of (a) *α*-cyclodextrin (b) *β*-cyclodextrin and its derivate.

via phase-solubility analysis as were the interactions of these excipients on drug solubility under conditions favoring oversaturation. The value of the apparent stability constant ($K_{1:1}$) is used to compare the affinity of drugs for different CDs [6]. A more precise method for evaluation of the solubilizing effects of cyclodextrins is to determine their complexation efficiency (*CE*). The utility number (U_{CD}) greater or equal to 1 indicates that solubilization is adequately provided by complexation with the cyclodextrin tested. Many of the processes of pharmaceutical interest such as complexation can be described in

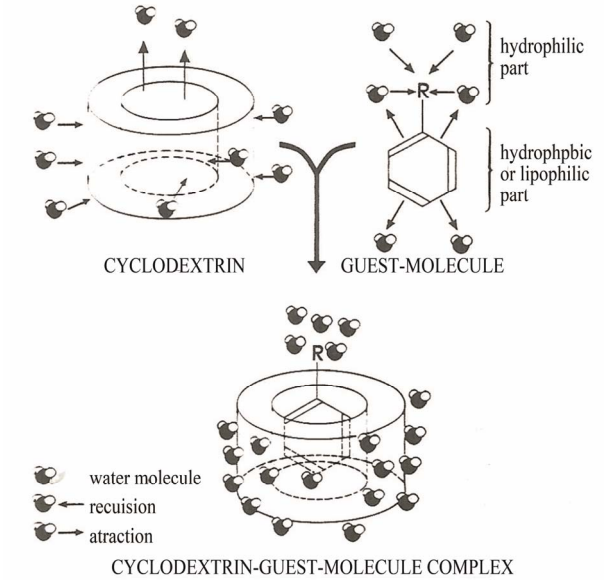

Figure 3. Shematic ilustration of the inclusion complex formation.

terms of changes of the Gibbs free energy. The standard free energy change is releated to standard entalpy change and entropy change. Several driving forces have been proposed to be important for the specific affinity of ligand molecules (CDs) for the guest molecules (drugs) [7].

2. Materials and Methods

2.1. Materials

Diazepam-(7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one) (Marsing & CoA, Denmark), Nitrazepam-(1,3-dihydro-7-nitro-5-phenyl-2H-1,4-benzodiazepin-2-on) (F.I.S.-Fabbrica Italiana Sintetici, Italy), Acidum hydrochloricum fumans, 37%, pro analysi, (Alkaloid, Macedonia), *α*-cyclodextrin, M_r = 972.86 g·mol^{-1} (Fluka, Chemika, Switzerland), *β*-cyclodextrin, M_r = 1135 g·mol^{-1} (Fluka, Chemika, Switzerland), 2-Hy-droxypropyl-*β*-cyclodextrin of molar substitution (MS) 0.6, M_r = 1383 g·mol^{-1} (Fluka, Chemika, Switzerland).

2.2. Phase Solubility Studies

Solubility measurements and the determination of saturation concentrations were carried out by adding excess amounts of diazepam and nitrazepam to water/cyclodextrin mixtures. Concentrations of these cyclodextrins were selected based on their solubility in water. Solutions with *α*-CD were prepared at concentrations from 1% to 14% (w/w) since it solubility is 14.5%. Solutions with *β*-CD were prepared at concentrations from 0.5% to 1.8% (w/w) (solubility is only 1.85%), while solutions with 2-HP-*β*-CD were prepared at concentrations from 1% to 40%

(w/w) (solubility more them 50%). The solubility of *β*-cyclodextrin in water about at room temperature. The diazepam/nitrazepam powder was added (Analytical balance type XS 205, Mettler Toledo GmbH, Germany) into dark, glass flasks containing already mentioned percentages of cyclodextrins (**Tables 1-6**). The samples were shaken for 24 hours on thermostated shaking bath (BDL, Type: GFL 1083, Czech Republic), to reach equilibrium. Preliminary studies were carried out using different equilibration periods confirming that equilibrium was reached within 24 h. Longer equilibration did not result in increased diazepam and nitrazepam solubility. Undissolved diazepam and nitrazepam were visualised before and after the equilibrium was reached [6]. These phase solubility experiments were repeated at different temperatures (15˚C, 25˚C and 37˚C ± 0.1˚C).

Table 1. Solubility of diazepam in aqueous solutions depending on the concentration of *α*-CD at 15˚C, 25˚C and 37˚C ± 0.1˚C (n = 3).

$S^a_{(\alpha\text{-CD})}$	$S_o{}^b_{(\text{diazepam})}$ (at 15˚C)	RSD[c]	$S_{CD}/S_D{}^d$	$S_o{}^b_{(\text{diazepam})}$ (at 25˚C)	RSD[c]	$S_{CD}/S_D{}^d$	$S_o{}^b_{(\text{diazepam})}$ (at 37˚C)	RSD[c]	$S_{CD}/S_D{}^d$
10.279	0.1849	1.65	1.30	0.2125	1.32	1.31	0.2404	1.79	1.45
30.837	0.2630	1.91	1.85	0.2947	1.54	1.82	0.3182	1.88	1.92
51.395	0.3698	1.73	2.60	0.4268	1.75	2.63	0.4467	1.74	2.69
102.790	0.5426	1.59	3.81	0.6891	1.78	4.25	0.7120	1.96	4.30
143.906	0.7112	2.01	5.00	0.8247	1.99	5.08	0.8551	1.98	5.16

[a]S—Concentration of cyclodextrin: *α*-CD, *β*-CD or 2-HP-*β*-CD (mmol·L^{-1}); [b]S_o—Concentration of drug: diazepam or nitrazepam (mmol·L^{-1}); [c]RSD—Relative standard deviation; [d]S_{CD}/S_D—Solubility enhancement factor calculated as the ratio of drug solubility in CD solution (S_{CD}) versus drug solubility value (S_D) measured in the absence of CD.

Table 2. Solubility of diazepam in aqueous solutions depending on the concentration of *β*-CD at 15˚C, 25˚C and 37˚C ± 0.1˚C (n = 3).

$S^a_{(\beta\text{-CD})}$	$S_o{}^b_{(\text{diazepam})}$ (at 15˚C)	RSD[c]	$S_{CD}/S_D{}^d$	$S_o{}^b_{(\text{diazepam})}$ (at 25˚C)	RSD[c]	$S_{CD}/S_D{}^d$	$S_o{}^b_{(\text{diazepam})}$ (at 37˚C)	RSD[c]	$S_{CD}/S_D{}^d$
4.405	0.2529	1.95	1.78	0.3267	1.80	2.01	0.3807	1.89	2.30
8.811	0.3986	2.05	2.80	0.4935	1.65	3.04	0.5391	1.95	3.25
13.216	0.5443	2.03	3.83	0.6379	1.85	3.93	0.6960	1.83	4.20
15.859	0.6044	1.89	4.25	0.7239	1.52	4.46	0.7944	1.78	4.79

Table 3. Solubility of diazepam in aqueous solutions depending on the concentration of 2-HP-*β*-CD at 15˚C, 25˚C and 37˚C ± 0.1˚C (n = 3).

$S^a_{(\text{2-HP-}\beta\text{-CD})}$	$S_o{}^b_{(\text{diazepam})}$ (at 15˚C)	RSD[c]	$S_{CD}/S_D{}^d$	$S_o{}^b_{(\text{diazepam})}$ (at 25˚C)	RSD[c]	$S_{CD}/S_D{}^d$	$S_o{}^b_{(\text{diazepam})}$ (at 37˚C)	RSD[c]	$S_{CD}/S_D{}^d$
7.231	0.4232	0.43	2.98	0.4921	0.57	3.03	0.5589	0.82	3.37
18.077	0.7031	0.71	4.94	0.8254	0.90	5.09	0.8288	1.11	5.00
36.153	1.3942	0.56	9.80	1.6719	0.61	10.30	1.8201	1.21	10.98
72.307	3.1189	1.05	21.93	3.3210	0.76	20.46	3.9403	0.72	23.77
144.613	6.4794	0.84	45.56	7.4464	0.95	45.89	7.6734	0.58	46.30
289.226	13.0852	1.07	92.00	15.0948	0.70	93.02	15.5565	0.77	93.85

Table 4. Nitrazepam solubility in different concentrations of *α*-CD at 15˚C, 25˚C and 37˚C ± 0.1˚C (n = 3).

$S^a_{(\alpha\text{-CD})}$	$S_o{}^b_{(\text{nitrazepam})}$ (at 15˚C)	RSD[c]	$S_{CD}/S_D{}^d$	$S_o{}^b_{(\text{nitrazepam})}$ (at 25˚C)	RSD[c]	$S_{CD}/S_D{}^d$	$S_o{}^b_{(\text{nitrazepam})}$ (at 37˚C)	RSD[c]	$S_{CD}/S_D{}^d$
10.279	0.1386	1.01	1.39	0.1717	1.46	1.49	0.1866	1.02	1.54
30.837	0.1991	1.80	2.00	0.2328	0.86	2.01	0.2417	0.91	2.00
51.395	0.2488	2.04	2.50	0.3032	1.65	2.62	0.3271	1.23	2.71
102.790	0.4330	1.06	4.35	0.4888	1.08	4.23	0.5318	1.68	4.40
143.906	0.5866	1.11	5.89	0.6978	1.34	6.04	0.7288	1.13	6.03

Table 5. Nitrazepam solubility in different concentrations of *β*-CD at 15˚C, 25˚C and 37˚C ± 0.1˚C (n = 3).

$S^{a}_{(\beta\text{-CD})}$	$S_o{}^{b}_{(\text{nitrazepam})}$ (at 15˚C)	RSD[c]	S_{CD}/S_D[d]	$S_o{}^{b}_{(\text{nitrazepam})}$ (at 25˚C)	RSD[c]	S_{CD}/S_D[d]	$S_o{}^{b}_{(\text{nitrazepam})}$ (at 37˚C)	RSD[c]	S_{CD}/S_D[d]
4.405	0.1493	0.54	1.50	0.1900	0.68	1.66	0.1934	1.24	1.60
8.811	0.2090	1.48	2.10	0.2631	1.22	2.28	0.2844	1.05	2.35
13.216	0.2648	0.94	2.66	0.3377	1.03	2.92	0.3448	1.55	2.85
15.859	0.3057	1.59	3.07	0.3868	1.84	3.35	0.4159	0.91	3.44

Table 6. Nitrazepam solubility in different concentrations of 2-HP-*β*-CD at 15˚C, 25˚C and 37˚C ± 0.1˚C (n = 3).

$S^{a}_{(\text{2-HP-}\beta\text{-CD})}$	$S_o{}^{b}_{(\text{nitrazepam})}$ (at 15˚C)	RSD[c]	S_{CD}/S_D[d]	$S_o{}^{b}_{(\text{nitrazepam})}$ (at 25˚C)	RSD[c]	S_{CD}/S_D[d]	$S_o{}^{b}_{(\text{nitrazepam})}$ (at 37˚C)	RSD[c]	S_{CD}/S_D[d]
7.231	0.2488	0.80	2.50	0.3015	1.40	2.61	0.3275	0.85	2.71
18.077	0.4081	1.34	4.10	0.4867	1.21	4.21	0.5190	1.12	4.29
36.153	0.8461	0.95	8.50	0.9954	1.48	8.62	1.0309	1.06	8.53
72.307	1.4397	1.60	14.46	1.8752	1.49	16.23	2.0050	1.58	16.59
144.613	3.0715	1.08	30.86	3.7327	1.82	32.31	3.9381	1.05	32.58
289.226	6.2744	1.25	63.04	7.4209	1.37	64.23	8.1337	1.10	67.29

After reaching equilibrium, the samples (all suspendsions) were filtered through a 0.2 μm pore size membrane filter (Cellulose acetate filter, Sartorius, Germany). The concentrations of dissolved substances in water/cyclodextrins mixtures were determined by absorption spectroscopy using Shimadzu UV-1601, UV-VIS spectrophotometer (Shimadzu, Japan) at 360 nm (diazepam), and 280 nm (nitrazepam) (Calibration curve for diazepam: concentration range: 15 - 60 $mg \cdot L^{-1}$, $R^2 = 0.9998$, calibration curve for nitrazepam: concentration range: 2 - 10.2 $mg \cdot L^{-1}$, $R^2 = 0.9995$). Aqueous solutions of diazepam/nitrazepam and cyclodextins were diluted with 0.1 $mol \cdot L^{-1}$ hydrochloric acid (which was previously used to develop the calibration curve) since the samples of diazepam and nitrazepam in *α*-CD and *β*-CD solution leave white sediment when in contact with 0.5% w/v H_2SO_4/CH_3OH [8]. To nullify the absorbance due to the presence of *α*-CD, *β*-CD, 2-HP-*β*-CD the apparatus was calibrated with the corresponding blank in every assay. Three replicates were made for each experiment (n = 3) and the results are presented as the mean values (**Tables 1-6**).

The changes in the solubility of diazepam and nitrazepam resulting from the addition of various concentrations of *α*-CD, *β*-CD and 2-HP-*β*-CD were used to plot phase-solubility diagrams and to evaluate the stoichiometry and apparent stability constants of the resultant complexes. The apparent stability constants ($K_{1:1}$) were estimated from the straight line of the phase solubility diagrams according to the following Equation of Higuchi and Connors [3,6]:

$$K_{1:1} = \frac{Slope}{S_o\left(1 - Slope\right)} \quad (1)$$

where $K_{1:1}$ is the apparent stability constant ($L \cdot mol^{-1}$), S_o is the saturation concentration of drug (diazepam or nitrazepam) in pure water, and *Slope* denotes the slope of the straight line. For 1:1 drug/cyclodextrin complexes the complexation efficiency (*CE*) can be calculated from the *Slope* of the phase-solubility diagram [3,9]:

$$CE = \frac{[D/CD]}{[CD]} = S_o \cdot K_{1:1} = \frac{Slope}{(1 - Slope)} \quad (2)$$

where [*D/CD*] is the concentration of dissolved drug-cyclodextrin complex, [*CD*] is the concentration of dissolved free cyclodextrin and *Slope* is the slope of the phase solubility profile [19]. Complexation efficiency (*CE*) was determined by measuring the solubility of given drugs by 5 (for *α*-CD), 4 (for *β*-CD) and 6 (for 2-HP-*β*-CD) concentrations of respective cyclodextrin in water. Determination of *CE* is a simple method for quick evaluation of the solubilizing effects of different cyclodextrins. The *CE* was used to calculate the drug:cyclodextrin ratio (*D*:*CD*), which can be correlated to the expected increase in formulation bulk [9]:

$$D : CD = 1 : \left(1 + \frac{1}{CE}\right) \quad (3)$$

In order to asses the efficacy of cyclodextrin as a complexing agent, the utility number (U_{CD}) was calculated according to Equation 4 [10]:

$$U_{CD} = \frac{K_{1:1}S_o}{1+K_{1:1}S_o}\frac{m_{CD}}{m_D}\frac{M_{\gamma D}}{M_{\gamma CD}} \quad (4)$$

where $K_{1:1}$ is the apparent stability constant of a complex with 1:1 stoichiometry, S_o is the intrinsic solubility of the drug, m_D is the drug dose, m_{CD} is the workable amount of cyclodextrin and molecular weights of cyclodextrin (M_{rCD}), and drug (M_{rD}).

2.3. Determination of Thermodinamic Parameters

The standard free energy of transfer (ΔG°_{tr}) represents the free energy of transfer of drug (solute) from pure water to cyclodextrin cavity. The values of Gibbs free energy change were calculated to understand transfer process of diazepam (nitrazepam) from pure water to aqueous solution of CDs. The ΔG°_{tr} value can be obtained using the following Equation:

$$\Delta G^\circ_{tr} = -RT \ln S/S_o \quad (5)$$

in which S/S_o is the ratio of molar solubility of drug in an aqueous solution of CD to that of molar solubility of drug in pure water [11].

The thermodynamic parameters results proved the solubilization effect of the carrier (cyclodextrin) on the drug (diazepam, nitrazepam). The thermodynamic parameters, *i.e.* the standard free energy change (ΔG°), the standard enthalpy change (ΔH°) and the standard entropy change (ΔS°), can be obtained from the temperature dependence of the apparent stability constant of the cyclodextrin complex [12]. The free energy of reaction is derived from the apparent stability constant using the relationship:

$$\Delta G^\circ = -2.303RT \log K_{1:1} \quad (6)$$

where $K_{1:1}$ is the apparent stability constant of a complex with 1:1 stoichiometry, R is equal to the gas constant, T is the absolute temperature.

The enthalpy (ΔH) values of solubility were measured directly from the temperature dependence of the saturation concentration. The enthalpies of reactions can thus be determined from $K_{1:1}$ obtained at various temperatures using the van't Hoff Equation [13]. The ΔH° values can be obtained from plot of log $K_{1:1}$ versus $1/T$ using the following relationship:

$$\log K_{1:1} = -\frac{\Delta H^\circ}{2.303R}\frac{1}{T} + C \quad (7)$$

where the *Slope* will provide the enthalpy data [12].

$$Slope = \frac{\Delta H^\circ}{2.3R} \quad (8)$$

The standard entropy change (ΔS°) for the complexation reaction can be calculated using the expression [13, 14]:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (9)$$

3. Results and Discussion

Solubility Study of Diazepam and Nitrazepam in the Presence of α-CD, β-CD and 2-HP-β-CD

The results of the change in diazepam and nitrazepam solubility in water, depending on α-CD, β-CD and 2-HP-β-CD concentration, are listed in **Tables 1-6** and **Figures 4-9**.

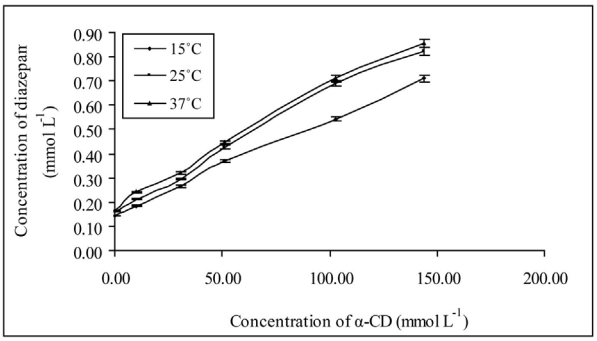

Figure 4. Phase solubility diagram of diazepam and α-CD in distilled water at different temperatures.

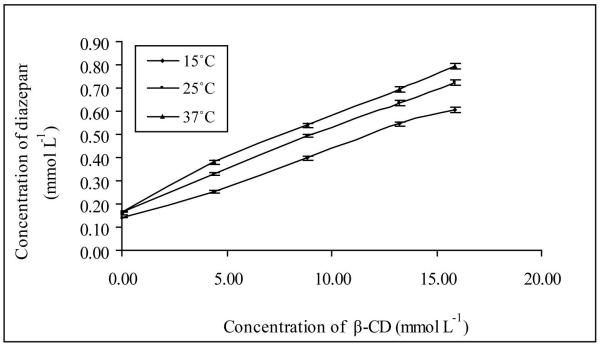

Figure 5. Phase solubility diagram of diazepam and β–CD in distilled water at different temperatures.

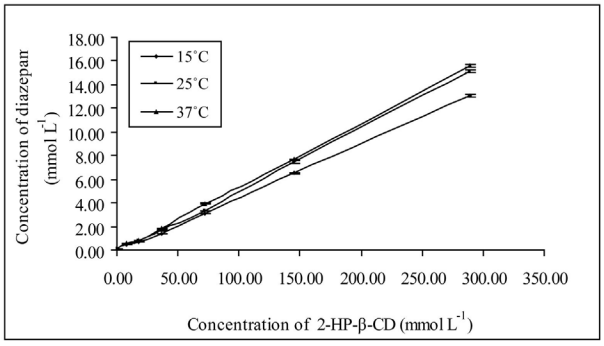

Figure 6. Phase solubility diagram of diazepam and 2-HP-β-CD in distilled water at different temperatures.

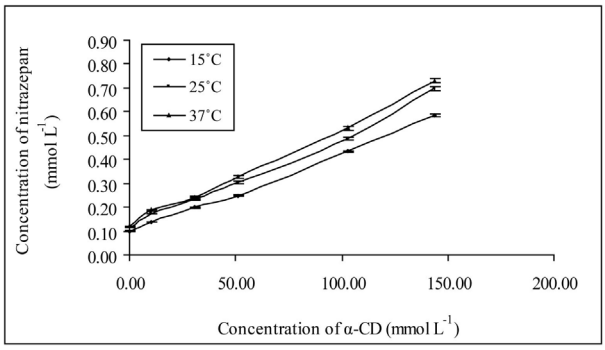

Figure 7. Phase solubility diagram of nitrazepam and α-CD in distilled water at different temperatures.

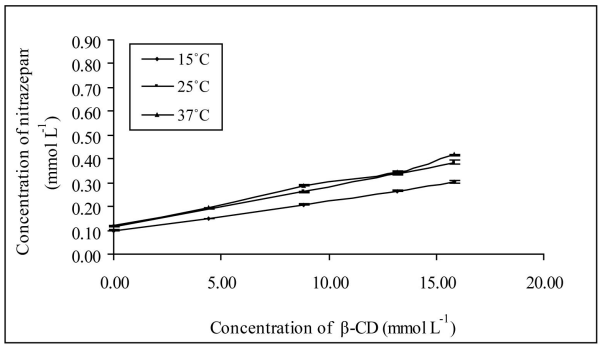

Figure 8. Phase solubility diagram of nitrazepam and β-CD in distilled water at different temperatures.

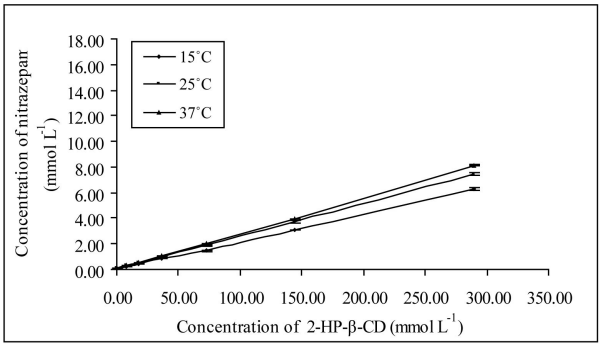

Figure 9. Phase solubility diagram of nitrazepam and 2-HP-β-CD in distilled water at different temperatures.

The solubility of diazepam and nitrazepam increased with increasing amount of CDs in water **(Tables 1-6)**. Hydroxypropyl-β-CD (in concentration of 1% - 40% w/w) increased the solubility of diazepam by 3.03 to 93.02 fold, β-cyclodextrin (0.5% - 1.8% w/w) increased the solubility by 2.01 to 4.46 fold and α-CD (1% - 14% w/w) increased diazepam solubility by 1.31 to 5.08 fold, at 25˚C. The solubility of nitrazepam was increased 64.23 fold at 40% (w/w) of 2-HP-β-CD (2.087 mg of nitrazepam /mL), 3.35 fold at 1.8% (w/w) β-CD (0.109 mg of nitrazepam /mL), and solubility of nitrazepam was increased 6.04 fold at 14% (w/w) of α-CD (0.196 mg of nitrazepam /mL) at 25˚C. The highest solubility of both drugs in water was achieved with 2-HP-β-CD, of all used cyclodextrins. α-CD achieved more significant increase in water solubility of nitrazepam than of diazepam.

The estimated values of *Slopes* of phase diagrams and $K_{1:1,}$ are presented in **Table 7**. The solubility of diazepam and nitrazepam increased linearly as function of chosen cyclodextrins. Linearity was characteristic of the A_{L-}type system and suggested that water-soluble complexes formed in the solution. The *Slope* value was lower than 1, thus indicating that the inclusion complexes in molar ratio of 1:1 formed between the guest (diazepam, nitrazepam) and host molecule (CD). Assuming 1:1 complexes were formed, the apparent stability constants ($K_{1:1}$) of the binary complexes were calculated using the linear regression analysis method, from the diagrams according to the following Equation mentioned above (Equation 1). The $K_{1:1}$ of complexes were ranged in the following order: 2-HP-β-CD > β-CD > α-CD, reflecting the greater affinity of modified cyclodextrin for the studied diazepam and nitrazepam compared to their parent α- and β-CDs. Small $K_{1:1}$ values (like diazepam/α-CD, nitrazepam/α-CD) indicate too weak interaction, whereas a larger value indicates the possibility of limited drug release from the complex (optimal value 100 - 1000 M^{-1}) [3]. The value of $K_{1:1}$ with nitrazepam/α-CD is slightly higher in comparison to the value of $K_{1:1}$ of diazepam/α-CD complex.

The *CE*, *D*:*CD* and U_{CD} of binary systems as a result of effect of the α-CD, β-CD and 2-HP-β-CD on diazepam and nitrazepam intrinsic solubility (S_o), at 25˚C, are presented in **Table 8**.

The aqueous solution of 2-HP-β-CD is most often a better solubilizer than β-CD and α-CD (**Table 8**). From our phase-solubility profile of diazepam with 2-HP-β-CD, the *CE* of 0.055 was calculated, indicating that approximately one of every 19 cyclodextrin molecules forms a complex with diazepam. The *CE* of nitrazepam with 2-HP-β-CD was calculated to be 0.026, indicating that approximately one of every 40 cyclodextrin molecules forms a complex with nitrazepam (25˚C ± 0.1˚C) [10].

Since all U_{CD} values are less than 1 (**Table 8**) the concentrations of selected cyclodextrins of 1% were not sufficient to achieved complete solubilization of 1 mg diazepam and nitrazepam/mL water. The concentration of 10% (w/w) 2-HP-β-CD would be required to dissolve 1 mg diazepam/mL of water (U_{CD} = 1.07). For the same concentration of diazepam dissolved in water, it would be necessary to use 15% (w/w) of β-CD (U_{CD} = 1.34), but the application of concentrations higher than 1.85% of this cyclodextrin are not possible due to its limited solubility in water [5]. The concentrations of 20% (w/w) 2-HP-β-CD (U_{CD} = 1.03) and 25% (w/w) of β-CD (U_{CD} = 5.1) would be adequate to dissolve 1 mg nitrazepam/mL

Table 7. Diazepam and nitrazepam-cyclodextrin *Slopes* and apparent stability constants.

	Diazepam			Nitrazepam		
			α-CD			
Temp. [e](°C)	*Slope*[f]	r[f]	$K_{1:1}$ (M^{-1})[g]	*Slope*[f]	r[f]	$K_{1:1}$ (M^{-1})[g]
15	0.0039	0.9977	27.5278	0.0033	0.9968	33.2631
25	0.0048	0.9932	29.7212	0.0039	0.9933	33.8868
37	0.0049	0.9937	29.7067	0.0041	0.9964	34.0614
			β-CD			
Temp. [e](°C)	*Slope*[f]	r[f]	$K_{1:1}$ (M^{-1})[g]	*Slope*[f]	r[f]	$K_{1:1}$ (M^{-1})[g]
15	0.0300	0.9963	217.4496	0.0130	0.9982	132.3249
25	0.0355	0.9988	226.8095	0.0170	0.9998	149.6808
37	0.0389	0.9954	244.1780	0.0180	0.9938	151.6543
			2-HP-*β*-CD			
Temp. [e](°C)	*Slope*[f]	r[f]	$K_{1:1}$ (M^{-1})[g]	*Slope*[f]	r[f]	$K_{1:1}$ (M^{-1})[g]
15	0.0452	0.9992	332.8400	0.0214	0.9988	219.6990
25	0.0521	0.9985	338.6966	0.0253	0.9998	224.6572
37	0.0535	0.9995	341.0033	0.0277	0.9994	235.7086

[e]Temperature; [f]Values obtained directly from the drug phase solubility diagram; [g]The $K_{1:1}$ calculated from *Slope* of phase solubility diagram according to Equation (1).

Table 8. The complexation efficiency, drug: cyclodextrin molar ratio and utility number of diazepam and nitrazepam.

Drug	Cyclodextrin[h]	*CE*[i]	Dose[j] (mg)	*D*:*CD*[k]	U_{CD}[l]
diazepam	*α*-CD	0.0048	1	1:208	0.0144
	β-CD	0.0368	1	1:28	0.0891
	2-HP-*β*-CD	0.0549	1	1:19	0.1072
nitrazepam	*α*-CD	0.0039	1	1:256	0.0113
	β-CD	0.0173	1	1:59	0.0421
	2-HP-*β*-CD	0.0260	1	1:40	0.0515

[h]*α*-cyclodextrin (*α*-CD), *β*-cyclodextrin (*β*-CD), 2-hydroxypropyl-*β*-cyclodextrin (2-HP-*β*-CD); [i]Complexation efficiency calculated from *Slope* of phase solubility diagram according to Equation (2); [j]Oral dosage (1 mg/mL - for a liquid dosage form); [k]The drug:cyclodextrin molar ratio is based on the calculated complexation efficiency according to Equation (3); [l]The utility number was calculated according to Equation (4). The U_{CD} was calculated for the values of $K_{1:1}$, S_o for diazepam and nitrazepam determined at 25˚C. The presented results for the U_{CD} are related to working concentration of 1 % cyclodextrin.

of water (for preparation of aqueous solution). Parenterally diazepam is administered in the form of a solution. Usually, two milliliter (2 mL) solution for injection (1 ampoule) contains 10 mg of diazepam. For 2-HP-*β*-CD, maximum concentration of 40% (w/w) in the formulation solution is workable due to increasing viscosity with the increase in 2-HP-*β*-CD concentration, resulting in the workable amount of 800 mg 2-HP-*β*-CD per ampoule if the fill volume of 2 mL is used. We calculated U_{CD} of 0.86 for diazepam/2-HP-*β*-CD, while for nitrazepam/2-HP-*β*-CD U_{CD} was been 0.417 at 25˚C, when both drugs are administered in 5 mg/mL. Based on these results, the application of 2-HP-*β*-CD at a concentration of up to 40% would be useful in aqueous solutions of diazepam and nitrazepam (5 mg/mL), since they would require concentrations in the upper range, especially in case of nitrazepam. As a complexing agent, the *α*-CD has the lowest ability for dissolving the tested benzodiazepines, since it is necessary to use about 75% (w/w) for dissolveing 1 mg of diazepam, and about 90% (w/w) for dissolveing 1 mg nitrazepam/mL aqueous solution.

The Gibbs free energy of transfer (ΔG_{tr}°) values of di-

azepam and nitrazepam from pure water to aqueous solutions with various concentrations of 2-HP-*β*-CD at 25˚C were calculated using the Equation (6). The obtained values of Gibbs free energy of transfer (ΔG_{tr}°) of diazepam and nitrazepam from aqueous solution to the cavity of 2-HP-*β*-CD in the presence of excess amount of investigated drugs, at 25˚C, are shown in **Table 9**.

The ΔG_{tr}° values provide information about whether the reaction condition is favorable or unfavorable for drug solubilization in the aqueous carrier solution. Negative Gibbs free energy of transfer values indicate favorable conditions. The ΔG_{tr}° values were all negative for 2-HP-*β*-CD at various concentrations (**Table 9**), thus indicating the spontaneous nature of diazepam and nitrazepam solubilization. These values decreased with increasing concentration of 2-HP-*β*-CD, thereby demonstrating that the reaction became more favorable as the concentration of 2-HP-*β*-CD increased.

The dependancies of log $K_{1:1}$ on the reciprocal temperatures (1/T:3.470 × 10^{-3} K^{-1} for 288.15 K, 3.354 × 10^{-3} K^{-1} for 298.15 K and 3.224 × 10^{-3} K^{-1} for 310.15 K), according to the van't Hoff equation, are given in **Figure 10** (for diazepam/CDs) and **Figure 11** (for nitrazepam/CDs). The corresponding thermodynamic values (**Table 10**) were calculated from the values of $K_{1:1}$ (ΔG°), using the values shown in **Figures 10** and **11** (ΔH°), and Equations 8 (ΔH°) and 9 (ΔS°).

Diazepam (nitrazepam) dissolution thermodynamics in

Table 9. Gibbs free energy of transfer (ΔG_{tr}°) for the solubilization process of diazepam and nitrazepam in aqueous solutions of 2-HP-*β*-CD at 25˚C.

Concentration of 2-HP-*β*-CD (mmol·L^{-1})	ΔG_{tr}° (for diazepam) (kJ·mol^{-1})[lj]	ΔG_{tr}° (for nitrazepam) (kJ·mol^{-1})[lj]
7.231	–2.7497	–2.3769
18.077	–4.0317	–3.5643
36.153	–5.7815	–5.3379
72.307	–7.4626	–6.9078
144.613	–9.4839	–8.6140
289.226	–11.2353	–10.3177

[lj]Values are mean of three determinations (n = 3).

Table 10. Thermodynamic parameters for complexation of diazepam and nitrazepam with cyclodextrins.

Drug-CD	ΔG° (kJ·mol^{-1})	ΔH° (kJ·mol^{-1})	ΔS° (J·mol^{-1})
Diazepam-*α*-CD	−8.4086	2.5200	36.6447
Diazepam-*β*-CD	−13.4474	3.9200	58.2492
Diazepam-2-HP-*β*-CD	−14.4415	0.8125	51.1628
Nitrazepam-*α*-CD	−8.7341	0.7916	31.9494
Nitrazepam-*β*-CD	−12.4169	4.5279	56.8329
Nitrazepam-2-HP-*β*-CD	−13.4235	2.3861	53.0268

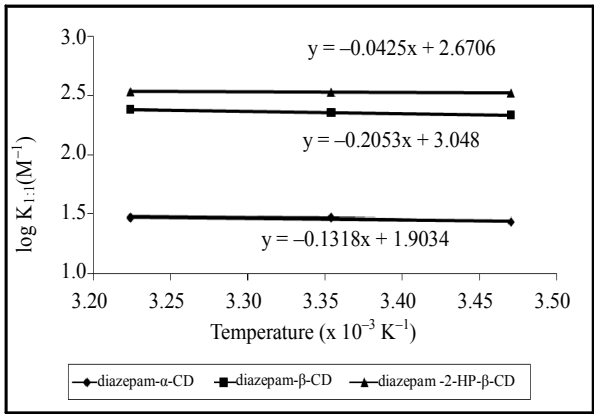

Figure 10. van't Hoff plot for diazepam solubility with *α*-CD, *β*-CD and 2-HP-*β*-CD.

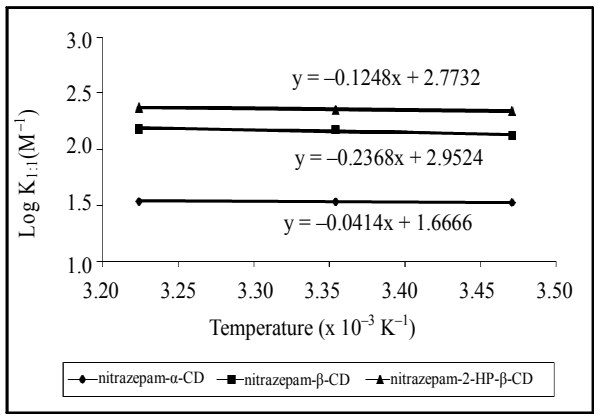

Figure 11. van't Hoff plot for nitrazepam solubility with *α*-CD, *β*-CD and 2-HP-*β*-CD.

aqueous 2-HP-β-CD were characterized by negative ΔG° value, indicative of spontaneous dissolution and positive ΔH° value, indicative of endothermic dissolution [13]. The relatively large ΔS° of + 51.04 $J \cdot K^{-1} \cdot mol^{-1}$ and + 53.14 $J \cdot K^{-1} \cdot mol^{-1}$ for diazepam and nitrazepam, respectively, can be attributed to the transfer of diazepam (nitrazepam) from aqueous medium to a more apolar site, such as the cavity of 2-HP-β-CD. This transfer involves breakdown of water structure around diazepam (nitrazepam), which creates a large positive ΔS° and a small positive ΔH°, apparently governed by hydrophobic interactions.

4. Conclusions

The increase in solubility of diazepam and nitrazepam displayed concentration dependency on CDs. The results of the phase-solubility showed a positive effect of 2-HP-β-CD on diazepam and nitrazepam in aqueous solutions. The presence of 2-HP-β-CD was proven to increase the solubility of diazepam (nitrazepam) by solubility enchancement factor, S_{CD}/S_D of about 93.0 (64.2) calculated as the ratio of diazepam (nitrazepam) solubility in 289.23 $mmol \cdot L^{-1}$ solution (S_{CD}) *versus* diazepam (nitrazepam) solubility value in the absence of 2-HP-β-CD at 25˚C. These $K_{1:1}$, *CE* and U_{CD} values show that 2-HP-β-CD can be very useful as solubilizer in the desired formulation. The calculated apparent stability constant was low for diazepam/α-CD and nitrazepam/α-CD, indicating that a relatively high amount of α-CD is required to achieve complexation of diazepam and nitrazepam. On the basis of the U_{CD} values, CDs are desirable for products with low dose of the drug. To prepare aqueous solution of nitrazepam (1 mg/mL), it would be sufficient to use 20% (w/w) 2-HP-β-CD, while for making the aqueous solution of diazepam of the same concentration, 10% (w/w) 2-HP-β-CD would be required.

Thermodynamic parameters derived from diazepam and nitrazepam solubility in the presence of various concentrations of CDs at several temperatures revealed that the solubility of diazepam and nitrazepam increased proportionally with an increase in temperature. Gibbs free energy values were all negative, indicating the spontaneous nature of diazepam (nitrazepam) solubilization and their values decreased in the following order: 2-HP-β-CD < β-CD < α-CD. The determined enthalpies for the interaction of diazepam and nitrazepam with CDs clearly indicate the interaction was endothermic. The positive value for ΔS° can be explained in term of hydrophobic effect which involves breakdown and removal of the structured water molecules inside CD cavity and around the non-polar substrate (diazepam, nitrazepam).

REFERENCES

[1] H. Möhler, M. J. Fritschy and U. Rudolph, "A New Benzodiazepine Pharmacology," *The Journal of Pharmacology and Experimental Therapeutics*, Vol. 300, No. 1, 2002, pp. 2-8.

[2] J. Hadžiabdić and S. Hadžović, "Influence of Particle Size on Water Solubility of Almost Insoluble Benzo-diazepines," *Pharmacia*, No. 16, 2005-2006, pp. 35-39.

[3] R. Arun, K. C. K. Ashok and V. V. N. S. S. Sravanthi, "Cyclodextrins as Drug CarrierMolecule: A Review," *Scientia Pharmaceutica*, Vol. 76, No. 4, 2008, pp. 567-598. doi:10.3797/scipharm.0808-05

[4] M. E. Brewster and T. Loftsson, "Cyclodextrins as Pharmaceutical Solubilizers," *Advenced Drug Delivery Reviews*, Vol. 59, No. 7, 2007, pp. 645-666. doi:10.1016/j.addr.2007.05.012

[5] M. Jug and M. B. Laćan, "Cyclodextrin-Based Pharmaceuticals," *Rad. MedicalSciences*, Vol. 32, No. 499, 2008, pp. 9-26.

[6] T. Higuchi and A. K. Connors, "Phase Solubility Techniques," In: C.N. Reilly, Ed., *Advences in Analytical Chemistry and Instrumentation*, Vol. 4, Wiley-Interscience, New York, 1965, pp. 117-212.

[7] C. T. Klein, D. Polheim, H. Viernstein, P. Wolschann, "A Method for Prediction of the Free Energies of Complexation between Cyclodextrin and Guest Molecules," *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, Vol. 36, No. 4, 2000, pp. 409-423. doi:10.1023/A:1008063412529

[8] Council of Europe, "The European Pharmacopoeia," 3rd Edition, Vol. 2, European Directorate for the Quality of Medicine (EDQM), Strasbourg, 1997, pp. 729-730.

[9] T. Loftsson, D. Hreinsdóttir and M. Másson, "The Complexation Efficiency," *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, Vol. 57, No. 1-4, 2007, pp. 545-552. doi:10.1007/s10847-006-9247-2

[10] J. H. Beijnen, S. C. van der Schoot, B. Nuijen, F. M. Flesch, A. Gore, D. Mirejovskyand and L. Lenaz, "Complexation Study of the Anticancer Agent EO-9 with 2-Hydroxypropyl-Beta-Cyclodextrin," *Drug Development and Industrial Pharmacy*, Vol. 34, No. 10, 2008, pp. 1130-1139. doi:10.1080/03639040801974261

[11] A. H. Al-Marzouqi, I. Shehatta, B. Jobe and A. Dowaidar, "Phase Solubility and Inclusion Complex of Itraconazole with β-Cyclodextrin Using Supercritical Carbon Dioxide," *Journal of Pharmaceutical Sciences*, Vol. 95, No. 2, 2006, pp. 292-304. doi:10.1002/jps.20535

[12] P. J. Sinko, "Martin's Physical Pharmacy and Pharmaceutical Sciences," 5th Edition, Lippincott Williams & Wilkins, Philadelphia, 2006, pp. 72-80.

[13] K. A. Connors, "Thermodynamics of Pharmaceutical Systems: An Introduction for Students of Pharmacy," John Wiley & Sons, Inc., Hoboken, 2002, pp. 52-55. doi:10.1002/0471234923.ch4

[14] C. Akbay, L. N. Gill and M. I. Warner, "Thermodynamic Studies of the Interaction of Molecular Micelles and Copolymerized Molecular Micelles with Benzodiazepinesand Alkyl Phenyl Ketones Using MEKC," *Electrophoresis*, Vol. 28, No. 11, 2007, pp. 1752-1761. doi:10.1002/elps.200600449

