Carbohydrate Polymers 77 (2009) 482–488

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

# Investigation of inclusion complex of trazodone hydrochloride with hydroxypropyl-β-cyclodextrin

Wieslawa Misiuk *, Magdalena Zalewska

*University of Bialystok, Department of Biology and Chemistry, Institute of Chemistry, Bialystok, Poland*

ARTICLE INFO

*Article history:*
Received 24 August 2008
Received in revised form 29 December 2008
Accepted 20 January 2009
Available online 6 February 2009

*Keywords:*
Trazodone hydrochloride
Hydroxypropyl-β-Cyclodextrin
Inclusion complex
Supramolecular structure
Spectroscopy

ABSTRACT

Inclusion complex of trazodone hydrochloride (TRD) with hydroxypropyl-β-cyclodextrin (HP-βCD) has been investigated by $^{1}$H NMR, $^{13}$CNMR, 2D NMR, FTIR and UV/visible spectroscopy. It was testified that the inclusion complex was formed between HP-βCD and trazodone. The stability constant $K_{1:1}$ and the 1:1 stoichiometry of complexation was determined. NMR analysis confirmed the inclusion and to provide information on the behaviour of TRD inside the cavity of HP-βCD. It was found that the fragment of TRD molecule, the benzene ring entered into the cavity of HP-βCD. Concerning the structure of the inclusion complex, a Cl– in orientation of trazodone in hydroxypropyl-β-cyclodextrin cavity has been confirmed by 2D NMR spectroscopy. Based on the enhancement of the absorbance of trazodone produced throught complex formation, a spectrophotometric method for the determination of trazodone in bulk aqueous solution in presence of HP-βCD was developed, which overcome the effect of condition change on the determination of trazodone. The linear relationship between the absorbance and trazodone concentration was obtained in the range of 5–30 μg ml$^{-1}$ with a correlation coefficient of 0.9998. The detection limit was 0.27 μg ml$^{-1}$.



## 1. Introduction

Cyclodextrins are cyclic oligosaccharides constituted by six (α-cyclodextrin), seven (β-cyclodextrin) and eight (β-cyclodextrin) glucopyranose units linked by α-(1,4) bonds (Szejtli, 1998) (Fig. 1). The form of cyclodextrin molecules resembles truncated cones with secondary hydroxyl groups located at the wider edge of the ring and the primary groups on the narrower edge, having a different cavity volume. Their most popular feature is the marked difference of polarity between the internal and external surfaces: the inner part is made apolar by the glycosidic oxygens and methine protons, whereas the external surface is polar by virtue of the presence of secondary and primary hydroxyls on the large and small rims, respectively, (Atwood, Davies, Macnicol, & Vogtle, 1996; Khan, Forgo, Stine & D'Souza, 1998). The cyclodextrin structure provides a molecule shaped like a segment of a hollow cone which is capable of forming stable, supramolecular structures with various molecules (Hazekamp & Verpoorte, 2006; Jullian, Miranda, Zapata-Torres, Mendizabal, & Olea-Azar, 2007; Liu & Zhu, 2006; Rajabi, Tayyari, Salari, & Tayyari, 2008; Sagiraju & Jursic, 2008; Spamer, Muller, Wessels, & Venter, 2002; Wang, Han, & Feng, 2007; Yong & Zhu, 2006; Zhu, Sun, & Wu, 2007), fitting partially or completely in the host molecular cavity.

Hydroxypropyl-β-cyclodextrin (HP-βCD) is a hydroxyalkyl β-cyclodextrin derivative which is widely studied in the field of drug encapsulation owing to its inclusion ability along with a high water solubility (Gould & Scott, 2005; Granero, Maitre, Garnero, & Longhi, 2008). In addition, toxicological studies pointed out that HP-βCD is well tolerated by the human body both by intravenous and oral administrations (Fromming & Szejtli, 1996).

Trazodone, known as 2-(3-(4-(3-chlorophenyl)piperazin-1-yl)propyl)-[1,2,4]triazolo[4,3-a]pyridine-3(2H)-one (Fig. 2) is a triazolopyridine derivative and belongs to the group of second-generation non-tricyclic antidepressants (Carda-Broch, Gil, Monferrer-Pons, & Esteve-Romero, 2007; El-Gindy, El-Zeany, Awad, & Shabana, 2001). Trazodone (TRD) has been shown to be effective in patients with major depressive disorders. It is generally more useful in depressive disorders associated with insomnia and anxiety. This drug does not aggravate psychotic symptoms in patients with schizophrenia or schizoaffective disorders. Its mechanism of action in humans is not clear. In animals, TRD selectively inhibits serotonin uptake by brain synaptosomes and enhances the behavioral changes induced by the serotonin precursor, 5-hydroxytryptophan (AHFS Drug information, America Society of Health – system pharmacists, 1998). The TRD metabolite 1-m-chlorophenyl-piperazine (m-CPP) is a potent post-synaptic serotonin agonist. It is sometimes referred to as a serotonin antagonist reuptake inhibitor (SARI) (Goeringer, Raymon, & Logan, 2000).

In this paper, the inclusion complex of HP-βCD with TRD was obtained by co-precipitation a widely used method for the prep-

* Corresponding author.
*E-mail address:* wiesmisi@uwb.edu.pl (W. Misiuk).

0144-8617/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2009.01.033

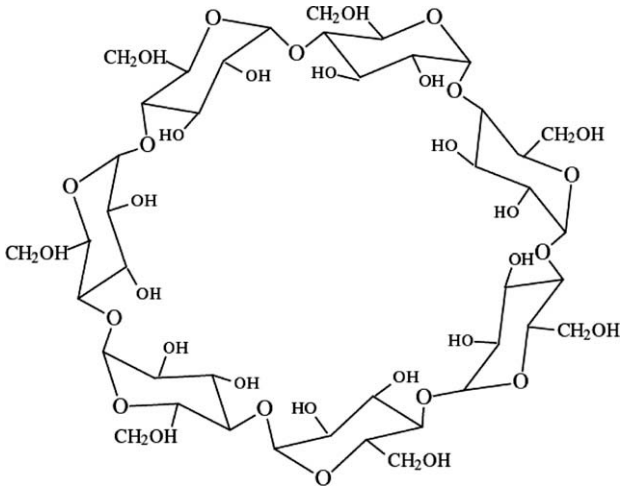

**Fig. 1.** Representation of the front view of β-cyclodextrin molecule.

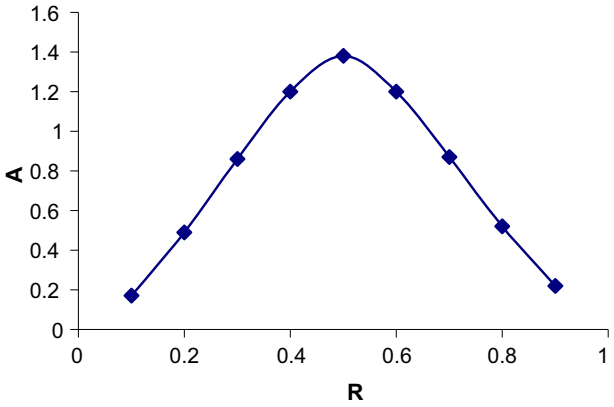


**Fig. 3.** Job's plot of TRD/HP-βCD complex.

aration of inclusion complex. The stability constant of inclusion complex of trazodone with HP-βCD was determined and the 1:1 stoichiometry of inclusion complexation was confirmed by the continuous variation Job's method (Job, 1928). In addition, the characteristics of the supramolecular structure of the 1:1 inclusion complex of trazodone with HP-βCD were investigated by different spectroscopic techniques, Fourier transformation-infrared spectroscopy (FT-IR), $^{1}$H NMR, $^{13}$C NMR and 2D NMR for understanding which fragment of TRD molecule was involved into the cavity of HP-βCD. Based on the enhancement of the absorbance of trazodone produced by inclusion complex formation, a sensitive spectrophotometric method for determination of TRD in bulk solution in presence of HP-βCD was developed. The linear relationship was obtained in the range of TRD 5–30 μg ml$^{-1}$ with correlation coefficient of 0.9998 and limit detection of 0.27 μg ml$^{-1}$.

## 2. Experimental section

### 2.1. Materials

TRD ($M_r$ = 340) and hydroxypropyl-β-cyclodextrin ($M_r$ = 1540) were purchased from Sigma. Other reagents and chemicals were of analytical reagent grade. All solutions were prepared using ultrapure water (MILLI Q).

### 2.2. Apparatus

Nicolet, Magna 550, serie II, Fourier Transform Infrared (FTIR) spectrometer; a Bruker AVANCE II spectrometer NMR at 400 MHz; UV/VIS Cecil CE 8020 spectrophotometer; DZF-6020 vacuum dryer and sonicator (precision apparatus factory).

### 2.3. Procedures

#### 2.3.1. Analytical procedure

A 1.0 ml portion of 5 × 10$^{-4}$ mol l$^{-1}$ trazodone was transferred accurately into 10 ml standard flask, 1 ml 10$^{-2}$ mol l$^{-1}$ HP-βCD and 2 ml buffer solution pH 5 were added sequentially, diluted to the mark with water and mixed well surged for 5 min by ultrasonic generator. This solution stood for 5–10 min at room temperature. The absorption spectrum of TRD–HP-βCD complex was recorded against reagent blank prepared with the same reagent concentration but no TRD. In addition, absorption spectra of TRD and HP-βCD were recorded according to the same procedure, respectively. All the absorbances at 245 nm were measured separately against a reagent blank.

#### 2.3.2. Preparation of the inclusion complex

The inclusion complex of TRD with HP-β CD at 1:1 molar ratio was prepared using the co-precipitation method. The accurately weighed HP-β CD was dissolved in distilled water to get a saturated solution. Then TRD solution in methanol was added slowly and a suspension was formed. The suspension was stirred at 40 °C for 30 min and maintained stirring at room temperature for 24 h. The obtained mass was filtered through 0.45 μm membrane filter and dried at 40 °C in an oven for 24 h. The dried complex was ground to fine powder and screened through an 80-mesh sieve.

Physical mixture of TRD and HP-βCD with 1:1 molar ratio was prepared by mixing exactly weighed amount of TRD and HP-βCD for 20 min in a mortar. The mixture was passed through an 80-mesh sieve before use.

#### 2.3.3. Job's plots

The stoichiometry of inclusion complex was determined by the continuous variation Job's method. The Job's plot (Fig. 3.) was determined from UV spectrophotometry data, according to the continuous variation method. Equimolar 5 × 10$^{-4}$ mol l$^{-1}$ solu-

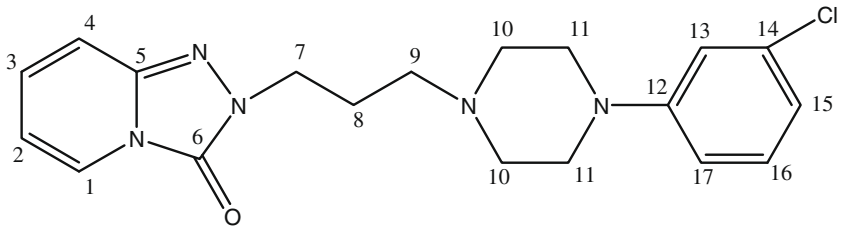


**Fig. 2.** Structure of trazodone with the corresponding carbon and proton numbering.

tions of TRD and HP-βCD were mixed to a standard volume (1 ml:9 ml; 2 ml:8 ml; 3 ml:7 ml and so on) varying the molar ratio but keeping the total concentration of the species constant. An analogous dilution set of TRD stock solution was carried out using ultrapure water. After stirring, the absorbance at $\lambda_{max}$ was measured for all solutions and the difference in absorbance in the presence and in absence of HP-βCD was plotted against R (R = [TRD]/{[TRD]+[HP-βCD]}. The shift of $\lambda_{max}$ around 245 nm of the UV-spectrum of TRD was observed to prepare the Job's plot. Spectra were obtained by Cecil CE 8020 UV–vis mini spectrophotometer (0.1 nm resolution). Each complex solution was measured in triplicate.

*2.3.4. Measurement of octanol–water partition coefficients*

2.5 ml of $10^{-4}$ mol $l^{-1}$ aqueous solutions of each compound (TRD and HP-βCD or its complexes) were respectively, mixed with the same volume of octanol at room temperature. The system was shaken vigorously until equilibrium. After centrifugation, the two phases were separated and the absorbances were measured at the appropriate wavelength.

*2.3.5. Characterization*

Infrared spectrum of the inclusion complex was obtained using a Nicole, Magna 550, serie II FTIR spectrometer according to potassium bromide disk method. The IR spectra of pure TRD, HP-βCD as well as their physical mixture of 1:1 molar ratio were also obtained by the same procedure for comparison. The scans were executed at a resolution of 8 $cm^{-1}$, from 4000 to 400 $cm^{-1}$.

$^{1}H$ NMR, $^{13}C$ NMR and 2D NMR spectra of the inclusion complex was obtained using a Bruker AVANCE II spectrometer NMR at 400 MHz using $D_2O$ as solvent, relaxation delay 2.0 s and mixing time equal 2.00 ms. The NMR spectra of pure TRD and HP-βCD were also achieved by the same procedure for comparison.

UV spectra of the inclusion complex, pure TRD and HP-βCD were obtained by applying UV/vis Cecil CE 8020 spectrophotometer. The scans were registered from 200 to 400 nm.

## 3. Results and discussion

*3.1. Absorption spectrum of trazodone–HP-βCD inclusion complex*

According to the procedure 2.3.1, the absorption spectra of TRD-HP-βCD complex, trazodone and HP-βCD were recorded, respectively. Some absorption spectra are given in Fig. 4. The obtained results showed that HP-βCD had no absorption in the range 225–400 nm. Trazodone was quite similar to trazodone–HP-βCD inclusion complex in absorption spectrum shape, but the absorbance of inclusion complex was higher than that of trazodone alone. Therefore, these facts were rationalized as being indicative of inclusion complex formation. On the absorption spectrum of TRD–HP-βCD inclusion complex appeared a peak and its absorption wavelengths was 245 nm. Obviously, it is fundamental that the spectrophotometric method is developed for determination of trazodone based on the measurement of changes in the absorbance at the $\lambda_{max}$ = 245 nm.

*3.2. Study of the formation and stability inclusion complex trazodone–HP-βCD*

Several preliminary studies were performed with the aim to assay the possible formation of inclusion complexes of trazodone with α-, β-, γ-, heptakis- (2,6-di-O-methyl)-β-CD and HP-βCD. The different cavities of the cyclodextrins enable them to discriminate among guest molecules. The influence of several cyclodextrins at different pH values on the absorption intensity of trazodone was studied. An important increase in the absorbance of trazodone is observed in the presence of HP-βCD.

The influence of the HP-βCD concentration on the absorption spectra of aqueous solutions $2 \times 10^{-4}$ mol $l^{-1}$ of trazodone was studied in the range $3 \times 10^{-4}$ to $4 \times 10^{-3}$ mol $l^{-1}$. The obtained results showed that the changes in the absorption intensity is significant in the complex. Upon inclusion in the HP-βCD cavity, generally the absorbance of the guest molecule is enhanced by shelding the excited species from non-radiative processes occurring in the bulk solution. It can be observed that in the complex, the intensity of absorbance increases when increasing the concentration of HP-βCD. Trazodone and its HP-βCD complex show quite similar absorption wavelength values. The absorption maximum of trazodone–HP-βCD complex shows a slight red shift of 2 nm as the concentration of HP-βCD is increased. Values of molar ratios HP-βCD/ analyte of 2–5 was selected for trazodone–HP-βCD for subsequently experiments, in order to guarantee the complexation of analyte.

The effect of pH on the inclusion of trazodone–HP-βCD has been tested, comparative tests at various pH values showed that absorbance of trazodone–HP-βCD changes with pH. The absorbances of inclusion complex were different under the pH tested. The obtained results showed that the absorbances of trazodone–HP-βCD inclusion complex were maximal at pH changing from 4.0 to 5.0. Therefore, pH of 5.0 buffer solution was used to control system pH. The obtained results are presented in Fig. 5.

The absorbance of TRD–HP-βCD was measured after standing for different times. The results showed that the trazodone reacts immediately with HP-βCD at room temperature. The absorbance was no longer changed after standing for 5–10 min. So, standing for 5 min was selected as the optimum. In addition, absorbance of the inclusion complex was stable at least 100 days at room temperature.

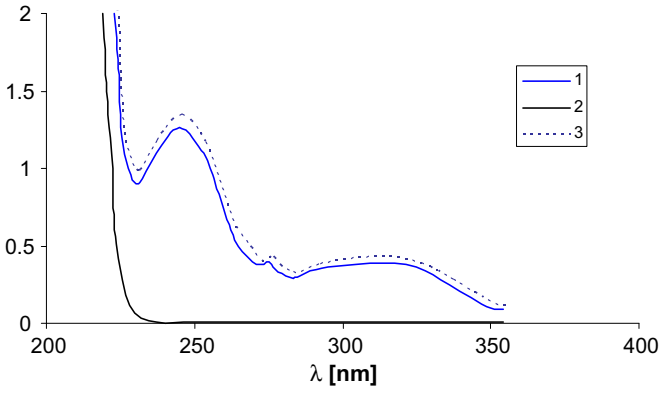


**Fig. 4.** UV spectra: TRD (1), HP-βCD (2), TRD/HP-βCD complex (3).

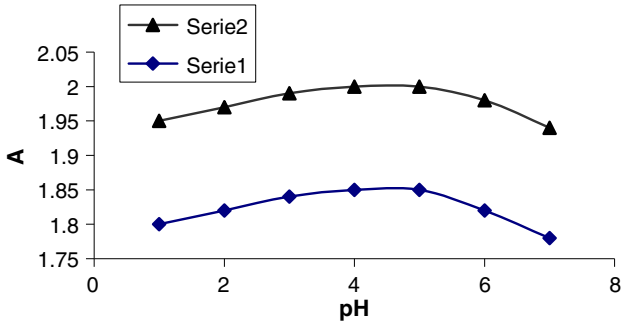


**Fig. 5.** Influence of pH on absorbance of TRD (1) and TRD/HP-βCD complex (2).

### 3.3. Stoichiometry of the complex and association constant

According to the continuous variation Job's method, when a physical parameter directly related to the concentration of the complex can be measured for a set of samples with continuously variable molar fraction of components. The maximum concentration of complex will be present in the sample where the molar ratio $R$ corresponds to the complexation stoichiometry. The maximum absorbance of TRD–HP-βCD was observed for $R = 0.5$, which indicates that the main stoichiometry is 1:1. This stoichiometry has been verified by Scatchard and Benesi–Hildebrand methods (Connors, 1987). The application of the Scatchard and Benesi–Hildebrand methods allows to graphically determine the stoichiometry of the system under study. Typical double-reciprocal plots for the studied complex is drawn. A linear relationship is obtained when $1/(A-A_0)$ is ploted against 1/HP-βCD ($R = 0.9992$ for trazodone–HP-βCD), indicating that the stoichiometry of the complex is 1:1.

Once the stoichiometry of the system is known, the association constant can also be calculated. In the case of the Scatchard method, the association constant is given by slope of the straight line $(A–A_0)/[HP\text{-}\beta CD]_0$ versus $(A–A_0)$ and the values obtained for trazodone–HP-βCD is 9605 l mol$^{-1}$ .

By the Benesi–Hildebrand's method, the association constant is determined by dividing the intercept by the slope of the straight line obtained in the double-reciprocal plot. The values $K_1$ obtained for trazodone – HP-βCD complex is similar.

An adequate estimation of formation constant can be made by using non-linear least-squares regression analysis (NRL). The formation constant calculated from the linear methods may be used, however, as a parameter estimate in the NLR method. Experimental data can be directly fitted according to the equation:

$$A = A_0 + \{(A_\infty - A_0)K_1[HP - \beta CD]_0/(1 = K_1[HP - \beta CD]_0)\}$$

The value of association constant for trazodone – HP-βCD inclusion complex obtained by NLR was of 9603 l mol$^{-1}$.

### 3.4. Measurement of octanol–water partition coefficient

The logarithm of the partition coefficient between $n$-octanol and water ($\log P$) is a leading physicochemical descriptor in many quantitative structures – activity relationship (QSAR) studies for modeling transports cross biological membranes, biochemical and pharmacological process and toxicity of organic compounds. Partition coefficients of TRD and its complex were determined by the shake–flask method (Leo, Hansch, & Elkins, 1971).

The octanol–water partition coefficient $P$ is defined as the ratio of a chemical's concentration in octanol phase to its concentration in the aqueous phase of a two-phase octanol–water. The logarithm $P$ is known as Hansch factor of sometimes lipophilicity. For this purpose, into a series of flasks, 2.5 ml of $10^{-4}$ mol/l aqueous solutions of each compound (guest and its complex) were, respectively, mixed with the same volume of octanol at room temperature. The system was vigorously shaken under sonication until equilibrium. After centrifugation, the two phases were separated and the absorbances were measured at appropriate wavelengths. The experimental results of partition coefficient measurements are following: $\log P$ for TRD (guest) is 0.87 and for TRD–HP-βCD (complex) $\log P$ is equal 0.48.

It can be shown that in the octanol–water solvent system, the hydrophilicity of trazodone is relatively low. As expected, this property was significantly modified with association of TRD–HP-βCD where the hydrophilicity was enhanced.

The relative hydrophilicity enhancement obtained via hydroxypropyl-β-cyclodextrin complexation can be expressed by the ΔlogP value defined as: $\Delta\log P = \log P(guest) – \log P(\text{complex})$.

According to the values of this quantity, it appears that during following the complexation of TRD, the hydrophilicity improved. On the basis of the results, it can be concluded that the hydrophilic properties and bioavailability of TRD could be improved by encapsulating it into HP-βCD.

### 3.5. Characterization of inclusion complex

#### 3.5.1. FT-IR analysis

The infrared (FTIR) spectra of wave number from 4000 to 400 cm$^{-1}$ of HP-βCD, TRD and the inclusion complex of TRD with HP-βCD were registered by FTIR spectrometer and the complete band assignments can be found in Table 1 and Fig. 6. In the spectrum of HP-βCD none of the bands in the range 500–1500 cm$^{-1}$ arise from a single type of molecular vibration due to strong coupling of vibrations from the macrocyclic, caused by neighbouring bonds vibrating with similar frequencies. The spectra of inclusion complex and that of HP-βCD are alike due to a combination of the following factors: (a) each HP-βCD molecule has a relatively large number of polar groups (O–H, C–O, etc.), giving rise to intense absorption bands; (b) both the host and guest molecules absorb coincidentally in most of the spectral regions; (c) there is an excess of free HP-βCD in the inclusion complex sample. However, the presence of TRD is undoubtedly confirmed by the bands at 1706, 1641, 1596 and 1541 cm$^{-1}$.

TRD showed a strong absorption band at 1705 cm$^{-1}$ for carbonyl stretching band due to five-membered condensed with benzene ring and the presence of $N$ atom. 2464 cm$^{-1}$ was noted for stretching vibration of =N– in tertiary amine group. The absorption peak in 1596, 1541 and 1492 cm$^{-1}$ split into triplet was corresponding to the C=C stretching vibration in the aromatic ring. The absorption band corresponding to the characteristic band of C–N stretching vibration in aromatic ring were observed at 1350, 1274 and 1230 cm$^{-1}$. Inclusion complex of TRD–HP-βCD did not show any new peaks, indicating no chemical bonds were created in the formed complex.

However, the spectrum of inclusion complex, whose band changed with the peak in 1099 cm$^{-1}$ disappeared, suggested that the band C–Cl of TRD was entrapped into the host cavities, during inclusion complexation. The band C–N–C stretching vibration in piperazine ring observed at 1260 and 1230 cm$^{-1}$ for the physical mixture, disappeared in the inclusion complex, indicating the restrict in infrared vibration after formation of inclusion complex. The C–H aromatic stretching vibration of pure TRD appears at 3071 cm$^{-1}$ and 3105 cm$^{-1}$ whereas it is disappeared in case of complex with HP-βCD.

These results indicated that the vibrating and bending of TRD molecule was restricted due to the formation of inclusion complex, the benzene ring and the substituent –Cl were inserted into the cavity of HP-βCD molecule.

#### 3.5.2. $^1$H NMR and $^{13}$C NMR spectroscopy

NMR (nuclear magnetic resonance) is the most effective method for studying space conformation of HP–βCD inclusion. One-dimentional $^1$H NMR spectra of TRD and TRD/HP-βCD complex were recorded with a 400 MHz Bruker AVANCE II spectrometer at 25 °C. Samples were suspended in $D_2O$ and degassed by bubbling $N_2$ directly in the NMR tubes. The chemical shifts (δ) are reported as ppm and are referenced to the residual water signal. $^1$H NMR and $^{13}$C NMR data of TRD and inclusion complex are shown in Table 2.

A structure of trazodone with the proton numbering used and a side view is presented in Fig. 2. The assignments and the chemical shift values of the various protons of trazodone are given in Table 2. A two-dimensional COSY($^1$H/$^1$H) spectrum of TRD and TRD/HP-βCD complex in $D_2O$ in the range 6.6–7.9 ppm and 2D MNR (HSQC, $^{13}$C: 20–130 ppm/$^1$H: 1.0–7.5 ppm) spectrum of TRD and inclusion complex were registered.

**Table 1**
Wavenumbers ($cm^{-1}$) and assignments for the bands observed in the FTIR spectra of trazodone and HP-βCD.

| Infrared bands ($cm^{-1}$) and assignments | | |
|---|---|---|
| Trazodone | HP-βCD | Inclusion complex |
| 3105 and 3071: ν (C–H) from aromatic ring | 3355: ν (O–H) | 3355: ν (O–H) |
| 2982, 2944 and 2854: ν (C–H) from $CH_2$ | 2927: ν (C–H) | 2936: ν (C–H) |
| 2464: ν (=N–) | 1459: δ (C–H) from $CH_2$ and $CH_3$ | 2535 and 2464: ν (=N–) |
| 1705: ν (C=O) | 1377: δ (C–H) from $CH_3$ | 1705: ν (C=O) |
| 1596, 1541 and 1492:ν(C=C) | 1334: coupled δ (C–C–H), δ (C–O–H),δ (H–C–H) | 1596: ν (C=C) from aromatic ring |
| From aromatic ring | 1261: coupled δ (O–C–H), δ (C–O–H), δ (C–C–H) | 1334: coupled δ (C–C–H), δ (C–O–H),δ (H–C–H) |
| 1351, 1274, 1260 and 1230: ν (C–N) | 1155 and 1081: ν (C–O), ν (C–C), δ (C–O–C), | 1156 and 1083: ν (C–O), ν (C–C), δ (C–O–C) |
| From aromatic ring | 1031: δ (O–C–H),δ (C–C–H), δ (C–C–O) | 1031: δ (O–C–H),δ (C–C–H), δ (C–C–O) |
| 1099: ν (C-Cl) | 947: skeletal vibration involving α-1,4 linkage | 944: skeletal vibration involving α-1,4 linkage |
| 884,778,764 and 744: δ (C–H) | 855: δ (C–C–H), ν (C–O), ν (C–C) | 744: δ (C–H) from aromatic ring |
| From aromatic ring | From anomeric vibration | |

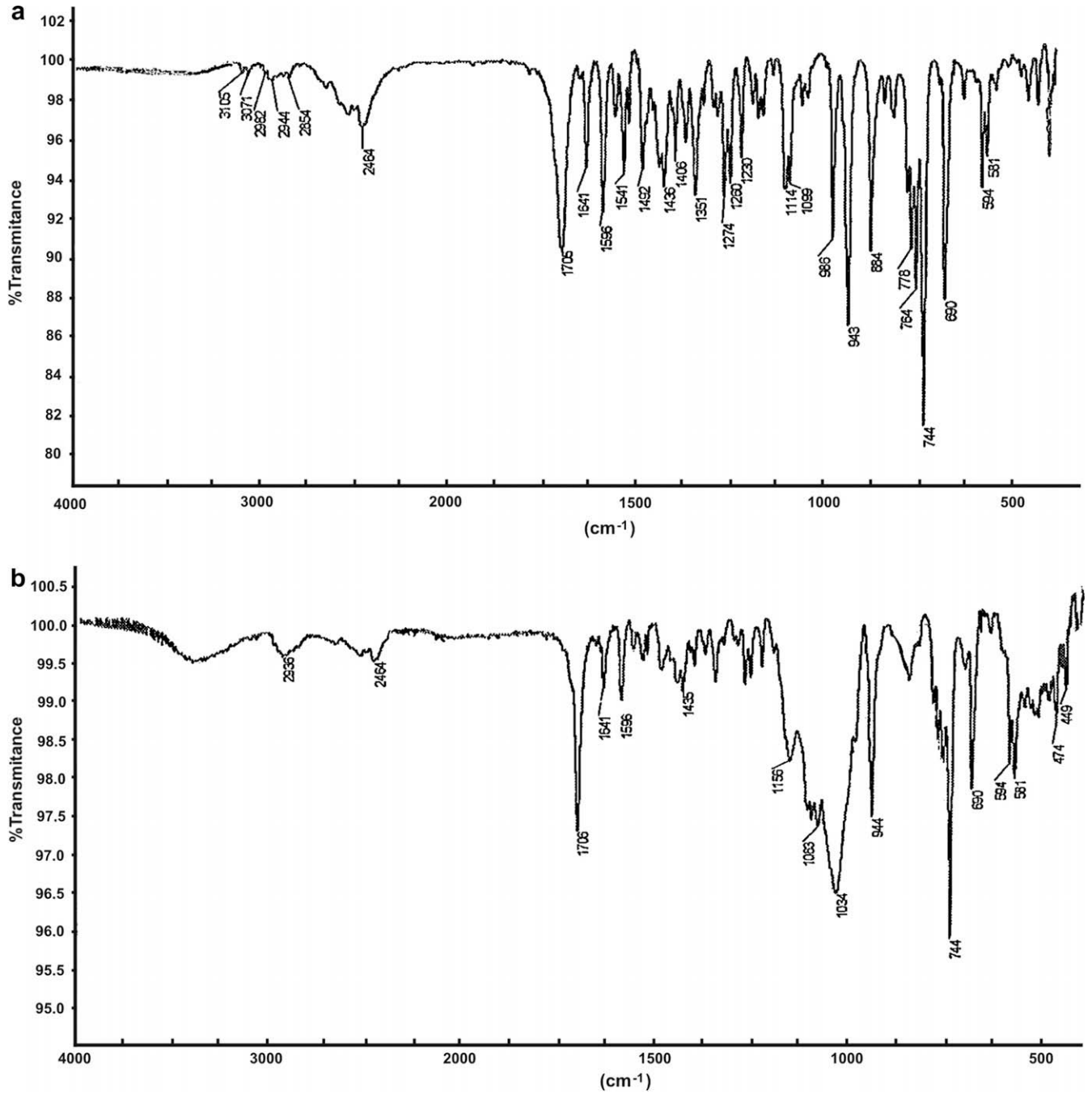


**Fig. 6.** FT-IR spectra: TRD (a), TRD/HP-βCD complex (b).

**Table 2**
Changed $^{1}$H NMR and $^{13}$C NMR chemical shifts of various protons and carbons of trazodone before and after forming inclusion complex from NMR spectra.

| No. H/C | TRD | | Inclusion complexTRD/HP-β-CD (1:1) | | Δδ | |
|---|---|---|---|---|---|---|
| | $^{1}$H NMR | $^{13}$C NMR | $^{1}$H NMR | $^{13}$C NMR | $^{1}$H NMR | $^{13}$C NMR |
| 1 | 7.73 | 123.15 | 7.72 | 123.16 | −0.01 | 0.01 |
| 2 | 6.72 | 112.53 | 6.67 | 112.51 | −0.05 | −0.02 |
| 3 | 7.30 | 132.26 | 7.26 | 132.24 | −0.04 | −0.02 |
| 4 | 7.16 | 114.19 | 7.13 | 114.20 | −0.03 | 0.01 |
| 5 | – | 142.90 | – | 142.90 | – | – |
| 6 | – | 149.02 | – | 149.04 | – | 0.02 |
| 7 | 4.09 | 42.90 | 4.06 | 42.99 | −0.03 | −0.01 |
| 8 | 2.27 | 23.02 | 2.23 | 23.03 | −0.04 | −0.01 |
| 9 | 3.21 | 53.95 | 3.18 | 53.96 | −0.03 | 0.01 |
| 10 | 3.37 | 51.46 | 3.36 | 51.49 | −0.01 | 0.03 |
| 11 | 3.39 | 46.35 | 3.38 | 46.50 | −0.01 | 0.15 |
| 12 | – | 150.24 | – | 150.50 | – | 0.26 |
| 13 | 6.98 | 116.79 | 6.94 | 116.83 | −0.04 | 0.04 |
| 14 | – | 134.54 | – | 134.53 | – | −0.01 |
| 15 | 6.91 | 121.37 | 6.88 | 121.26 | −0.03 | −0.11 |
| 16 | 7.22 | 130.68 | 7.20 | 130.69 | −0.02 | 0.01 |
| 17 | 6.89 | 115.31 | 6.86 | 115.23 | −0.03 | −0.08 |

Based on the data it was observed that the formation of the inclusion complex of trazodone with HP-βCD was indicated by upfield shifts observed for all the resonances of the ring protons of trazodone. Additionally, if the integrals of the proton resonances of the trazodone were compared to those for the cyclodextrin studied, it was evident that in solution, the cyclodextrin to trazodone was at least 10 to 2 higher, indicating fairly complexation formation.

2D NMR spectra have become an important tool to prove complex formation. With the shift of the signal of TRD we can infer the inclusion complex formation. Sometimes the use of derivatized cyclodextrins such as HP-βCD brings complications to the interpretation of 2D NMR spectra and in some works a model complex with native βCD is prepared for characterization. In such case an analogues inclusion mode is assumed on the basis of size similarity between cavities (6 Å).

Based on the NMR data a model for the inclusion of TRD into HP-βCD cavity can be suggested and the proposed stoichiometry of the 1:1 TRD–HP-βCD inclusion complex was confirmed. Using two-dimensional NMR experiment, it was possible to infer the complex formation and that the chloro–phenyl ring of trazodone was situated into the torus cavity of the hydroxypropyl-β-cyclodextrin proving complexation, evidencing a relatively good penetration.

### 3.6. Analytical parameters

The spectrophotometric determination of trazodone, in both, the absence and the presence of HP-βCD, involves the construction of the corresponding calibration curves. The analytical and statistical parameters of the determinations procedures are summarized in Table 3. The calibration curve of trazodone is shown in Fig. 7. The linear relationship between the absorbance and trazodone concentration was obtained in the range of 5–30 μg ml$^{-1}$ in the presence HP-βCD with a correlation coefficient of 0.9998. The detection limit was 0.27 μg ml$^{-1}$. As can be appreciated, the calibration sensitivity in presence of HP-βCD, significantly improves with respect to those without HP-βCD, while the limit of detection is quite similar in presence or absence of HP-βCD. The repeatability of the methods have been analyzed by measuring 10 identical solutions of each analyte and of each complex in similar conditions as employed in the concentration of calibration curves. The relative error percentage upon the average of concentration found of each analyte is inferior to 2% in all cases. The proposed method with HP-βCD is characterized by high accuracy and good sensitivity and low limit of detection.

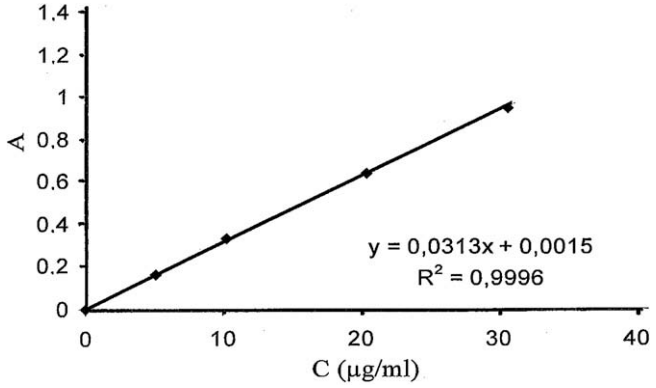


**Fig. 7.** The calibration absorption curve of trazodone.

**Table 3**
Analytical and statistical parameters for the determination of trazodone, as free analyte and of its complex with HP-βCD, $\lambda_{max}$ = 245 nm.

| | Trazodone | |
|---|---|---|
| | Water solution | HP-βCD |
| Linear range (μg mL$^{-1}$) | 5–30 | 5–30 |
| ε (L mol$^{-1}$ cm$^{-1}$) | $8.2 \times 10^{3}$ | $1.28 \times 10^{4}$ |
| Slope | 0.0238 | 0.0313 |
| Intercept | 0.0124 | 0.0015 |
| Correlation coefficient ($R$) | 0.9991 | 0.9998 |
| R.S.D.[a] (%) | 0.78 | 0.62 |
| LOD (μg mL$^{-1}$) | 0.31 | 0.27 |
| LOQ (μg mL$^{-1}$) | 1.58 | 1.55 |

[a] Relative standard deviations for 10 individual replicates, $n$ = 10.

## 4. Conclusion

Trazodone was encapsulated by HP-βCD, forming an inclusion complex and the ratio of 1:1 the complex was valued by the continuous variation Job's method. The results showed that the inclusion process was occurred. Its structure was characterized by FT-IR, $^{1}$H NMR, $^{13}$C NMR and 2D NMR (COSY and HSQC), which all verified the inclusion complex formation between HP-βCD and trazodone. The obtained data suggests that a part of trazodone molecule, a

benzene ring with – Cl substituent is included inside HP-βCD cavity.

Based on the enhancement of the absorbance of trazodone produced through complex formation, a sensitive method for the determination of TRD in bulk solution in presence of HP-βCD was developed. The principal advantage of the method is lower detection limit, high accuracy and good sensitivity.

## References


AHFS Drug Information, America Society of Health – System pharmacists, Inc. (1998). Bethesta, MD, US.

Atwood, J. L., Davies, J. E. D., Macnicol, D. D., & Vogtle, F. (1996). In Comprehensive Supramolecular Chemistry. Vol. 3. UK: Pergamon Press.

Carda-Broch, S., Gil, M. T., Monferrer-Pons, L., & Esteve-Romero, J. S. (2007). Determination of trazodone in urine and pharmaceuticals using micellar liquid chromatography with fluorescence detection. *Journal of Chromatography A, 1156*, 254–258.

Connors, K. A. (1987). Binding constants: A measurement of molecular complex stability. New York: John Wiley and Sons.

El-Gindy, A., El-Zeany, B., Awad, T., & Shabana, M. M. (2001). Spectrophotometric, spectrofluorimetric and LC determination of trazodone hydrochloride. *Journal of Pharmaceutical and Biomedical Analysis, 26*, 211–217.

Fromming, K. H., & Szejtli, J. (1996). Pharmacokinetics and toxicology of cyclodextrins. In Szejtli., Szente, L. (Eds.), Proc. Of the 8th Int. Symposium on cyclodextrins(pp. 33–45). (Chapter 3).

Goeringer, K. E., Raymon, L., & Logan, B. K. (2000). Postmortem forensic toxicology of trazodone. *Journal of Forensic Sciences, 45*, 850–856.

Gould, S., & Scott, R. C. (2005). 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD): A toxicology review. *Food and Chemical Toxicology, 43*, 1451–1459.

Granero, G. E., Maitre, M. M., Garnero, C., & Longhi, M. R. (2008). Synthesis, characterization and in vitro release studies of a new acetazolamine–HP-β-CD-TEA inclusion complex. *European Journal of Medicinal Chemistry, 43*, 464–470.

Hazekamp, A., & Verpoorte, R. (2006). Structure elucidation of the tetrahydrocannabinol complex with randomly methylated β-cyclodextrin. *European Journal of Pharmaceutical Sciences, 29*, 340–347.

Jullian, C., Miranda, S., Zapata-Torres, G., Mendizabal, F., & Olea-Azar, C. (2007). Studies of inclusion complexes of natural and modified cyclodextrin with (+) catechin by NMR and molecular modeling. *Bioorganic & Medicinal Chemistry, 15*, 3217–3224.

Khan, A. R., Forgo, P., Stine, K. J., & D'Souza, V. T. (1998). Methods for selective modifications of cyclodextrins. *Chemical Reviews, 98*, 1977–1996.

Leo, A., Hansch, C., & Elkins, D. (1971). Partition coefficients and their uses. *Chemical Reviews, 71*, 525–616.

Liu, L. X., & Zhu, S. Y. (2006). Preparation and characterization of inclusion complexes of prazosin hydrochloride with β-cyclodextrin and hydroxypropyl-β-cyclodextrin. *Journal of Pharmaceutical and Biomedical Analysis, 40*, 122–127.

Job, P. (1928). *Ann. Chim., 9*, 113–203.

Rajabi, O., Tayyari, F., Salari, R., & Tayyari, S. F. (2008). Study of interaction of spironolactone with hydroxypropyl-β-cyclodextrin in aqueous solution and in solid state. *Journal of Molecular Structure, 878*, 78–83.

Sagiraju, S., & Jursic, B. S. (2008). NMR spectroscopic study of cyclodextrin inclusion complexes with A–007 prodrugs. *Carbohydrate Research, 343*, 1180–1190.

Spamer, E., Muller, D. G., Wessels, P. L., & Venter, J. P. (2002). Characterization of the complexes of fluorosemide with 2-hydroxypropyl-β-cyclodextrin and sulfobutyl ether-7-β-cyclodextrin. *European Journal of Pharmaceutical Sciences, 16*, 247–253.

Szejtli, J. (1998). Introduction and general overview of cyclodextrin chemistry. *Chemical Reviews, 98*, 1743–1753.

Wang, H. Y., Han, J., & Feng, X. G. (2007). Spectroscopic study of orange G – β-cyclodextrin complex and its analytical application. *Spectrochimica Acta, 66*, 578–585.

Yong, G. L., & Zhu, J. F. (2006). The preparation of inclusion complex of ipriflavone and β-cyclodextrin. *Chinese Additional Machine Leader, 8*, 817–818.

Zhu, X., Sun, J., & Wu, J. (2007). Study on the inclusion interactions of β-cyclodextrin and its derivative with dyes by spectrofluorometry and its analytical application. *Talanta, 72*, 237–242.