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Thermochimica Acta 459 (2007) 111–115
thermochimica acta
www.elsevier.com/locate/tca

Binding constants of inclusion complexes of nitroimidazoles with β-cyclodextrins in the absence and presence of PVP

Renu Chadha [a,*], D.V.S. Jain [b], Amit Aggarwal [a], Surjit Singh [c], Deepika Thakur [a]

[a] *University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160014, India*
[b] *Depatment of Chemistry, Panjab University, Chandigarh 160014, India*
[c] *GND University, Amritsar, Panjab, India*

Received 30 January 2007; received in revised form 13 April 2007; accepted 19 April 2007
Available online 25 April 2007

Abstract

Thermodynamics of complexation of 5-nitroimidazoles with β-cyclodextrin and its methylated and hydroxypropyl derivatives in water and in 0.25% polyvinylpyrrolidone are determined by solution calorimetry. A 1:1 stoichiometry was established. The equilibrium constant (K) for all the nitroimidazoles fall in the range 1000–1900 M^{-1} suitable for use of cyclodextrins as drug carriers. The complexation ability is significantly enhanced by methylation of the β-cyclodextrin. The stability constant increased in the order metronidazole < ornidazole < tinidazole < secnidazole. The presence of polyvinylpyrrolidone enhances the stability constants.

Keywords: Cyclodextrin; Calorimetry; Nitroimidazole; Thermodynamic parameters; Polyvinylpyrrolidone

1. Introduction

The complexation of different drugs with cyclodextrins (CDs) has been extensively studied in recent years [1–5]. But for a variety of reasons including cost, production capabilities and toxicology, the amount of cyclodextrin that can be incorporated into drug formulations is limited. Even under ideal conditions, cyclodextrin complexation results in a 4–10-fold increase in the formulation bulk which limits their use in solid oral dosage forms. Recently, attention has been focused on the use of water-soluble polymers to enhance the efficiency of drug cyclodextrin complexation [6–11]. This solubilization enhancement may be due to increase in the apparent stability constant (K_c) of the drug–CD complex [8,12–15].

Calorimetry is most suited for the determination of thermodynamic parameters for drug–cyclodextrin complexation [16–19]. Nitroimidazoles are well-established efficient agents against anaerobic bacteria (Bacteroides species) and protozoans, such as *Trichomonas*, *Entamoeba* species and *Giardia*. But these therapeutic agents possess low intrinsic solubility. Several ester and hemi ester prodrugs of these agents have been prepared in an effort to enhance their water solubility [20]. One of the other promising approaches in this respect is to encapsulate the drug in the hydrophobic cavity of cyclodextrin. This study is undertaken to determine the binding constants of inclusion complexes of these agents with β-cyclodextrins (β-CDs) in the absence and presence of polyvinylpyrrolidone (PVP).

2. Experimental

Nitroimidazoles (M/s AARTI Drugs Ltd., Mumbai, India) were gift samples. All the samples were stored as received in air-tight plastic containers inside desiccators. These were used without further purification. All the drugs were sieved and fractions with particle size less than 150 μm were used throughout the study. β-Cyclodextrin (β-CD), hydroxypropyl β-cyclodextrin (HPβ-CD), methyl β-cyclodextrin (M β-CD) and polyvinylpyrrolidone (M_W = 40,000) were obtained from E. Merck (Germany), Fluka (Switzerland), Aldrich and Hi media, respectively. Phosphate buffer of pH 3.0 was prepared according to given procedure [21]. The solutions were freshly prepared with triple-distilled water and pH values were measured with a pH meter (Elico, India) calibrated with standard buffers of pH 4.0, 7.0 and 9.2.

* Corresponding author. Tel.: +91 172 2534104; fax: +91 172 2541142.
E-mail address: renukchadha2004@yahoo.co.in (R. Chadha).

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doi:10.1016/j.tca.2007.04.016

2.1. Calorimetric studies

Heat flux calorimeter model C-80 (SETARAM, France) was used for thermal measurements. The performance of the calorimeter was tested by measuring the enthalpy of a solution of potassium chloride (17.301 kJ/mol) in triple-distilled water, which has a known enthalpy of solution of 17.322 kJ/mol. The precision of any individual measurement was better than ±0.02 kJ/mol for three consecutive experiments.

Complexation thermodynamics of the nitroimidazoles with β-CDs and its derivatives were determined by measuring the enthalpy of solution of the drugs in pure buffer and in aqueous solutions of cyclodextrins at pH 3 in the absence and presence of 0.25% PVP. The solutions of β-CD, HPβ-CD and methyl β-CD were prepared over a range of concentrations (0.705–7.048, 0.508–6.540 and 0.458–7.633 mM, respectively) in buffer solution of pH 3. The sample cell contained a weighed amount of drug and 5 ml of desired solution partitioned by a displaceable lid. After stabilization, the calorimetric block containing the vessels was rotated by 180° several times to displace the lid between the drug and solution, leading to their mixing [22].

The enthalpy of interaction between drug and CD was calculated from the following equation:

$$\Delta_{\mathrm{sol}} H_{\mathrm{int}} = \Delta_{\mathrm{sol}} H(\mathrm{CD}) - \Delta_{\mathrm{sol}} H \tag{1}$$

where $\Delta_{\mathrm{sol}}H(\mathrm{CD})$ is molar enthalpy of solution of drug in buffered aqueous solution of CDs and $\Delta_{\mathrm{sol}}H$ is molar enthalpy of solution of drug in buffer. To determine the stoichiometry, stability constant K and other thermodynamic parameters, the interaction enthalpy per litre ($\Delta H_{\mathrm{int}}(\mathrm{l})$) was calculated from the equation:

$$\Delta_{\mathrm{sol}} H_{\mathrm{int}}(\mathrm{l}) = \Delta_{\mathrm{sol}} H_{\mathrm{int}}[a] \tag{2}$$

where a is the molar concentration of nitroimidazole. The stoichiometry of the reaction between the drugs and CDs was established by calculating the enthalpy of interaction per mole ($\Delta_{\mathrm{sol}}H_{\mathrm{int}}(\mathrm{m})$) of the drug and CD.

$$\Delta_{\mathrm{sol}} H_{\mathrm{int}}(\mathrm{m}) = \frac{\Delta_{\mathrm{sol}} H_{\mathrm{int}}(\mathrm{l})}{[a] + [b]} \tag{3}$$

where a and b are the molarity of the drug and CD, respectively.

Plots of $\Delta_{\mathrm{sol}}H_{\mathrm{int}}(\mathrm{m})$ against the molar ratio of CD to CD + drug for all the nitroimidazoles with the β-cyclodextrin, methyl β-cyclodextrin and hydroxypropyl β-cyclodextrin have a minimum at 0.5, indicating 1:1 stoichiometry of the complex. The enthalpy of interaction [$\Delta_{\mathrm{sol}}H_{\mathrm{int}}(\mathrm{l})$] is proportional to the product of molarity (M) of drug–CD complex formed and the enthalpy of binding ΔH°:

$$\Delta_{\mathrm{sol}} H_{\mathrm{int}}(\mathrm{l}) = \Delta H^{\circ} M \tag{4}$$

At equilibrium between nitroimidazole (N), cyclodextrin (CD) and their complex CD:N and assuming their activities are equal to their respective molarities we can write:

$$K = \frac{[\mathrm{CD:N}]}{[\mathrm{CD}][\mathrm{N}]} = \frac{M}{(b - M)(a - M)} \tag{5}$$

By solving the above equation, we get

$$M = \frac{(a + b + 1/K) - \sqrt{\{(a + b + 1/K)^2 - 4ab\}}}{2} \tag{6}$$

The enthalpy of interaction per litre $\Delta H_{\mathrm{int}}(\mathrm{l})$ is then given by

$$\Delta H_{\mathrm{int}}(\mathrm{l}) = \Delta H^{\circ} M = \frac{\Delta H^{\circ}[(a + b + 1/K) - \sqrt{\{(a+b+1/K)^2 - 4ab\}}]}{2} \tag{7}$$

The thermodynamic parameters (ΔH° and K) were computed by iterative non-linear least square regression program prepared in our laboratory.

2.2. Solubility studies

Excess amount of the drug (1 g) was weighed into 25-ml glass flask with 10 ml of CD solution. The resulting suspension was allowed to equilibrate in water bath shaker at 37 °C for 24 h. The suspension was then filtered through a 0.45 μm membrane filter (Millipore) and the filtrate analyzed spectrophotometrically at λ_{max} of the drug. Three replicates were made for each experiment and the results are presented as the mean values (±0.015 mM).

2.3. Proton NMR studies

Bruker WM 300 was used for studying the proton NMR spectra of the inclusion complexes in deuterated dimethyl sulfoxide solution.

3. Results

3.1. NMR studies of drug–cyclodextrin complex

Evidence of a drug:CD complex in solution was based on the modification of the proton NMR spectrum of the drug molecule [23,24]. The spectrum had no new peaks but chemical shifts occurred. The effect of inclusion of the drug in the CD cavity on the chemical shift was observed by comparing the spectrum of the pure drugs and their inclusion complexes in deuterated dimethyl sulfoxide. Changes in the chemical shifts of various protons suggest interaction between the host and the guest. The chemical structures of the drugs suggest that the imidazole ring is the potential group to penetrate the cyclodextrin cavity while the nitro group and the side chain (R) bearing the hydroxyl group will partly remain outside the cyclodextrin cavity. Length and width of all the nitroimidazoles is less than cavity dimensions so that the whole molecule and a part R group can penetrate into the cavity suggesting 1:1 stoichiometry as shown in Fig. 1. The proposed structure is supported by a small downfield shift observed for H-2 and H-4 protons of the imidazole ring. This indicates that both the protons lie close to the oxygen atom of the CD cavity. Large downfield shifts were observed in protons of side chain on the imidazole ring in all the drug molecules, which lie outside the CD cavity. This may be attributed to the

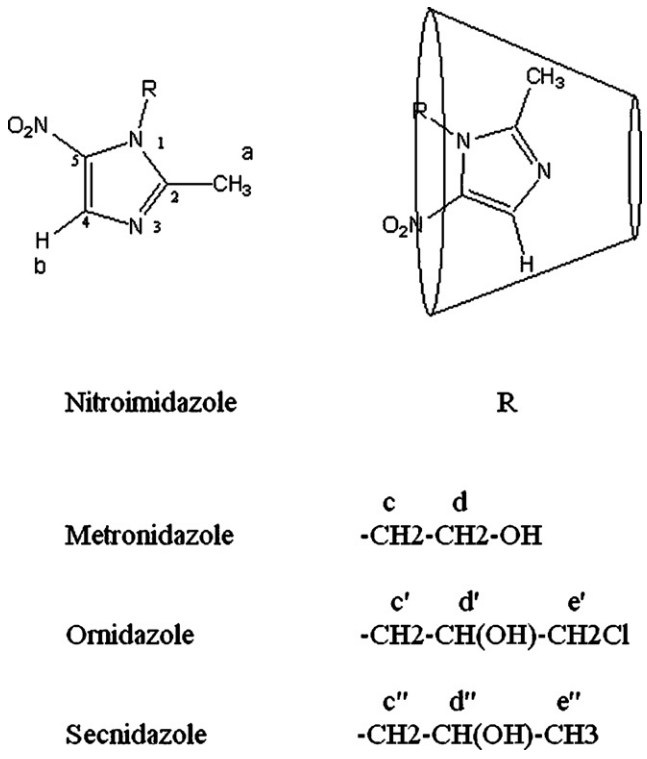

Fig. 1. Structural formula of nitroimidazoles and proposed structure of cyclodextrin complex.

interaction of methyl, methylene and methine protons of the drug with the oxygen atom of the CD outside the cavity. This trend was also found for hydroxypropyl and methyl CD. However no correlation is observed between the magnitude of the chemical shift and the type of β-CD.

3.2. Calorimetric studies of drug–cyclodextrin complex

The enthalpies of solution of all the drugs in pure buffer (pH 3) are given in Table 1. These are independent of the concentration of the drugs. The enthalpy of solution of all nitroimidazoles in β-CD and its methyl and hydroxypropyl derivatives were determined. The enthalpy of solution ($\Delta_{sol}H$(CD)) of all the nitroimidazoles in CDs is less endothermic in magnitude than molar enthalpy of solution ($\Delta_{sol}H$) of pure drug in buffer solution without any CD.

Equilibrium constants and molar enthalpies of binding for all the nitroimidazoles based on 1:1 binding model as described in Section 2 are given in Table 2. The free energy (ΔG°) and entropy of inclusion (ΔS°) are also given in Table 2.

The solubility of nitroimidazoles in presence of 5% cyclodextrins containing 0.25% PVP was also determined (Table 3). Solubility in all the cases increases from 3 to 15% due to increased value of K.

4. Discussion

The values of K for all the nitroimidazoles fall in the range 1000–1900 M^{-1}, optimum values for the use of CDs as drug carriers [25]. The association constant K increases in the order HPβ-CD < β-CD < Mβ-CD. The complexation ability is significantly enhanced by methylation, which enlarges the cavity of the CD, makes the environment around it hydrophobic and allows for increased adaptability of the CD towards a guest, through enhanced flexibility. However, hydroxyl groups in case of HPβ-CD make the extended part of the cyclodextrin cavity partially hydrophilic. Thus all the nitroimidazoles bind to HPβ-CD less strongly than to β-CD [26,27]. This is also accompanied by less exothermic value of ΔH°. However, polarity of hydroxyl groups on the substituents makes the solvation changes more significant. Thus the complexation reactions between HPβ-CD and all the nitroimidazoles are accompanied by more positive entropy change and are more entropically driven [28]. The enthalpic gain is obtained predominantly through van der Waal's interaction of the methyl group introduced in the dimethyl β-CD. Thus increase in complex stability in Mβ-CD is predominantly enthalpic in origin. The more exothermic value of ΔH° in case of Mβ-CD is more or less compensated by lower entropy of reaction [19].

The stability constants increase in the order metronidazole, ornidazole, tinidazole, secnidazole. This may be explained in terms of deprotonation at this pH value of the cationic form of the nitroimidazoles. The inclusion complex formation is proportional to the hydrophobic character of the guest molecule. The association constant is due to the contribution of both ionic and nonionic species. At pH 3.0 metronidazole (pK_a 2.5) is mostly present in the protonated form whereas secnidazole is present in unprotonated form (pK_a 1.16). The fraction of the protonated species of the drugs is in the order metronidazole > ornidazole > tinidazole > secnidazole.

All the nitroimidazoles bind to the cyclodextrins with a favorable enthalpic term ($\Delta H^{\circ} < 0$) and even larger contribution from entropic term ($\Delta S^{\circ} > 0$). Negative enthalpy changes are due to van der Waals interactions arising from the precise matching in size and shape between host and guest molecules. The entropic gain arising from desolvation of the CD cavity upon inclusion of the guest is quite significant and is due to release of water molecules from the cavity and the induced dehydration of peripheral hydroxyl group of CD [29]. Therefore, a combination of hydrophobic effect ($\Delta S^{\circ} > 0$), van der Waals forces

Table 1
Enthalpy of solution of 5-nitroimidazoles at pH 3

Metronidazole		Tinidazole		Ornidazole		Secnidazole	
mM	ΔH_{sol} (kJ/mol)	mM	ΔH_{sol} (kJ/mol)	mM	ΔH_{sol} (kJ/mol)	mM	ΔH_{sol} (kJ/mol)
9.35	34.0 ± 0.02	4.05	45.42 ± 0.02	4.56	33.56 ± 0.02	8.65	34.74 ± 0.02
5.85	34.0 ± 0.02	6.48	45.40 ± 0.02	7.29	33.53 ± 0.02	10.81	34.75 ± 0.02

Table 2
Thermodynamic parameters of inclusion complexes of nitroimidazoles and cyclodextrins in absence and presence of 0.25%PVP

System	K (M^{-1})	ΔH° (kJ/mol)	ΔG° (kJ/mol)	ΔS° (J/mol)
MET + β-CD	1159 ± 02	−6.56 ± 0.02	−18.2 ± 0.01	37.5 ± 0.07
MET + β-CD + PVP	1423 ± 46	−7.96 ± 0.03	−18.7 ± 0.08	34.7 ± 0.26
MET + HPβ-CD	1030 ± 25	−6.17 ± 0.02	−17.9 ± 0.07	37.8 ± 0.23
MET + HPβ-CD + PVP	1318 ± 42	−7.09 ± 0.03	−18.5 ± 0.08	36.9 ± 0.28
MET + Mβ-CD	1481 ± 59	−8.20 ± 0.03	−18.8 ± 0.10	34.2 ± 0.11
MET + Mβ-CD + PVP	1930 ± 115	−9.194 ± 0.04	−19.5 ± 0.15	30.9 ± 0.47
TIN + β-CD	1448 ± 71	−7.96 ± 0.02	−18.8 ± 0.13	34.9 ± 0.46
TIN + β-CD + PVP	1655 ± 39	−8.25 ± 0.03	−19.1 ± 0.06	35.0 ± 0.22
TIN + HPβ-CD	1314 ± 36	−7.21 ± 0.02	−18.5 ± 0.07	36.5 ± 0.24
TIN + HPβ-CD + PVP	1599 ± 75	−7.95 ± 0.04	−19.0 ± 0.12	35.7 ± 0.41
TIN + Mβ-CD	1767 ± 70	−9.37 ± 0.03	−19.3 ± 0.13	31.9 ± 0.35
TIN + Mβ-CD + PVP	1948 ± 50	−10.15 ± 0.03	−19.5 ± 0.07	30.3 ± 0.21
ORN + β-CD	1398 ± 48	−7.60 ± 0.02	−18.7 ± 0.09	35.7 ± 0.29
ORN + β-CD + PVP	1569 ± 59	−7.99 ± 0.07	−18.9 ± 0.10	35.4 ± 0.39
ORN + HPβ-CD	1253 ± 31	−6.91 ± 0.02	−18.4 ± 0.06	37.0 ± 0.22
ORN + HPβ-CD + PVP	1481 ± 195	−7.53 ± 0.09	−18.8 ± 0.35	36.4 ± 0.04
ORN + Mβ-CD	1653 ± 98	−9.18 ± 0.04	−19.1 ± 0.15	32.0 ± 0.51
ORN + Mβ-CD + PVP	1947 ± 77	−9.98 ± 0.05	−19.5 ± 0.10	30.8 ± 0.36
SEC + β-CD	1509 ± 66	−8.30 ± 0.03	−18.9 ± 0.11	34.1 ± 0.37
SEC + β-CD + PVP	1812 ± 55	−9.13 ± 0.02	−19.4 ± 0.08	32.9 ± 0.26
SEC + HPβ-CD	1408 ± 54	−8.02 ± 0.03	−18.7 ± 0.09	34.4 ± 0.33
SEC + HPβ-CD + PVP	1628 ± 72	−8.23 ± 0.04	−19.1 ± 0.11	34.9 ± 0.38
SEC + Mβ-CD	1841 ± 72	−9.95 ± 0.03	−19.4 ± 0.10	30.4 ± 0.34
SEC + Mβ-CD + PVP	2339 ± 130	−11.33 ± 0.05	−20.0 ± 0.14	27.8 ± 0.44

Table 3
Solubility of 5-nitroimidazoles in 5% cyclodextrin solutions in presence and absence of 0.25% PVP at pH 3.0

System	In absence of PVP		In presence of 0.25% PVP	
	Solubility (mg/ml)	Molar solubility (mM)	Solubility (mg/ml)	Molar solubility (mM)
MET + β-CD	11.25 ± 0.15	65.8 ± 0.9	12.44 ± 0.16	72.7 ± 0.9
MET + HPβ-CD	15.03 ± 0.20	87.9 ± 1.2	16.04 ± 0.21	93.8 ± 1.2
MET + Mβ-CD	17.43 ± 0.22	101.9 ± 1.3	17.94 ± 0.23	104.9 ± 1.3
TIN + β-CD	8.54 ± 0.11	34.6 ± 0.4	9.05 ± 0.12	36.6 ± 0.5
TIN + HPβ-CD	9.55 ± 0.12	38.7 ± 0.5	10.68 ± 0.13	43.2 ± 0.6
TIN + Mβ-CD	9.99 ± 0.13	40.4 ± 0.5	11.25 ± 0.14	45.5 ± 0.6
ORN + β-CD	18.95 ± 0.24	86.3 ± 1.1	20.05 ± 0.26	91.3 ± 1.2
ORN + HPβ-CD	19.68 ± 0.25	89.7 ± 1.1	21.49 ± 0.28	97.9 ± 1.3
ORN + Mβ-CD	21.24 ± 0.27	96.8 ± 1.2	24.03 ± 0.31	109.5 ± 1.4
SEC + β-CD	43.55 ± 0.55	235.4 ± 2.9	44.68 ± 0.57	241.5 ± 3.1
SEC + HPβ-CD	49.44 ± 0.63	267.2 ± 3.4	52.04 ± 0.66	281.3 ± 3.6
SEC + Mβ-CD	50.24 ± 0.64	271.6 ± 3.5	54.27 ± 0.69	293.4 ± 3.4

($\Delta H^\circ < 0$), and the solvent reorganization could account for such a thermodynamic pattern [15]. A correlation between ΔH° and ΔS° is nearly linear showing enthalpy–entropy compensation in the complexes of all the nitroimidazoles with CDs.

The pharmaceutical dosage form should contain as little cyclodextrin as possible. The addition of a third component such as alcohol, amino acids or water-soluble polymers enhance the efficiency of drug–cyclodextrin complexation. This solubilization enhancement is synergistic. The molar enthalpy of solution of drug in CD and PVP mixture is less endothermic than in the solution of CD alone. PVP does not change the stoichiometry of the complex formed.

The magnitude of stability constant increases in the presence of PVP. Higher negative ΔH° values as compared to those in the absence of PVP is ascribed to stronger binding between the host and the guest as a consequence of ternary complexation. The complexation is also accompanied by a positive entropy change. However, the magnitude is less than in the absence of PVP. This suggests that the ternary complex is more ordered than the binary complex. It is suggested that PVP coats the

drug–CD inclusion complex and interacts with cyclodextrin by means of multiple intermolecular hydrogen bonding resulting in higher exothermic enthalpy of binding and more ordered ternary complex. The geometry of the drug–CD complex does not change in presence of PVP as only the imidazole ring of the nitroimidazoles enters the CD cavity and the side chain remains outside it.

Acknowledgement

The financial assistance provided by University Grants Commission is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tca.2007.04.016.

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