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A Comparative Study of Molecular Interactions of Three Tetracycline Derivatives with Aqueous β-Cyclodextrin Solution at Different Temperatures

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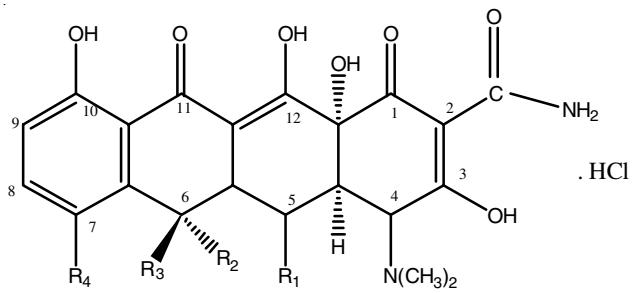
A comparative study of complexation behaviour of three tetracycline derivatives *viz*. doxycycline hydrochloride, oxytetracycline hydrochloride and minocycline hydrochloride with β-cyclodextrin (β-CD) has been done with the help of various thermodynamic and spectroscopic methods. Density (ρ) and conductivity measurements have been carried out for binary drug/water and ternary drug/water/(β-CD) systems at three different temperature *viz*. 305.15, 310.15 and 315.15 K. The interactions of these tetracycline derivatives with β-cyclodextrin in aqueous solutions are further studied by means of fluorescence spectroscopy and UV-visible spectroscopy. From the measured density (ρ) data, partial molar volume (Φ_v°), partial molar volume expansibility (φ_e°), Hepler's constant $(\partial^2\varphi_v^\circ/\partial T^2)_P$ and partial molar volume of transfer ($\Delta\varphi_v^\circ$) have been obtained. From the conductance studies, the energetically favourable interactions are interpreted in the form of free energy change (ΔG) and the apparent association constant (K_a) was estimated from the fluorescence data.

Keywords: Doxycycline hydrochloride, Oxytetracycline hydrochloride, Minocycline hydrochloride, Cyclodextrin, Fluorescence.

INTRODUCTION

Tetracyclines are a group of broad spectrum antimicrobial drugs that has been used in the treatment of manifold infectious diseases such as respiratory tract infection, urinary tract infection, chlamydia, *etc*. in humans and veterinary medications and also as an additive in animal nutrition to facilitate growth. Tetracycline exists in various derivative forms. These derivatives are quite frequently used in the clinical practices based on the functionality of their molecular structure [1-3]. The basic structure of tetracycline comprises of hydronaphthacene system containing four fused rings as shown in Fig. 1. The various analogues differ mainly by the substituent at fifth, sixth and seventh position of tetracycline backbone. In general, these analogues show contrast in their efficacy and toxicity. These drugs are used orally or intravenously. Today 70 % of chemical entities entering the drug discovery program are not sufficiently soluble in the physiological medium therefore having a low efficacy. This leads to the need of taking a higher dose of the drug in order to completely cure the disease resulting in increased toxicity in the body which affects human immunity system and body becomes more prone to diseases. Thermodynamic



Doxycycline	R_1= OH,	R_2= CH_3,	R_3= H,	R_4=H
Oxytetracycline	R_1= OH,	R_2= CH_3,	R_3= OH,	R_4=H
Minocycline	R_1= H,	R_2 = H,	R_3= H,	R_4= $N(CH_3)_2$

Fig. 1. Basic structure of three tetracycline analogues

study of drug-superamolecular interactions can be exploited to explain important phenomenon in biological processes involving bio-membranes, blood and biofluids.

Cyclodextrins (CD's) belong to the family of macrocyclic compounds having six to eight D-glucose moieties linked in a cyclic manner by α-1,4-glycosidic bonds (Fig. 2), with a hydrophilic outer surface and a lipophilic central cavity, hence making them able to host suitable guest molecules [4-7]. The host guest

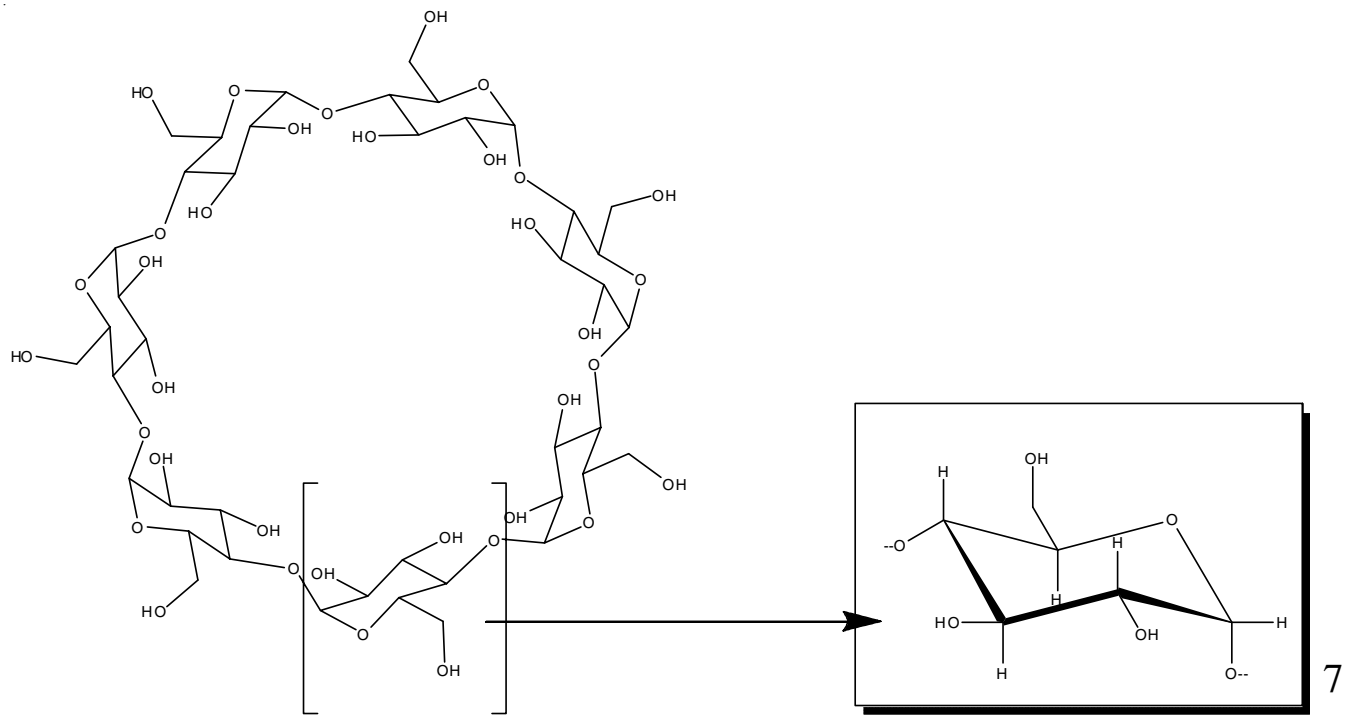


Fig. 2. Molecular structures of β-cyclodextrin

complexes of cyclodextrins have wide application spectrum as the complexation of guest molecules resulting in the change in their physical and chemical properties like solubility, bioavailability, chemical and photochemical stability of pharmaceuticals [8-10]. Cyclodextrins also possess ability to either reduce or eliminate the bad taste and odour of drug [11-13].

The present studies are aimed to give an insight of the molecular interactions prevailing between some tetracycline derivatives and β-cyclodextrin in aqueous medium and to see how small changes in the structure of drug changes the complexation capacities of these drugs by employing conventional *viz*. volumetric and conductance as well as spectroscopic techniques *i.e*. UV-visible and fluorescence studies. Moreover, the effect of temperature has been analyzed by studying all the systems at 305.15, 310.15 and 315.15 K.

EXPERIMENTAL

Doxycycline hydrochloride (CAS Number: 10592-13-9), oxytetracycline hydrochloride (CAS Number: 2058-46-0), minocycline hydrochloride (CAS Number: 13614-98-7) and β-cyclodextrin (CAS Number: 7585-39-9) of purity > 97 %, were purchased from Alfa Aesar and used as such without further purification.

Deionized water was obtained from a Millipore-Elix system. The conductivity (κ) and the pH of water collected was (1-2 × 10^{-7} S cm^{-1}) and (6.8-7), respectively (at 25 ºC) was used for the preparation of solutions. Drug solutions over a concentration range (0.001 to .01 mol kg^{-1}) were made by weight using high precision (± 0.0001 g) digital balance. The solutions were gently stirred with on a magnetic stirrer before the measurements. Density for different solutions has been measured by an Anton Paar Density and Sound Analyzer-5000 (DSA-5000). The instrument was calibrated with deionized water obtained from a Millipore-Elix system for the temperature range investigated. The conductance of different concentrations of both the systems was measured with ELICO-CM 183EC digital conductivity meter. The conductivity cell was calibrated with 0.01 mol dm^{-3} KCl sample solution supplied by Merck Chem. Ltd. The temperature was maintained constant to ± 0.1 ºC by circulating water from a thermostat through a double walled vessel containing the solution. The reproducibility of conductance measurement is estimated to be ± 0.5 %. The UV-visible spectra were recorded with UV-visible spectrophotometer (Varian Cary-100 Bio) using quartz cells. For the experiments with binary drug/water system, the concentration of drug was kept at 0.1 mM while for drug/β-CD/water ternary system, the concentration of drug was kept constant at 0.1 mM and the concentration of β-CD varied from 0 to 0.3 mM, both in the sample and in the reference cell. Fluorescence measurements were performed by Perkin Elmer LS 55 Fluorescence Spectrometer.

RESULTS AND DISCUSSION

Thermodynamics and conductivity studies: Tetracycline analogues are known to exist in zwitter ionic form in aqueous solution with dimethylic group protonated and adjacent hydroxyl group deprotonated. So, it has both a polar part and non-polar part in its structure. At a slightly higher concentration, the molecules start aggregating which exists in equilibrium with non aggregated molecules. The effect of cyclodextrin on the critical aggregation concentration has been studied with the help of molar conductance plots. Specific conductivity (κ) at three different temperatures *viz*., 298, 308 and 318 K for binary drug/water and ternary drug/β-CD/water system has been plotted as a function of drug concentration [D] (Fig. 3) for three tetracycline derivatives *viz*. doxycycline hydrochloride, oxytetracycline hydrochloride, minocycline hydrochloride, respec-

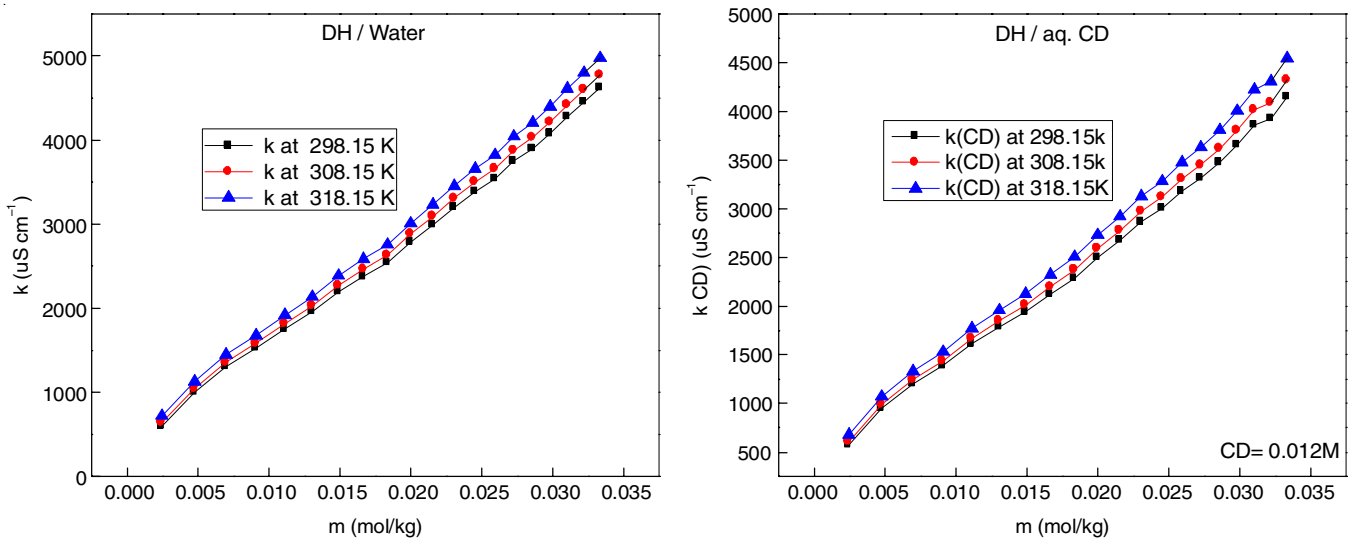


Fig. 3. Variation of specific conductivity (κ) *vs.* drug concentration at different temperatures for doxycycline hydrochloride in the absence and presence of β-cyclodextrin

tively. There is seen a monotonic increase in the specific conductance values with respect to temperature. This can be attributed to increased thermal energy of the molecules with temperature. On comparing the two drug/water and drug/β-CD/water systems (Fig. 4) for all three tetracycline derivatives at 298 K, it is seen that in the presence of β-CD, the system shows lower conductivity values as compared to pure drug/water system. This can be accounted by the fact that the mobility of associated drug is comparatively less than that of free drug which points towards the interaction of drug molecule with cyclodextrin moiety [14]. The energetics of these interactions is estimated in terms of change in free energy according to the following equation [15].

$$\Delta G = RT \ln (X_{cac}) \quad (1)$$

where R is the universal gas constant, T is the temperature and X_{cac} is the mole fraction of critical aggregation concentration. The critical aggregation concentration has been determined from the molar conductance plots shown in Fig. 4a-c. The values of ΔG for the three tetracycline analogues are listed in Table-1. The negative shift in the ΔG values suggested that the molecular interactions between these tetracycline analogues and cyclodextrin solution are energetically favourable. Moreover, a decrease in ΔG values as we replace the solvent with aqueous cyclodextrin solution is more for doxycycline hydrochloride and oxytetracycline hydrochloride as compared to minocycline hydrochloride. So, it suggested that the doxycycline and oxytetracycline interacts to a larger extent with cyclodextrin as compared to minocycline which interacts to a smaller extent.

TABLE-1
VALUES OF CRITICAL AGGREGATION CONCENTRATION AND GIBBS FREE ENERGY OF AGGREGATION IN THE ABSENCE AND PRESENCE OF β-CYCLODEXTRIN

Temp. (K)	X_{cac} (drug/W)	ΔG (kJ/ mol)	X_{cac} (drug/β-CD/W)	ΔG (kJ/ mol)
Doxycycline hydrochloride				
298	0.0121	-10.930	0.0110	-11.17
308	0.0137	-10.980	0.0120	-11.32
318	0.0150	-11.103	0.0134	-11.40
Oxytetracycline hydrochloride				
298	0.0120	-10.95	0.0114	-11.08
308	0.0134	-11.04	0.0122	-11.28
318	0.0146	-11.17	0.0131	-11.46
Minocycline hydrochloride				
298	0.0122	-10.91	0.0120	-10.95
308	0.0136	-11.00	0.0134	-11.04
318	0.0149	-11.12	0.0144	-11.21

W = Water

Volumetric studies: The measured experimental values of density given in Table-2 were used to evaluate different parameters which throw light on the structural rearrangement of these solutions. Volumetric properties such as apparent

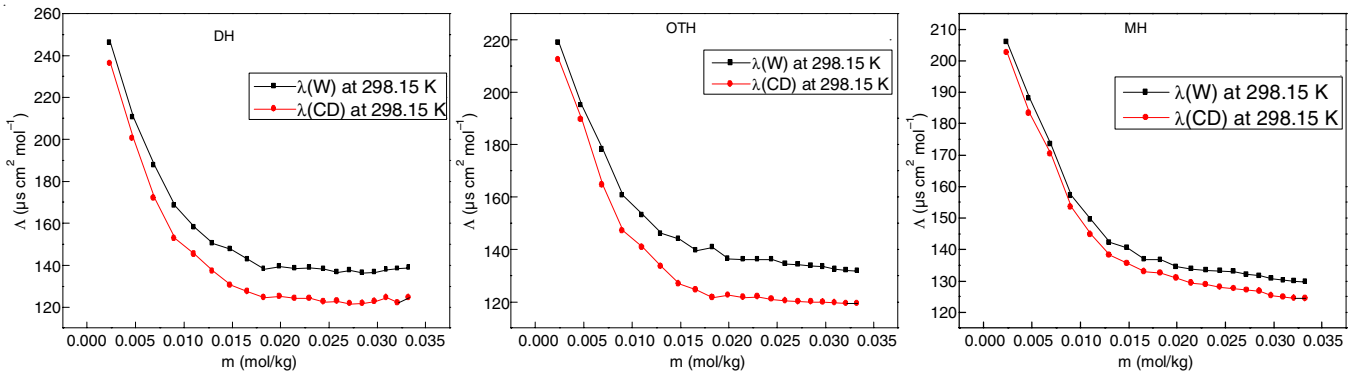


Fig. 4. Comparison of molar conductance of three tetracycline analogues in the absence and presence of β-cyclodextrin

TABLE-2
EXPERIMENTAL VALUES OF DENSITY (ρ) AND PARTIAL MOLAR VOLUME FOR DOXYCYCLINE HYDROCHLORIDE (DH) IN DIFFERENT COMPOSITIONS OF AQUEOUS β-CYCLODEXTRIN (β-CD) AT 305.15-315.15 K, RESPECTIVELY

Temp. (K)	Conc. (mol Kg^{-1})	Density (ρ_o) (g cm^{-3})				Partial molar volume (φ_v^o) (cm^3 mol^{-1})			
		DH/water	DH/2 mM aq. β-CD	DH/4 mM aq. β-CD	DH/6 mM aq. β-CD	DH/water	DH/2 mM aq. β-CD	DH/4 mM aq. β-CD	DH/6 mM aq. β-CD
305.15	–	0.994988	0.995652	0.996346	0.997014	367.2800	370.7200	371.9000	376.1500
	0.001	0.995105	0.995765	0.996458	0.997121	367.0826	370.5713	371.8653	376.1359
	0.002	0.995223	0.995879	0.996569	0.997228	366.9500	370.4593	371.8033	376.1146
	0.003	0.995341	0.995993	0.996681	0.997335	366.7910	370.3473	371.7412	376.0933
	0.004	0.995459	0.996107	0.996793	0.997441	366.6451	370.2104	371.6791	376.0719
	0.005	0.995578	0.996221	0.996905	0.997548	366.4727	370.1032	371.6169	376.0546
	0.006	0.995697	0.996336	0.997017	0.997655	366.3035	369.9613	371.5382	376.0376
	0.007	0.995816	0.996451	0.997130	0.997762	366.1220	369.8278	371.4642	376.0207
	0.008	0.995936	0.996566	0.997242	0.997869	365.9575	369.6995	371.3931	376.0003
	0.009	0.996055	0.996681	0.997354	0.997976	365.7823	369.5417	371.3240	375.9807
	0.010	0.996176	0.996797	0.997467	0.998083	365.5996	369.4028	371.2661	375.9638
310.15	–	0.993380	0.994072	0.994786	0.995494	367.6800	371.2200	372.6000	377.0500
	0.001	0.993498	0.994186	0.994898	0.995601	367.5395	371.1024	372.5037	377.0275
	0.002	0.993617	0.994300	0.995010	0.995708	367.3726	370.9610	372.4219	376.9962
	0.003	0.993735	0.994415	0.995123	0.995815	367.1760	370.8129	372.3335	376.9551
	0.004	0.993854	0.994530	0.995235	0.995922	367.0139	370.6680	372.2384	376.9189
	0.005	0.993974	0.994645	0.995348	0.996030	366.8419	370.5561	372.1466	376.8827
	0.006	0.994093	0.994760	0.995461	0.996137	366.6895	370.4111	372.0481	376.8547
	0.007	0.994213	0.994875	0.995574	0.996244	366.5202	370.2755	371.9600	376.8173
	0.008	0.994334	0.994991	0.995687	0.996351	366.3772	370.1458	371.8660	376.7864
	0.009	0.994454	0.995107	0.995800	0.996459	366.2189	370.0089	371.7790	376.7598
	0.010	0.994575	0.995223	0.995914	0.996566	366.0399	369.8967	371.6672	376.7263
315.15	–	0.991483	0.992212	0.992956	0.993704	369.0800	372.6200	374.1000	378.6500
	0.001	0.991601	0.992326	0.993068	0.993811	368.9203	372.5290	373.9700	378.5947
	0.002	0.991720	0.992440	0.99318	0.993917	368.6606	372.31910	373.8589	378.5340
	0.003	0.991839	0.992555	0.993292	0.994024	368.4664	372.0763	373.7149	378.4700
	0.004	0.991958	0.992670	0.993405	0.994131	368.2639	371.8991	373.5873	378.4175
	0.005	0.992078	0.992786	0.993518	0.994238	368.0383	371.6890	373.4267	378.3518
	0.006	0.992198	0.992901	0.993631	0.994346	367.8831	371.5445	373.3154	378.2877
	0.007	0.992318	0.993017	0.993745	0.994453	367.6698	371.4093	373.1618	378.2245
	0.008	0.992439	0.993133	0.993858	0.994560	367.4569	371.2306	373.0433	378.1743
	0.009	0.992560	0.993249	0.993972	0.994668	367.2663	371.1216	372.9484	378.1107
	0.010	0.992682	0.993366	0.994086	0.994775	367.0517	370.9330	372.8204	378.0575

molar volume (φ_v), limiting apparent molar volume (φ_v°), standard partial molar volume of transfer ($\Delta\varphi_v^\circ$), partial molar volume expansibility (φ_e°), are considered a precise mean for the understanding of interactions in solutions. The apparent molar volume can be considered to be the sum of geometric volume of solute molecule and changes in the solvent volume due to its interaction with the solute. Apparent molar volume of doxycycline, oxytetracycline and minocycline hydrochloride in water and different compositions of aqueous β-cyclodextrin used as modified solvents at a particular temperature was calculated from densities of solution (ρ) and density of solvent (ρ^o) using the following expression [16]:

$$\varphi_v = M_2/\rho_o + 1000\ (\rho_o - \rho)/m\ \rho\rho_o \quad (2)$$

where M_2 represents the molecular weight of solute and m is the molal concentration of aqueous solution. It is evident from Table-2 that the values of partial molar volume are high and positive which decreases with the extent of H-bonding for the drug in water. The structural effects of β-CD give a favourable support in the molecular interactions. The apparent molar volume (φ_v), were plotted against the drug concentration (m) for all three tetracyclines are shown in Fig. 5a-c in accordance with the eqn. 3 [17] as given below:

$$\varphi_v = \varphi_v^\circ + s_v m \quad (3)$$

where φ_v° is the limiting apparent molar volume of solute molecules in the specific solvent. The φ_v° values can give relevant information concerning the hydration properties of drug and solute-solvent interactions and S_v is a semi-empirical parameter which depends on solvent, solute and temperature. Its value for large organic solutes is not of much significance. The values of φ_v° have been calculated from the intercept and slope of the linear plot of eqn. 3 by the least-square fitting method. The partial molar volume can be considered to be total structural volume change brought about by the solute-solvent interactions. It has the contribution from mainly four type of molecular interactions.

$$\varphi_v = \varphi_v(\text{INT}) + \varphi_v(\text{Elect.}) + \varphi_v(\text{Hphobic}) + \varphi_v(\text{HB}) \quad (4)$$

where φ_v(INT) is the intrinsic partial molar volume of the solvent, φ_v(Elect.) is due to the electrostatic interaction between the polar part of the drug and solvent molecules, φ_v(Hphobic) is the contribution from the hydrophobic interactions which are mainly due to non-polar part of the solute particle and φ_v(HB) is due to the hydrogen bonding between solute and solvent molecules. It is evident from Table-2 that the values of φ_v° for all the three tetracycline analogues in water and different compositions (2, 4 and 6 mM) of aqueous β-cyclodextrin solution are positive and high. This suggests that the solute-

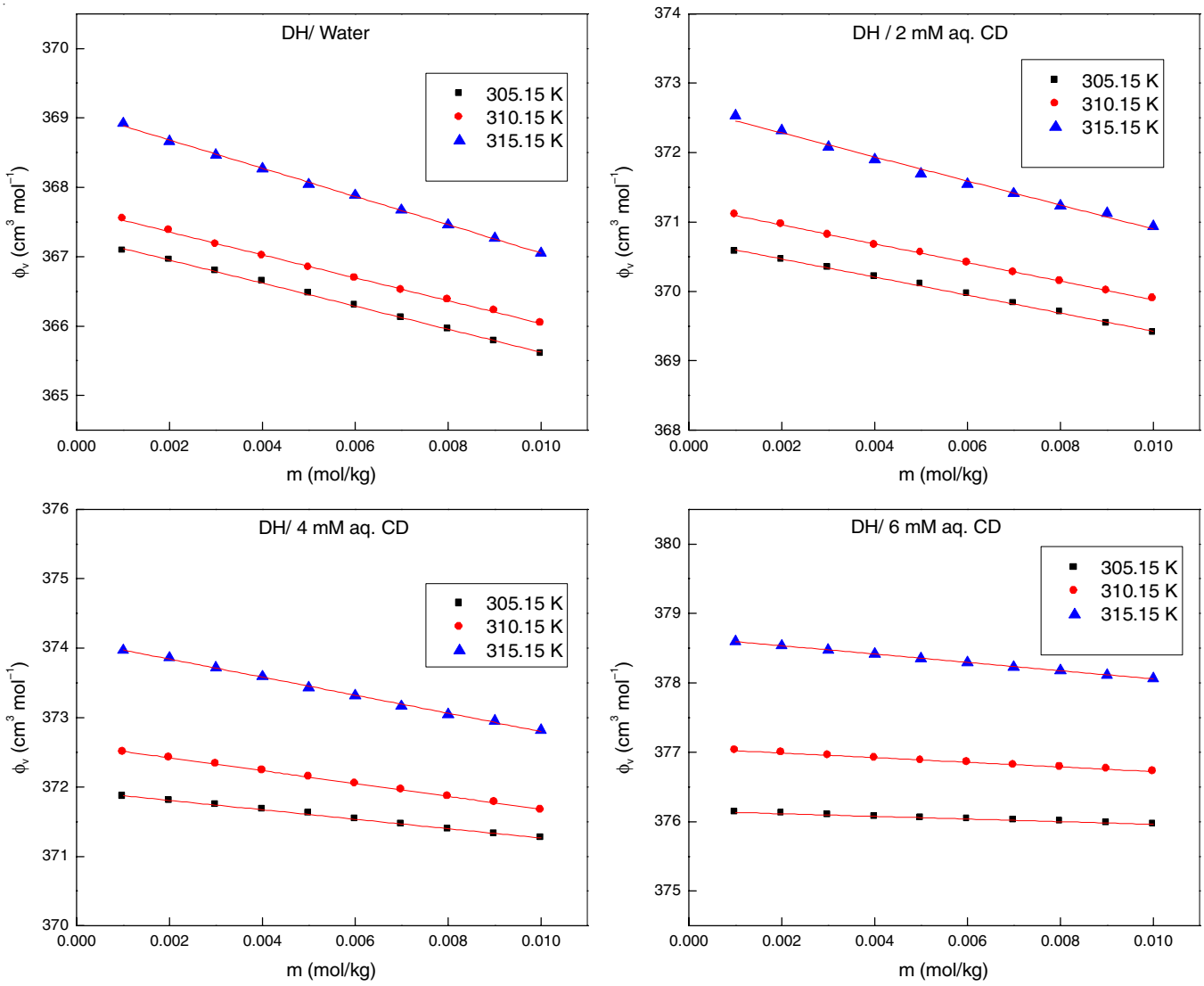


Fig. 5. Plot of partial molar volume *vs.* drug concentration for doxycycline hydrochloride in water and different compositions of aqueous β-cyclodextrin solution at different temperatures

solvent interactions for these tetracycline derivatives in water and different composition (2, 4 and 6 mM) of aqueous β-cyclodextrin solution are high. The order of φ_v° for all three drugs in water and different composition (2, 4 and 6 mM) of aqueous β-cyclodextrin solution increases with increase in the concentration of β-cyclodextrin as:

(Drug/water) < (drug/2 mM aqueous β-CD) < (Drug/4 mM aqueous β-CD) < (Drug/6 mM aqueous β-CD)

As the hydrophobic (non-polar) part of drug molecule does not orient the surrounding water molecules as does the next shell of water molecules. Thus, the nearest water molecules interacts more strongly *via* H-bonding with the neighbouring water molecules that leads to the stiffening of water structure around the solute and a decrease in partial molar volume [18]. In the present system, φ_v° increases with temperature as well as with increase in the concentration of β-cyclodextrin in the system. The observed trend can be attributed to the fact that the hydrophobic moiety of drug molecule is released from the water cavity (ice like structure) by partial or complete encapsulation by the host β-CD molecule, and as the concentration of β-CD is increased in the system the solute-solvent interaction increases. The driving forces for the complex formation include release of enthalpy-rich water molecules from the cavity, hydrogen bonding, van der Waals interaction, charge transfer interaction, dipole interactions and hydrophobic interactions. All these interactions being energetically favourable therefore the complex is formed spontaneously [19]. Increasing φ_v° values for all the three drugs implies that all the three tetracycline derivatives interact with β-cyclodextrin to some extent, but the degree of interaction is different. Doxycycline and oxytetracycline interacts fairly, while small interactions of β-CD with minocycline hydrochloride. The shift in the partial molar volume with increasing concentration of β-cyclodextrin has been shown in Fig. 6. These results further made evident with the help of partial molar volume expansibility values and a quantitative comparison of these interactions was given by Hepler′s constant values. Temperature dependence of φ_v° can be expressed by the following equation [20]:

$$\varphi_v^\circ = a_0 + a_1T + a_2T^2 \quad (5)$$

Separate relations were formed for each temperature in different solvents and solved for the values of a_o, a_1 and a_2 by the method of elimination. The limiting apparent molar volume

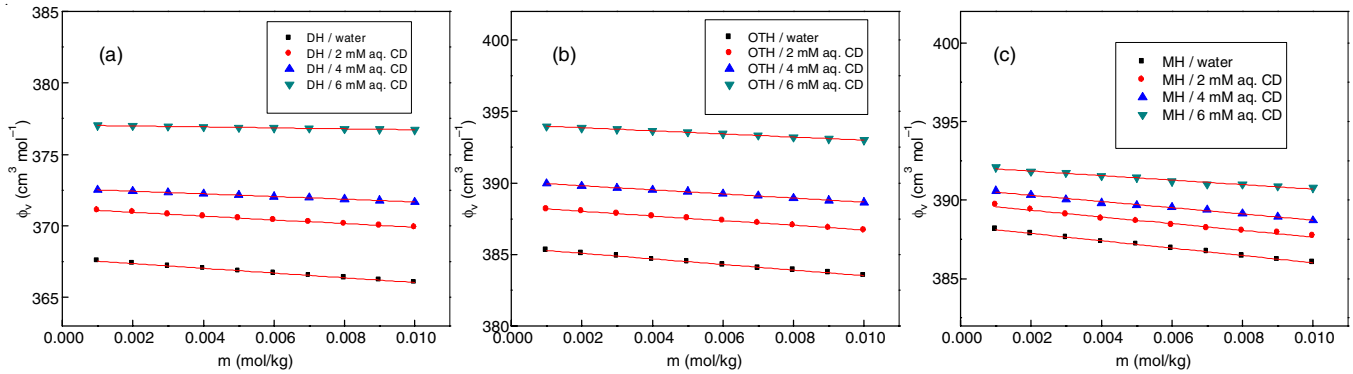


Fig. 6. Comparison of partial molar volume of three tetracyclines in different concentration of β-cyclodextrin at 310.15 K

expansibility defined as $\varphi_v^\circ = [\partial\varphi_v^\circ/\partial T]_p$ was calculated by the following relation:

$$\varphi_E^\circ = a_1 + 2a_2T \quad (6)$$

The variation of φ_E° with temperature for tetracycline analogues in water and different composition (2, 4 and 6 mM) of aqueous β-cyclodextrin solution are shown in Fig. 7. If φ_E° increases with increase in temperature caging effect is present and *vice-versa* [21]. The sign of φ_E° values is found to provide important information regarding the size of the solute and its hydrophobicity. Negative values of φ_E° suggest that there are weak solute-solute interactions and the solute is hydrophobic while positive values of φ_E° suggest that there are strong solute-solute interactions and the solute is hydrophilic. Fig. 7 showed that φ_E° increases with increase in temperature for doxycycline in water and different composition (2, 4 and 6 mM) of aqueous β-cyclodextrin solution, indicating thereby the presence of "caging effect". Although, it is seen that as the concentration of β-cyclodextrin increases, the magnitude of this increase in the value of φ_E° with increase in temperature shifts to a lower value suggesting that this "caging effect" is reduced as we replaced water with aqueous β-cyclodextrin solution, and the caging is further decrease as we go on increasing the concentration of β-cyclodextrin in present solution. It is also noticed that the value of φ_E° is slightly negative for all the three drugs in water at 305.15 K indicating a poor solubility of these drugs in water. This value increases and becomes positive in the presence of β-cyclodextrin except for minocycline hydrochloride for which it remains still negative for lower concentration of β-cyclodextrin specifying that minocycline is not effectively encapsulated by the cyclodextrin cavity. The basic structure of all three drugs is same except for the functional group present at positions C6 and C7. So from the above results, it can be concluded that the encapsulation of the drug is hindered due to the presence of a bulky group at position C7 in case of minocycline hydrochloride. In order to get more precise results, various alternative methods has been explored.

Further qualitative information on hydration of solutes can be obtained from the criteria proposed by Hepler [22], called hydrophobicity criteria, It is suggested that the structure breaking solutes are accompanied by the negative $[\partial^2\phi_v^\circ/\partial T^2]_p$ values. Correspondingly, the positive values of $[\partial^2\phi_v^\circ/\partial T^2]_p$ are associated with the structure-making solutes. Strongly hydrated solutes are known as kosmotropes (structure makers), while weakly hydrated ones are chaotropes (structure breakers) [22]. A drug interacts with water to yield the intermolecular H-bonding between them. It is well known that the formation of H-bond results in a decrease in the partial molar volume due to shortening of the inter-atomic distance.

Table-3 shows that in the present investigation Hepler′s constant has very small positive values. The positive values suggest that all three tetracycline derivatives are kosmotropes, *i.e.* structure makers in water. But as we replace our solvent with aqueous β-cyclodextrin solution the structures making effect of doxycycline is reduced and go on decreasing with increase in the concentration of β-cyclodextrin in the system. There is comparatively lesser effect on solvation of minocycline hydrochloride in the presence of β-cyclodextrin.

These results were also supported by transfer volume and partial molar adiabatic compressibility trends. High positive values of $\Delta_{tr}\varphi_v^\circ$ indicate that the ion-hydrophilic and hydrophilic-hydrophilic interactions seem to be dominating over hydrophobic-

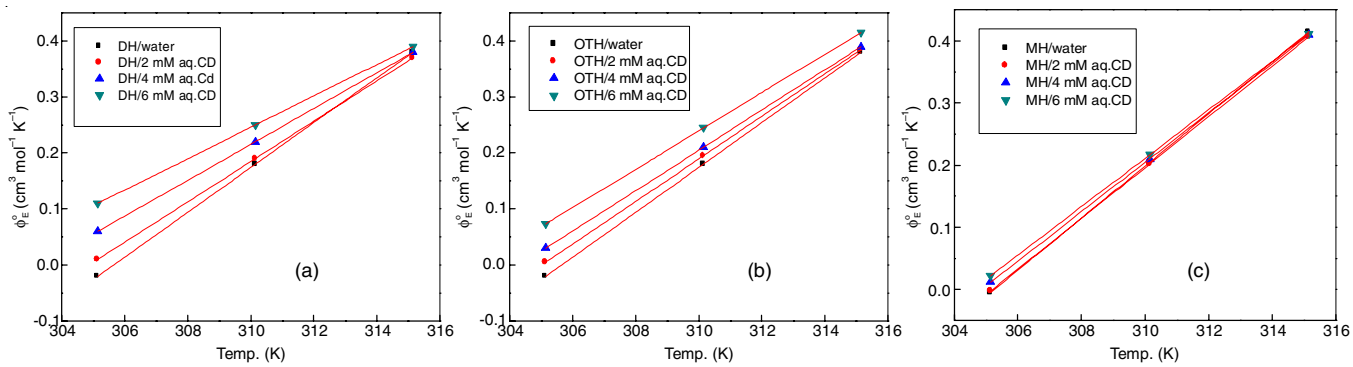


Fig. 7. Variation of partial molar expansibility with temperature for three tetracycline analogues

TABLE-3
APPARENT MOLAR EXPENSIBILITY (φ_E^o), TRANSFER APPARENT MOLAR VOLUME ($\Delta_{tr}\varphi_v^o$) AND HEPLER'S CONSTANT $[\partial^2\phi_v^o/\partial T^2]_p$ FOR DOXYCYCLINE HYDROCHLORIDE, OXYTETRACYCLINE HYDROCHLORIDE AND MINOCYCLINE HYDROCHLORIDE IN WATER AND DIFFERENT COMPOSITIONS (2, 4 AND 6 mM) OF AQUEOUS β-CYCLODEXTRIN SOLUTION AT DIFFERENT TEMPERATURES (*i.e.*, 305.15, 310.15 AND 315.15 K)

Temp. (K)	Doxycycline hydrochloride			Oxytetracycline hydrochloride			Minocycline hydrochloride		
	φ_E^o (cm^3 mol^{-1} K^1)	$\Delta_{tr}\varphi_v^o$	Hepler's constant	φ_E^o (cm^3 mol^{-1} K^1)	$\Delta_{tr}\varphi_v^o$	Hepler's constant	φ_E^o (cm^3 mol^{-1} K^1)	$\Delta_{tr}\varphi_v^o$	Hepler's constant
Drug/Water									
305.15	- 0.02	–	0.04	-0.02	–	0.04	-0.005	–	0.042
310.15	0.18	–		0.18	–		0.205	–	
315.15	0.38	–		0.38	–		0.415	–	
Drug/ 2mM aq. β-cyclodextrin									
305.15	0.01	3.44	0.036	0.005	2.79	0.038	-0.002	1.46	0.041
310.15	0.19	3.54		0.195	2.89		0.202	1.46	
315.15	0.37	3.54		0.385	2.94		0.407	1.435	
Drug/ 4mM aq. β-cyclodextrin									
305.15	0.06	4.62	0.032	0.03	4.63	0.036	0.012	2.32	0.04
310.15	0.22	4.92		0.21	4.63		0.21	2.37	
315.15	0.38	5.02		0.39	4.73		0.41	2.37	
Drug/ 6mM aq. β-cyclodextrin									
305.15	0.11	8.37	0.028	0.073	8.12	0.034	0.022	3.69	0.039
310.15	0.25	9.37		0.245	8.62		0.217	3.79	
315.15	0.39	9.57		0.415	8.87		0.412	3.81	

hydrophobic and ion-hydrophobic interactions in the ternary system with the manifestation of solute-solvent interactions in the system. For the detection of mode of action and confirmation of above results few spectroscopic studies are as follow:

UV-visible spectroscopic studies (mode of interaction): The λ_{max} values for all three tetracycline analogues and their literature values have been reported in Table-4. The observed values were in close proximity with the literature values. The UV absorption spectra of the aqueous solution of doxycycline hydrochloride, oxytetracycline hydrochloride and minocycline hydrochloride in the presence of β-CD as well as absence of β-CD at room temperature are shown in Fig. 8a-c. The absorption shows a hyperchromic shift in the intensity for all three drugs in the presence of β-cyclodextrin. The magnitude of the intensity shift has been reported in terms of the molar extinction coefficient (Table-4). As there is no change in λ_{max} value, it is suggested that this change in intensity is not due to the active involvement of an auxochrome, rather it is due to the protection of molecule from quenching which could be caused due to the collision of the drug molecule with other molecules and

TABLE-4
EXTINCTION COEFFICIENTS AND λ_{max} FOR DOXYCYCLINE, OXYTETRACYCLINE AND MINOCYCLINE IN THE ABSENCE AND PRESENCE OF CYCLODEXTRIN

Drug	Literature values of λ_{max} (nm)	Reported λ_{max} (nm)	Molar extinction coefficient (cm^{-1} M^{-1}) in water	Molar extinction coefficient (cm^{-1} M^{-1}) in 0.1 mM aq. CD	Molar extinction coefficient (cm^{-1} M^{-1}) in 0.3 mM aq. CD
Doxycycline hydrochloride	351	350	15,400	19,300	23,400
Oxytetracycline hydrochloride	354	355	15,200	17,700	21,300
Minocycline hydrochloride	352	352	15,500	16,400	17,900

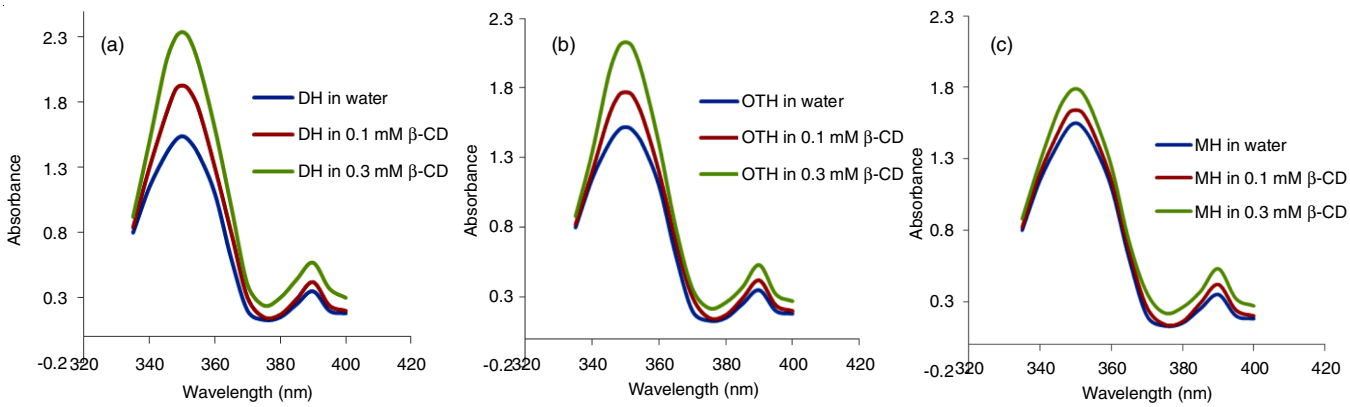


Fig. 8. UV-visible spectra of aqueous solution of (a) doxycycline hydrochloride, (b) oxytetracycline hydrochloride, (c) minocycline hydrochloride in the presence and absence of β-cyclodextrin

heating brought about by these collisions. This clearly indicates the presence of molecular interaction between these drugs and β-CD forming an inclusion coordinated compound by intermolecular non-covalence force [19]. This process of partial encapsulation or inclusion induces structural modifications in the system. Thus the drug is isolated from the hydrophobic environment which causes free movement of electrons into different energy levels and hence absorbs more UV light. Thus in the presence of β-CD the probability of electronic transition of drug is increased resulting in an increase in the magnitude of molar absorption coefficient. This magnitude of absorption coefficient increase is maximum in case of doxycycline, but very small in case of minocycline indicating that the bulky group at C7 hinders the encapsulation of drug by cyclodextrin cavity.

Fluorescence studies (apparent association constant): The estimation of the strength of complex formed between the drug and β-cyclodextrin has been done with the help of fluorescence studies in terms of apparent association constant K_a. Variation in the fluorescence intensity of three tetracycline analogues on the addition of β-cyclodextrin has been shown in Fig. 9. The fluorescence spectra were recorded for a constant concentration of doxycycline which was 0.1 mM and the concentration of β-cyclodextrin was gradually increased by dropwise addition of 0.015 M β-CD with the help of a micropipette. The observed spectra clearly shows that the addition of β-cyclodextrin to doxycycline solution results in a significant enhancement of fluorescence signal [23-25]. As the concentration of cyclodextrin is increased stepwise the intensity of the fluorescence signal of doxycycline goes on increasing. It is well known that the intensification of luminescent processes of a fluorescent guest molecule partially or totally encapsulated by the cyclodextrin cavity is due to the shielding of guest molecule from quenching and non-radioactive decay processes (which may occur due to heat produced by collision in the bulk solution [26,27]). Also on encapsulation cyclodextrin cavity provides an apolar environment for the doxycycline molecule and hence increases the quantum yield of the fluorescence of doxycycline. Thus, all these observations indicates that doxycycline has been able to get into the cyclodextrin cavity spontaneously in aqueous solution and a self-inclusion (drug:β-CD) complex has been formed.

As the formation of drug:β-CD complex is evident the strength of this complex can be quantitatively estimated in terms of the apparent association constant [24,25] by applying the modified Benesi-Hildebrand equation (eqn.1) [27,28]:

$$F_o/F - F_o = 1/\alpha + 1/[CD]\ K_a \quad (7)$$

where F and F_o are the steady state fluorescence intensities at 350 nm in the presence of β-CD as well as absence of β-CD respectively; K_a is the association constant and α is a constant.

As can be seen from these plots (Fig. 10), the dependence of $F_o/(F - F_o)$ on the reciprocal value of β-cyclodextrin concentration is linear and K_a value can be calculated from the slope [28].

The K_a values for doxycycline hydrochloride, oxytetracycline hydrochloride and minocycline hydrochloride were calculated to be 272, 202 and 90 M^{-1}, respectively. The affinity of doxycycline and oxytetracycline by cyclodextrins is moderate. This moderate value is favourable from pharmaceutical point of view, since it is known that a high affinity between the drug

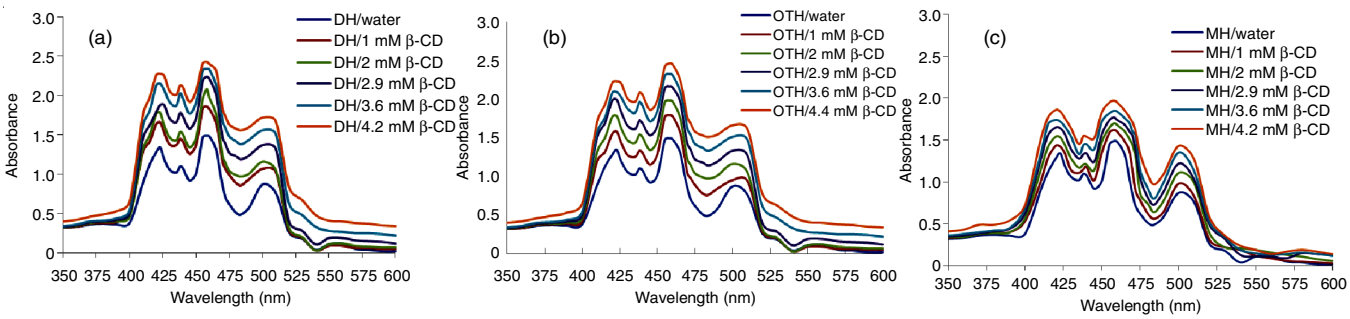


Fig. 9. Emission fluorescence spectra of aqueous solution of (a) doxycycline hydrochloride, (b) oxytetracycline hydrochloride, (c) minocycline hydrochloride at constant concentration (0.1 mM) with step wise increasing concentration of β-cyclodextrin

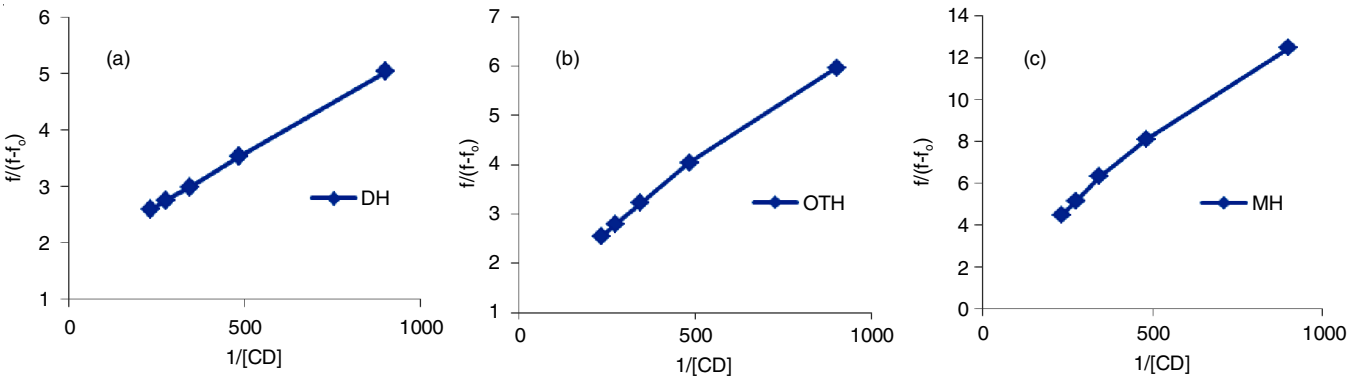


Fig. 10. Benesi-Hildebrand plot for (a) doxycycline hydrochloride (DH)/β-CD/water system at a constant doxycycline concentration 0.1 mM; (b) oxytetracycline hydrochloride (OTH)/β-CD/water system at a constant oxytetracycline concentration 0.1 mM; (c) minocycline hydrochloride (MH)/βCD/water system at a constant minocycline concentration 0.3 mM

and cyclodextrin can cause a difficult delivery of therapeutic compound to the organism. And also as the fit of double reciprocal plot is linear, the stoichiometry of drug:β-CD complex is presumptively 1:1. On the basis of the above results, a 3D scheme (Fig. 11) has been proposed for the mode of inclusion in the aqueous system.

Conclusion

The effect of β-cyclodextrin on the solvation behaviour of three tetracycline analogues (*viz.* doxycycline hydrochloride, oxytetracycline hydrochloride and minocycline hydrochloride) in aqueous medium has been studied and compared by the means of thermodynamic studies. The conductance data revealed the energetically favourable interactions between drug and cyclodextrin molecules in aqueous media. In the volumetric studies, the structural changes were evaluated in terms of the solute-solute and solute-solvent interactions of these tetracyclines both in binary drug/water and ternary drug/water/β-CD systems. From different parameters, it was suggested that in water non-polar interactions of drug molecules dominate over the polar interactions, but in aqueous β-cyclodextrin solution non-polar effects are reduced due to partial encapsulation of non-polar entity in β-cyclodextrin cavity. The second derivative of temperature $[\partial^2\phi_v^\circ/\partial T^2]_p$ shows the structure making property of these tetracycline analogues in aqueous β-cyclodextrin solution. The mode of interaction has been proposed on the basis of UV-visible and fluorescence studies. The stoichiometry, association constant and stability parameters for inclusion

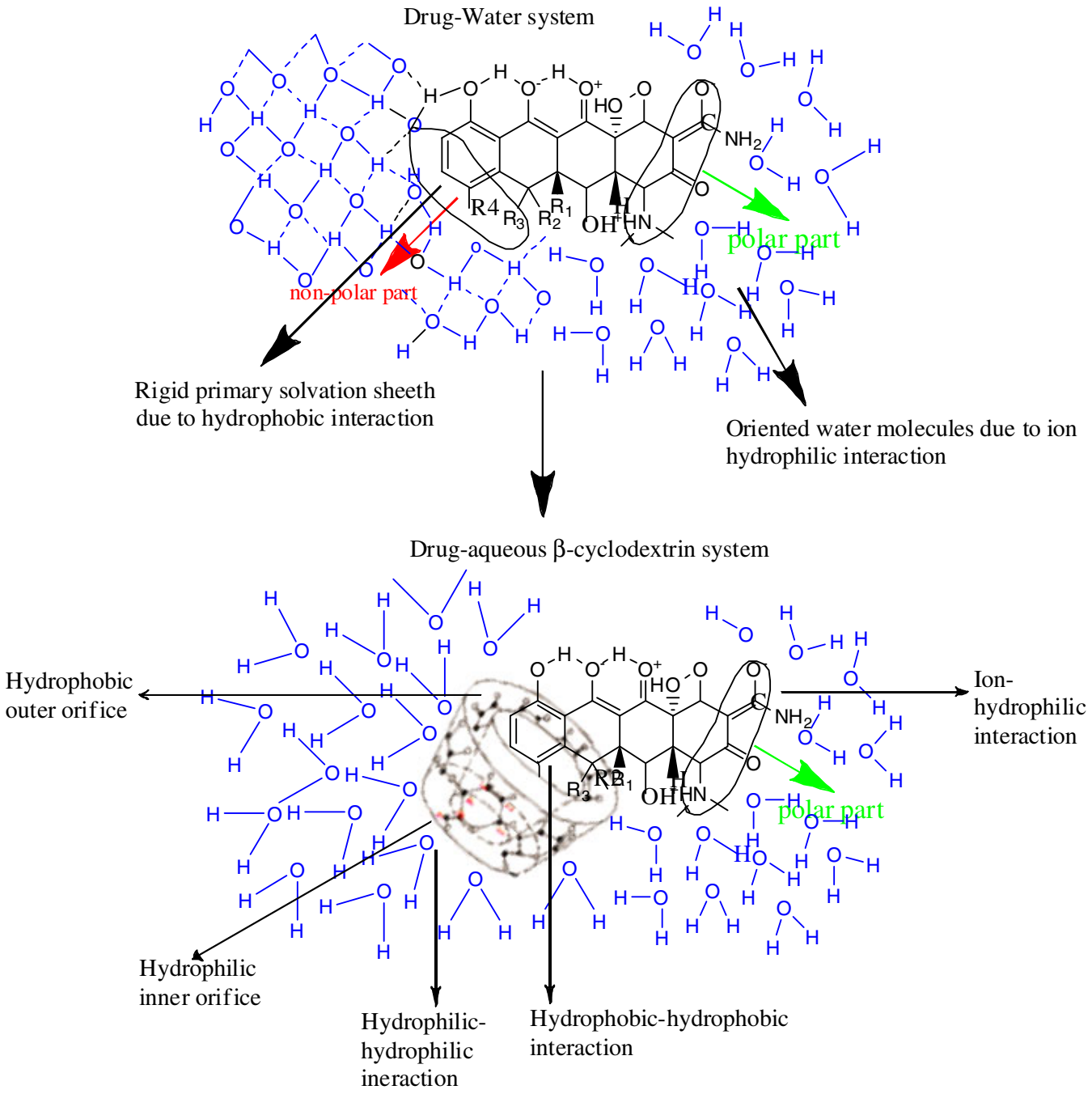


Fig. 11. Molecular interactions in drug-water and drug-water-β-CD systems

complex were evaluated with the help of these fluorescence intensities for different concentrations of β-cyclodextrin. It was suggested that these drug molecules are partially encapsulated by the cyclodextrin moiety, although the molecular interactions are greater in case of doxycycline hydrochloride and least in case of minocycline hydrochloride.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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