CHAPTER- VIII

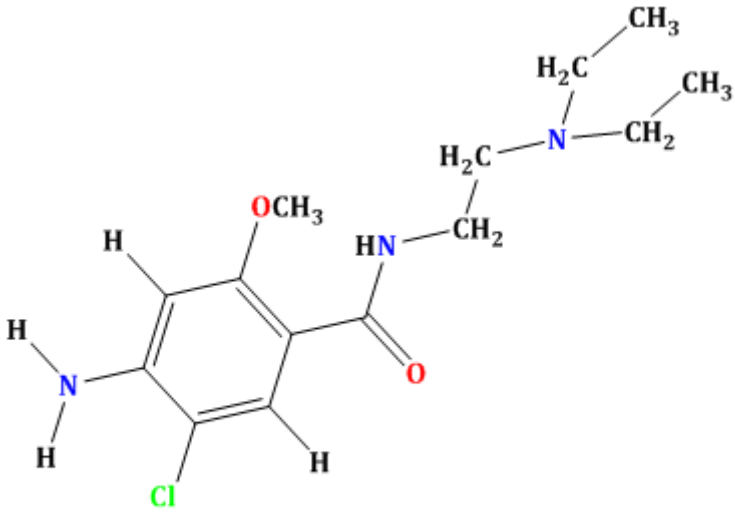
SUBSISTENCE OF HOST-GUEST INCLUSION COMPLEXES OF METOCLOPRAMIDE HYDROCHLORIDE WITH α- AND β-CYCLODEXTRIN MOLECULES PROBED BY PHYSICO-CHEMICAL INVESTIGATION

VII.1. INTRODUCTION

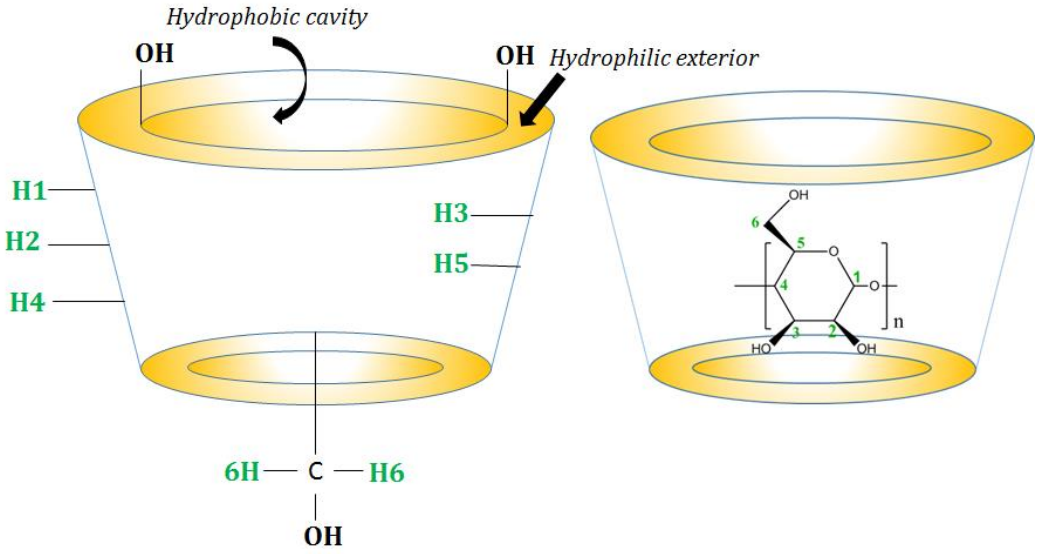
Enhancement of drug-delivery performance using formulations based on cyclodextrin (CD)-drug inclusion complexes is well known because of the enhanced solubility, bioavailability and stability of drug molecules after complexation with CD. Cyclodextrins (CDs) are cyclic oligosaccharides containing six (α-CD), seven (β-CD) and eight (γ-CD) glucopyranose units which are bound together by α-(1–4) linkages forming a truncated conical structure, which allows CDs to form host–guest ICs with different sized guest molecules. Owing to lack of free rotation about the bonds connecting the glucopyranose units, the cyclodextrins are not perfectly cylindrical molecules but the torroidal or cone shaped with a hydrophobic cavity and a hydrophilic surface [**Scheme VIII.1**] [1]. The primary hydroxyl groups are located on the narrow side of the cone shape, while the secondary hydroxyl groups are located on the wider edge due to the presence of specific architecture of the cyclodextrin. The hydrophobic cavity of the cyclodextrin is capable of trapping the hydrophobic parts present in the molecules within to produce stable host-guest inclusion complexes through various interactions, such as hydrogen-bonding, van der Waals, and hydrophobic interactions [2-8]. As a result, the solubility of the hydrophobic and amphiphilic compounds increases. Many advantages of drug- complexation with Cyclodextrins have been reported in scientific literature which includes increased solubility, enhanced bioavailability, improved stability, masking of bad test or odour, reduced volatility, transformation of liquid or gas into solid form reduced side effect and the possibility of a drug release system, etc [9]. Due to these abilities, cyclodextrins are of great interests in pharmaceutical chemistry [10–13], agriculture [14], cosmetics [15, 16], food [17], drug delivery [18-21] and industries [22-24].

Metoclopramide hydrochloride (MP) is a white crystalline, odorless substance, freely soluble in water. Chemically, it is 4-amino-5- chloro-N-[2-(diethylamino) ethyl]-2-methoxy benzamide monohydrochloride, and is used as an anti-emetic in the treatment of some forms of nausea and vomiting and to treat heartburn caused by gastroesophageal reflux in people who have used other medications without relief of symptoms. MP have a greater impact on the treatment of disorders of the gastrointestinal tract. MP is prokinetic agents in gastroenterology. Prokinetic drugs enhances the response to acetylcholine of tissue in upper gastrointestinal tract causing enhanced motility and accelerated gastric emptying without stimulating gastric, biliary, or pancreatic secretions; increases lower oesophageal sphincter tone [25]. It is also used to treat slow gastric emptying in people with diabetes, also called diabetic gastroparesis which can cause nausea, vomiting, heartburn, loss of appetite, and a feeling of fullness after meals. Metoclopramide Hydrochloride blocks dopamine receptors and (when given in higher doses) also blocks serotonin receptors in chemoreceptor trigger zone of the Central Nervous System. Since metoclopramide has been confirmed as an effective drug in treating and preventing various types of disease hence the stabilization and regulatory release of this drug is of great concern in pharmacology. Thus to protect these drugs from external effects and to reduce side effects for their regulatory release, it is crucial to investigate whether they can be encapsulated into the CD molecule.

In this work, the inclusion complex (IC) formation of metoclopramide hydrochloride (MP) with both α and β-cyclodextrins (CDs) was studied in detail based on physicochemical and spectroscopic measurements. The factors affecting the inclusion process were discussed. Enhanced fluorescence and absorption characteristics served as an aid for better understanding the inclusion mechanism, including the size/shape-fit, hydrophobicity. Especially, detailed spatial information in solution has been studied by ^{1}H NMR. The related mechanisms proposed to explain the inclusion process.



(a)



(b)

Scheme VIII.1: Molecular structures of (a) metoclopramide hydrochloride and (b) cyclodextrin molecule with interior and exterior protons (n = 6, 7 for α-CD and β-CD respectively).

VII.2. EXPERIMENTAL SECTION

VIII.2.1 Reagents

Metoclopramide Hydrochloride, α-cyclodextrin and β-cyclodextrin of high purity grade were purchased from Sigma-Aldrich and used as received. Purity of Metoclopramide Hydrchloride, α-cyclodextrin and β-cyclodextrin were ≥98.0%, ≥98.0% and ≥97.0% respectively.

VIII.2.2 Instrumentations

UV-visible spectra were recorded by JASCO V-530 UV/VIS Spectrophotometer, with an uncertainty of wavelength resolution of±2 nm. All the absorption spectra were recorded at 25°C ±1°C. The measuring temperature was held constant by an automated digital thermostat.

The surface tension experiments were accomplished by platinum ring detachment technique using digital tensiometer K9, KRÜSS, Germany at the experimental temperature. Accuracy in the measurement was ±0.1 mNm^{-1}. Temperature was maintained at 298.15 K by circulating auto-thermostat water through a double-wall glass vessel containing the solution.

Specific conductivities of the experimental solutions were measured by Mettler Toledo Seven Multi conductivity meter with uncertainty ±1.0 $\mu S\ m^{-1}$. The experiments were carried out in an auto-thermostat water bath maintaining the temperature at 298.15 K and using the HPLC grade water with specific conductance of 6.0 $\mu S\ m^{-1}$. The cell was calibrated using a 0.01M aqueous KCl solution.

Steady state fluorescence emission study was carried out in bench top spectrofluorimeter from Photon Technologies International (Quantamaster-40, USA) with excitation and emission slit widths fixed at 3.0 nm and 2.0 nm respectively. Samples were taken in Hellma quartz cuvette of optical length 1.0 cm.

^1H NMR spectra were recorded in D_2O at 300 MHz in Bruker Avance 300 MHz instrument at 298 K. Signals are cited as δ values in ppm using residual protonated solvent signal as internal standard (HDO: δ 4.79 ppm). Data are reported as chemical shift.

Fourier transform infrared spectra were recorded in a Perkin Elmer FT-IR spectrometer according to the KBr disk method. KBr disks were made in 1:100 ratios of sample and KBr. FTIR studies were carried out in the scanning range of 4000–400 cm^{-1} at room temperature.

VIII.2.3 Preparation of MP:α-CD and MP:β-CD inclusion complexes

Prior to the start of the experimental work solubility of the chosen CDs and MP have been precisely checked and it was observed that the selected drug freely soluble in all proportion of CD solution. The two solid ICs (MP+ α-CD and MP + β-CD) have been prepared in 1:1 molar ratio of MP and CD. For each complex, 1.0 millimole MP and 1.0 millimole CD were dissolved in 30 mL water separately and stirred for 2 hours. Then the aqueous solution of MP was added drop wise to the aqueous solution of CD. The resulting mixture was stirred for an additional 24 h at room temperature and filtered. The filtrate was concentrated by evaporating, and the resulting precipitate was collected and dried at 50°C for 12 h. The white inclusion complexes were used to characterize by different physical and spectroscopic methods.

VII.3. RESULTS AND DISCUSSION

VIII.3.1 Job plot demonstrate the Stoichiometry

To establish the stoichiometry of the complex, the continuous variation method (Job's plot) was used to follow the changes in absorbance [26-28]. The absorbance of a set of solutions of MP with α and β-CD was/were measured by using UV-visible spectroscopy. The plot of ΔA ×R against R represents the job plot where ΔA is the differences in absorbance of MP with and without CDs and R= [MP]/ [MP] + [CD] and is presented in **Figure VIII.1a** and **VIII.1b**. Absorbance values were calculated at λ_{max}=272 nm for all the solutions at 298.15 K. In this method, the total molar concentration of the two binding partners ([MP] + [CD]) is kept constant but their mole fraction are varied in the range of 0-1 (**Table VIII.1 and VIII.2**) [29, 30].

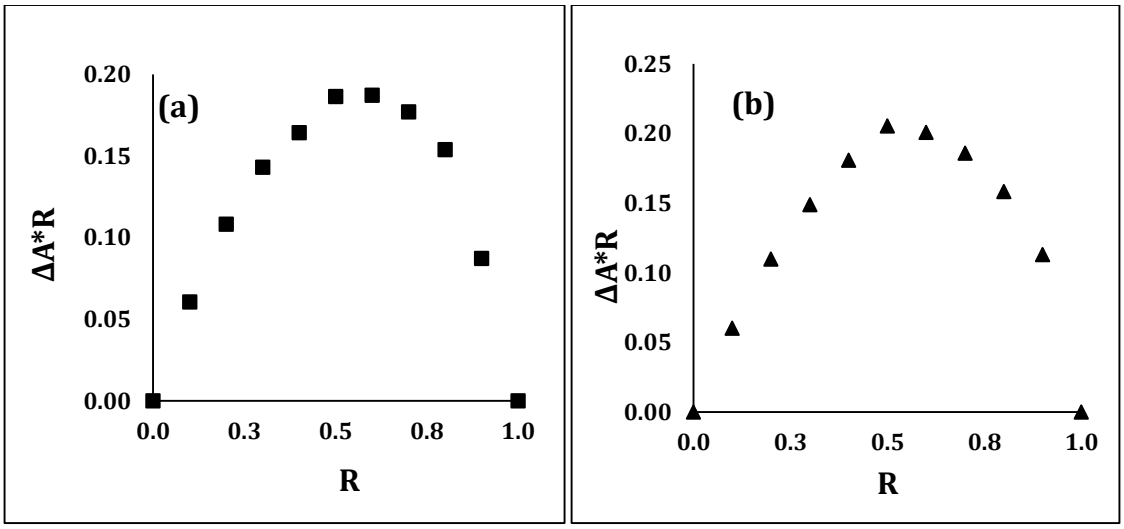


Figure VIII.1: Job plots of (a) MP:α-CD system and (b) MP:β-CD system at λ_{max} = 272 nm at 298.15 K. R = [SS]/([MP] + [CD]), ΔA = absorbance difference of MP with and without CD.

According to this method, the value of R at the maxima on the curve provides the stoichiometry of IC, thus, the ratio of guest and host is 1:2 if R ≈ 0.33; 1:1 if R ≈ 0.5; 2:1 if R ≈ 0.66 etc. The maxima for each of the two plots in **Figure VIII.1a** and **VIII.1b** were the found at R ≈ 0.5, which indicate 1:1 stoichiometry of the host-guest inclusion complexes.

Table VIII.1: Data for the Job plot performed by UV-Vis spectroscopy for aqueous MP:α-CD system at 298.15K[a]

MP (mL)	α-CD (mL)	MP (μM)	α-CD (μM)	$R=\frac{[MP]}{[MP]+[\alpha-CD]}$	Absorbance (A)	ΔA	ΔA*R
0	3	0	50	0.0	0.00000	0.7114	0.0000
0.3	2.7	05	45	0.1	0.10455	0.6068	0.0607
0.6	2.4	10	40	0.2	0.16964	0.5417	0.1083
0.9	2.1	15	35	0.3	0.23445	0.4769	0.1431
1.2	1.8	20	30	0.4	0.30051	0.4109	0.1643
1.5	1.5	25	25	0.5	0.33845	0.3729	0.1865
1.8	1.2	30	20	0.6	0.39919	0.3122	0.1873
2.1	0.9	35	15	0.7	0.45848	0.2529	0.1770
2.4	0.6	40	10	0.8	0.51901	0.1924	0.1539
2.7	0.3	45	05	0.9	0.61435	0.0970	0.0873
3.0	0	50	0	1.0	0.71136	0.0000	0.0000

[a] Standard uncertainties in temperature *u* are: *u*(T) = ±0.01 K.

Table VIII.2: Data for the Job plot performed by UV-Vis spectroscopy for aqueous MP:β-CD system at 298.15K[a]

MP (mL)	β- CD (mL)	MP (μM)	β- CD (μM)	$R=\frac{[MP]}{[MP]+[\beta-CD]}$	Absorbance (A)	ΔA	ΔA*R
0	3	0	50	0.0	0.0000	0.7114	0.0000
0.3	2.7	05	45	0.1	0.1086	0.6028	0.0603
0.6	2.4	10	40	0.2	0.1624	0.5490	0.1098
0.9	2.1	15	35	0.3	0.2151	0.4963	0.1489
1.2	1.8	20	30	0.4	0.2590	0.4523	0.1809
1.5	1.5	25	25	0.5	0.3007	0.4107	0.2054
1.8	1.2	30	20	0.6	0.3766	0.3348	0.2009
2.1	0.9	35	15	0.7	0.4457	0.2657	0.1860
2.4	0.6	40	10	0.8	0.5133	0.1981	0.1584
2.7	0.3	45	05	0.9	0.5856	0.1257	0.1131
3.0	0	50	0	1.0	0.7114	0.0000	0.0000

[a] Standard uncertainties in temperature *u* are: *u*(T) = ±0.01 K.

VIII.3.2 Surface tension study

Surface tension (γ) study provides significant evidence regarding the formation and the stoichiometry of the host-guest inclusion complex [31-33]. MP behaves like surfactant molecule, which is reflected in the lower γ value of its aqueous solution compared to pure aqueous media [34,35]. CDs in contrast, because of having hydrophobic outer surface and hydrophilic rims, hardly show any change in γ while dissolved in aqueous medium for a wide range of concentration [36,37]. In the present study γ of aqueous MP was measured with increasing concentration of α and β-CD at 298.15K (**Table VIII.3** and **VIII.4**). In both cases there were progressively rising trend of γ with increasing concentration of α and β-CD (**Figure VIII.2a** and **VIII.2b**), may be as a result of encapsulation of the MP molecule from the surface of the solution into the

hydrophobic cavity of CDs forming host-guest ICs (**Scheme VIII.2**) [30]. Both the plots also demonstrate that there are single noticeable breaks in each curve (**Figure VIII.2a** and **VIII.2b**), which not only reveal the formation of IC but also specify the 1:1 stoichiometric ratio for each of the ICs formed [31,32].

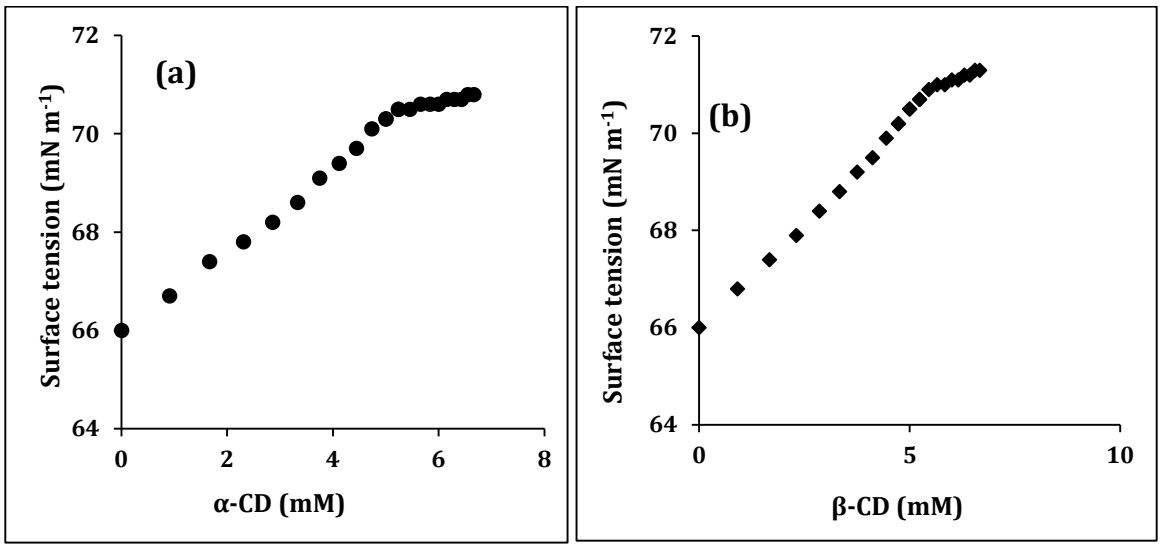


Figure VIII.2: Variation of surface tension of aqueous MP with increasing concentration of (a) α-CD and (b) β-CD solution respectively at 298.15 K.

Table VIII.3: Data for surface tension and conductivity study of aqueous MP:α-CD system at 298.15K[a]

Conc. of MP (mM)	Conc. of α-CD (mM)	Surface tension (mN m^{-1})	Conductuvity (mS m^{-1})
10	0	66	1.280
9.091	0.909	66.7	1.163
8.333	1.667	67.4	1.075
7.692	2.308	67.8	0.997
7.143	2.857	68.2	0.929
6.667	3.333	68.6	0.870
6.250	3.750	69.1	0.812
5.882	4.118	69.4	0.761
5.556	4.444	69.7	0.719
5.263	4.737	70.1	0.684
5.000	5.000	70.3	0.652
4.762	5.238	70.5	0.643
4.545	5.455	70.5	0.635
4.348	5.652	70.6	0.628
4.167	5.833	70.6	0.621
4.000	6.000	70.6	0.615
3.846	6.154	70.7	0.610
3.704	6.296	70.7	0.605
3.571	6.429	70.7	0.599
3.448	6.552	70.8	0.595
3.333	6.667	70.8	0.591

[a] Standard uncertainties in temperature u are: u(T) = ±0.01 K.

Table VIII.4: Data for surface tension and conductivity study of aqueous MP:*β*-CD system at 298.15K[a]

Conc. of MP (mM)	Conc. of *β*-CD (mM)	Surface tension (mN m^{-1})	Conductuvity (mS m^{-1})
10	0	66	1.280
9.091	0.909	66.8	1.160
8.333	1.667	67.4	1.070
7.692	2.308	67.9	0.988
7.143	2.857	68.4	0.917
6.667	3.333	68.8	0.856
6.250	3.750	69.2	0.799
5.882	4.118	69.5	0.750
5.556	4.444	69.9	0.708
5.263	4.737	70.2	0.675
5.000	5.000	70.5	0.640
4.762	5.238	70.7	0.630
4.545	5.455	70.9	0.621
4.348	5.652	71.0	0.613
4.167	5.833	71.0	0.604
4.000	6.000	71.1	0.598
3.846	6.154	71.1	0.586
3.704	6.296	71.2	0.579
3.571	6.429	71.2	0.572
3.448	6.552	71.3	0.565
3.333	6.667	71.3	0.560

[a] Standard uncertainties in temperature *u* are: *u*(T) = ±0.01 K.

The values of γ and corresponding concentrations of MP and CDs at each break have been listed in **Table VIII.5**, which also point out that at each break point the concentration ratio of host and guest is about 1:1, establishing the formation of 1:1 ICs between MP and CDs [30, 35].

Table VIII.5: Values of surface tension (γ) at the break point with corresponding concentrations of MP and CD at 298.15 K[a]

	Conc. of MP/mM	Conc. of CD/mM	γ^a/mN.m^{-1}
α-CD	4.80	5.20	70.4
β-CD	4.46	5.36	70.7

[a] Standard uncertainties (u): temperature: u(T) = ±0.01 K, surface tension: $u(\gamma)$ = ±0.1 mN·m^{-1}

VIII.3.3 Conductivity study

Conductivity (κ) study is an essential tool to elucidate the inclusion phenomenon in solution phase [38,39]. It identifies the formation as well as the stoichiometry of the ICs [34,36]. In the present study the conductivity of aqueous solution of MP was measured with continuous addition of α and β-CD (**Table VIII.3** and **VIII.4**).

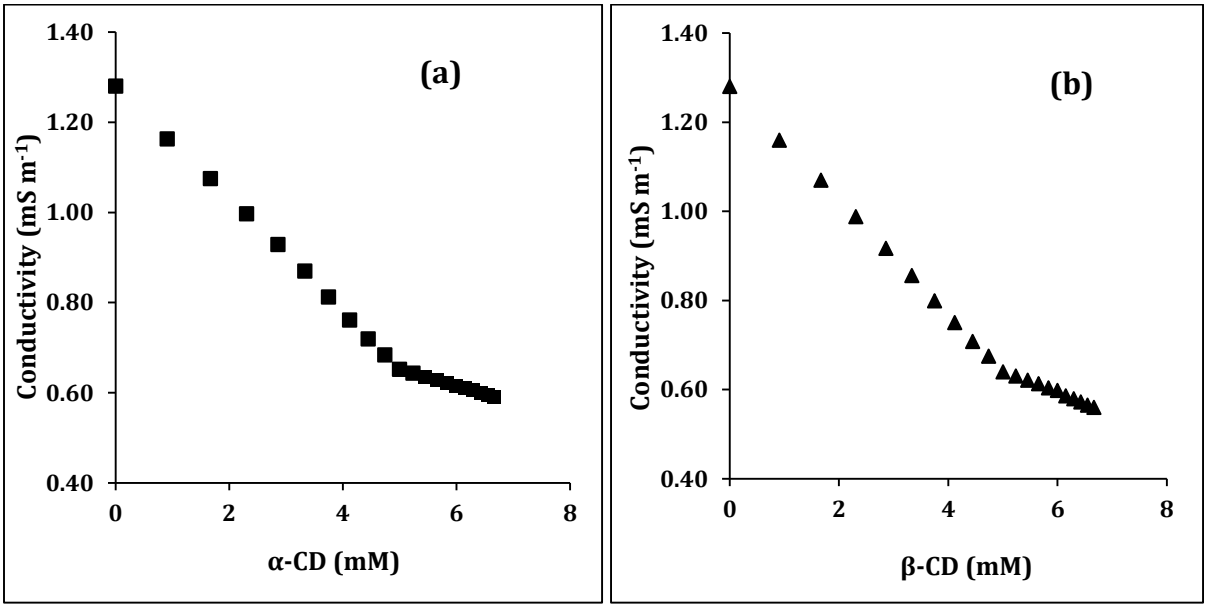


Figure VIII.3: Variation of surface tension of aqueous MP with increasing concentration of (a) α-CD and (b) β-CD solution respectively at 298.15 K.

In aqueous solution MP has substantial conductance value which show gradually decreasing trend of κ (**Figure VIII.3a** and **VIII.3b**) with the successive addition of aqueous solution of CDs, may be because of less number of free MP molecules in the medium due to encapsulation into the cavity of CDs [40.41]. Thus, the conductivities of the solutions are noticeably affected by the inclusion phenomenon (Scheme VIII.2) [39]. At certain concentrations of α and β-CD single breaks were found in each conductivity curve signifying the formation of 1:1 IC (**Figure VIII.3a** and **VIII.3b**) [30,34]. The values of κ and corresponding concentrations of MP and CDs at each break have been listed in **Table VIII.6**, which inform that the ratio of the concentrations of MP and each CD at the break point is roughly 1:1, suggesting that MP-CD IC is equimolar, *i.e.*, the host-guest ratio is 1:1 [36,39]. There always exists a dynamic equilibrium between the host and guest molecule. At the break point most of the guest molecules are inserted in the cavity of CD i.e., at this point maximum inclusion takes place than before. After this point the concentration of CD is more than the drug molecule and the equilibrium is shifted toward the ICs.

Table VIII.6: Values of conductivity (κ) at the break point with corresponding concentrations of MP and CD at 298.15 K[a]

	Conc. of MP/mM	Conc. of CD/mM	κ^a/mS.m^{-1}
α-CD	5.05	4.95	0.651
β-CD	5.01	4.99	0.659

[a] Standard uncertainties (u): temperature: u(T) = ±0.01 K, conductivity: $u(\kappa)$ = ±0.001 mS·m^{-1}

VIII.3.4 Association constants from UV-vis spectroscopy

Spectrophotometric titration is carried out to determine the molecular encapsulation behavior of MP with CDs in aqueous solution [42-45]. The absorption spectral data of MP with various concentrations of CD are given in **Table VIII.7** and **VIII.8**. The absorption intensity of MP gradually increased with the stepwise addition of

CDs [**Figure VIII.4a** and **VIII.5a**][30]. This change might be partly attributed to the shielding of chromophore groups of MP in the CD cavity [28].

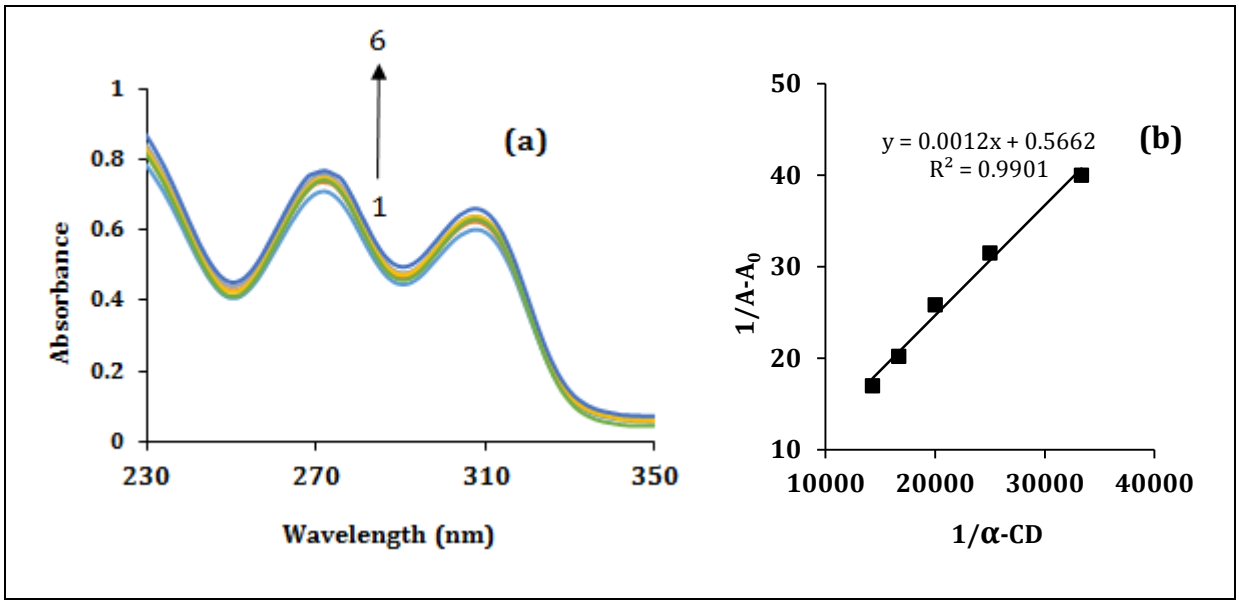


Figure VIII.4: (a) Absorption spectra of MP (50 μM) in different α-CD concentrations (μM): 1) without α-CD, 2) 30 μM , 3) 40 μM, 4) 50 μM, 5) 60 μM, 6) 70 μM. (b) Benesi–Hildebrand plot of $1/A–A_0$ vs. 1/[α-CD] for 1:1 complexation of MP with α-CD.

Table VIII.7: Data for the Benesi-Hildebrand double reciprocal plot performed by UV-Vis spectroscopy for MP:α-CD system

[MP] /μM	[α-CD] /μM	A_0	A	$A- A_0$	1/[α-CD] /M^{-1}	$1/A- A_0$	Intercept	Slope	K_a /M^{-1}
50	30		0.73636	0.02500	33333	40.00000			
50	40		0.74309	0.03173	25000	31.51592			
50	50	0.71136	0.75008	0.03872	20000	25.82645	0.5662	0.0012	472
50	60		0.76079	0.04943	16667	20.23063			
50	70		0.77018	0.05882	14286	17.00102			

[a] Standard uncertainties in temperature are: (T) = ±0.01 K.

In this case, the association constant for the formation of MP: CD complexes are determined by analyzing the changes in the absorbance of MP with the CDs concentration. The association constant K_a and stoichiometry of the inclusion complex of MP with both CDs can be determined by the Benesi-Hildebrand equation [**Figure VIII.4b** and **VIII.5b**][46]:

$$\frac{1}{A-A_0} = \frac{1}{\Delta\varepsilon[MP]K_a}.\frac{1}{[CD]} + \frac{1}{\Delta\varepsilon[MP]}$$

where [CD] and [MP] refer to the total concentration of cyclodextrin and metoclopramide drug respectively, Δϵ is the change in molar extinction coefficient of the chromophore MP as the MP molecules go from the polar aqueous environment to the apolar cavity of α or β-CD making the ICs [36]. A-A_0 denotes the absorption changes of MP on the addition of CDs. The values of K_a for each of the complexes were evaluated by dividing the intercept by the slope of the straight line of the double reciprocal plot (**Table VIII.7** and **VIII.8**). The change of absorbance (A-A_0) was measured as a function of concentration of α and β-CD molecule to find out the association constant (K_a). The good linearity of the plot shows the formation of a 1:1 complex between MP and CDs.

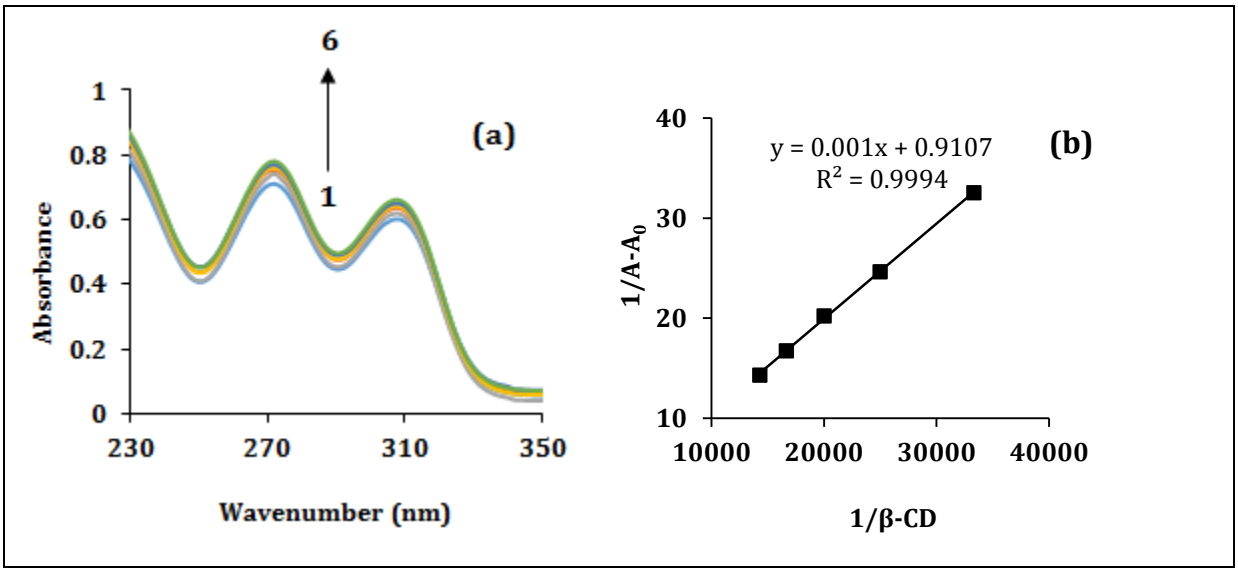


Figure VIII.5: (a) Absorption spectra of MP (50 μM) in different β-CD concentrations (μM): 1) without β-CD, 2) 30 μM , 3) 40 μM, 4) 50 μM, 5) 60 μM, 6) 70 μM. (b) Benesi–Hildebrand plot of 1/A–A_0 vs. 1/[β-CD] for 1:1 complexation of MP with β-CD

Table VIII.8: Data for the Benesi-Hildebrand double reciprocal plot performed by UV-Vis spectroscopy for MP:β-CD system at 298.15K

[MP] /μM	[β-CD] /μM	A_0	A	$A - A_0$	$1/[\beta\text{-CD}]$ /M^{-1}	$1/(A - A_0)$	Intercept	Slope	K_a /M^{-1}
50	30		0.74208	0.03072	33333	32.55208			
50	40		0.75195	0.04059	25000	24.63661			
50	50	0.71136	0.76078	0.04942	20000	20.23472	0.9107	0.001	911
50	60		0.77105	0.05969	16667	16.75322			
50	70		0.78121	0.06985	14286	14.31639			

[a] Standard uncertainties in temperature *u* are: *u*(T) = ±0.01 K.

VIII.3.5 Association constant from fluorescence spectroscopy

The supramolecular interaction of MP with CDs was been investigated by spectrofluorimetry [47-49]. As shown in **Figure VIII.6a** and **VIII.7a**, with an increase in the CDs (α-CD and β-CD) concentration, however, the fluorescence intensity of MP was enhanced accompanied by a slight hypsochromic shift of the emission peak [**Table VIII.9** and **VIII.10**]. These findings indicated the formation of MP–CDs inclusion complexes. Molecules partially or fully encapsulated in the CD cavity often exhibit an enhancement in their fluorescence intensity. This is because the cyclodextrin's cavity offers a protective microenvironment which can shield the excited singlet species from quenching and nonradiative decay process occurring in the bulk aqueous solution [50,51].

The association constants (K_a) of both the complexes were calculated from fluorescence data using the modified Benesi–Hildebrand equation [46]:

$$\frac{1}{I - I_0} = \frac{1}{[I' - I_0]K_a} \cdot \frac{1}{[CD]} + \frac{1}{I' - I_0}$$

Where I and I_0 represent the fluorescence intensities of MP in the presence and absence of CDs, respectively; [CD] represent the concentrations of both CDs; K_a is the association constant of the complexes and I' denotes the fluorescence intensity when all MP

molecules are essentially complexed with CDs. The double reciprocal plots $1/(I-I_0)$ vs. 1/[CD] for MP complexed with α-CD and β-CD (shown in **Figure 6b** and **7b**) exhibit good linearity, implying that the inclusion complexes have a stoichiometric ratio of 1:1.

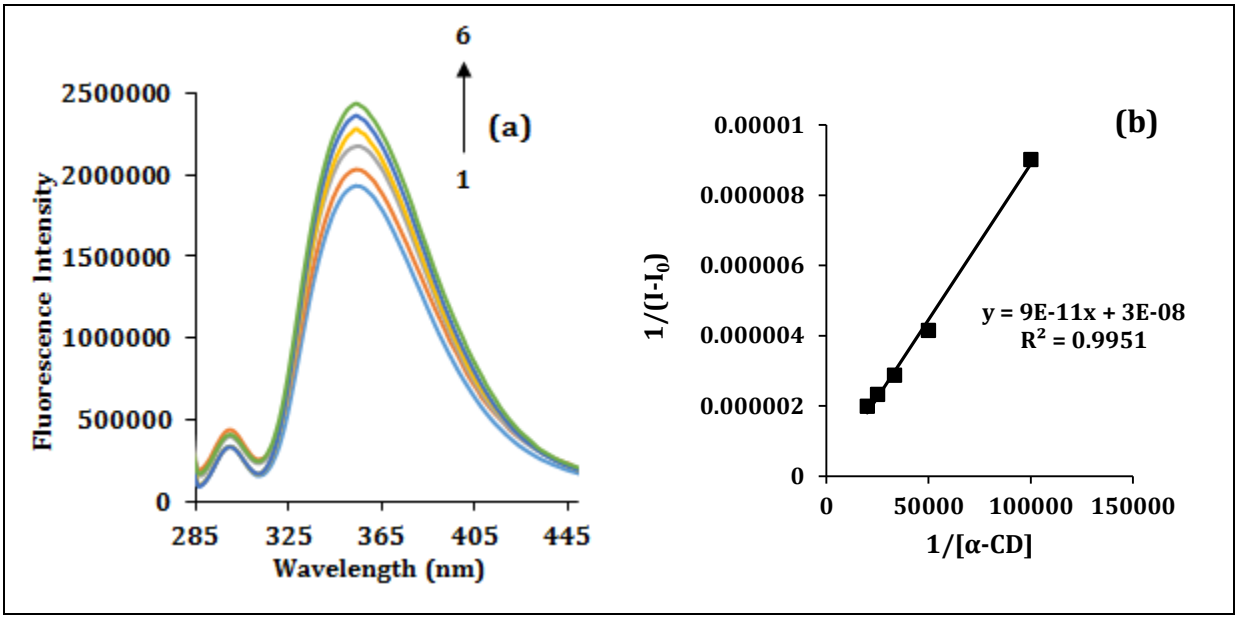


Figure VIII.6: (a) Fluorescence emission spectra of MP (5 μM) in different α-CD concentrations (μM): 1) without α-CD, 2) 10 μM , 3) 20 μM, 4) 30 μM, 5) 40 μM, 6) 50 μM. (b) Benesi–Hildebrand plot of $1/I-I_0$ vs. 1/[α-CD] for 1:1 complexation of MP with α-CD.

Table VIII.9: Data for the Benesi-Hildebrand double reciprocal plot performed by fluorescence spectroscopy for MP:α-CD system at 298.15K[a]

[MP] /μM	[α-CD] /μM	I_0	I	I- I_0	1/[α-CD] /M^{-1}	1/ I- I_0	Intercept	Slope	K_a /M^{-1}
5	10		2042391	110928	100000	9.01×10^{-6}			
5	20		2172112	240649	50000	4.16×10^{-6}			
5	30	1931463	2278802	347339	33333	2.88×10^{-6}	3×10^{-6}	9×10^{-6}	333
5	40		2360301	428838	25000	2.33×10^{-6}			
5	50		2433963	502499	20000	1.99×10^{-6}			

[a] Standard uncertainties in temperature *u* are: *u*(T) = ±0.01 K.

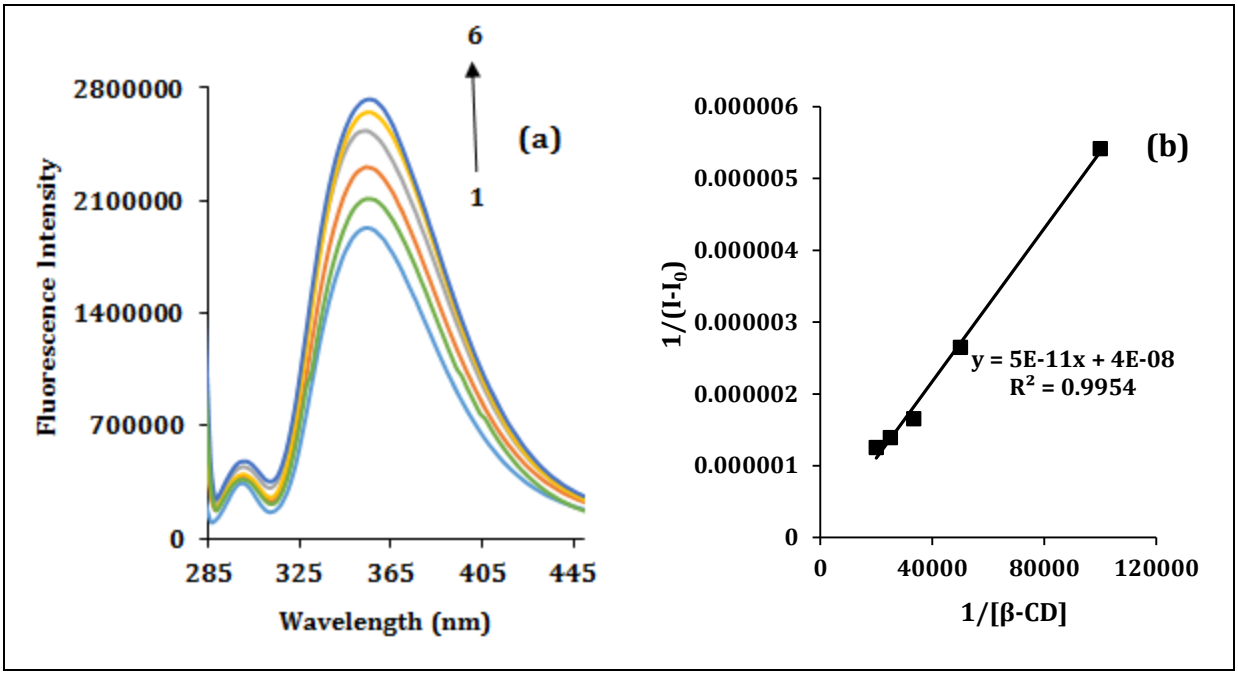


Figure VIII.7: (a) Fluorescence emission spectra of MP (5 μM) in different β-CD concentrations (μM): 1) without β-CD, 2) 10 μM , 3) 20 μM, 4) 30 μM, 5) 40 μM, 6) 50 μM. (b) Benesi–Hildebrand plot of $1/I-I_0$ vs. $1/[\beta\text{-CD}]$ for 1:1 complexation of MP with β-CD.

Table VIII.10: Data for the Benesi-Hildebrand double reciprocal plot performed by fluorescence spectroscopy for MP:β-CD system at 298.15K[a]

[MP] /μM	[β-CD] /μM	I_0	I	I- I_0	1/[β-CD] /M^{-1}	1/ I- I_0	Intercept	Slope	K_a /M^{-1}
5	30		2116069	184606	100000	$5.42\times10^{-}$			
5	40		2309301	377838	50000	$2.65\times10^{-}$			
5	50	1931463	2535769	604306	33333	$1.65\times10^{-}$	4×10^{-8}	5×10^{-11}	800
5	60		2648717	717254	25000	$1.39\times10^{-}$			
5	70		2727701	796238	20000	$1.26\times10^{-}$			

[a] Standard uncertainties in temperature *u* are: *u*(T) = ±0.01 K.

The association constant of MP:β-CD complex is higher than MP:α-CD complex [**Table VIII.11**] which indicates that the capability of β-CD to form an inclusion complex

with MP is higher than probably due to its larger cavity size than that of α-CD i.e the size of its cavity is more appropriate to encapsulate the drug molecules.

Table VIII.11: Values of Association constants (K_a) obtained by Benesi–Hildebrand method both from UV-vis spectroscopy and Fluorescence spectroscopy and corresponding free energy change (ΔG^0) of the MP:CD inclusion complexes at 298.15K[a]

	$K_a \times 10^{-2}$/ M^{-1}[b]	ΔG^0/KJ mol^{-1}[b]	$K_a \times 10^{-2}$/ M^{-1}[b]	ΔG^0/KJ mol^{-1}[b]
	UV-vis spectroscopy		Fluorescence spectroscopy	
MP:α-CD	4.72	-3.85	3.33	-2.98
MP:β-CD	9.11	-5.48	8.00	-5.15

[a]Standard uncertainties in temperature *u* are: *u*(T) = ±0.01 K.

[b]Mean errors in K_a = ±0.02 × 10^{-3} M^{-1}; ΔG^0 = ±0.01 kJ mol^{-1}.

VIII.3.6 The thermodynamics of inclusion process

The thermodynamic parameters ΔG for the binding of guest molecule to cyclodextrin cavity can be calculated from the association constant 'K' by using the following equation

$$\Delta G = - RT \ln K_a$$

The thermodynamic parameters ΔG for the binding of guest molecules (MP) to CD cavity are given in **Table VIII.11**. The negative value of ΔG suggests that the inclusion process proceeded spontaneously at 298.15 K.

VII.3.7 ^{1}H-NMR analysis of inclusion complexes

NMR study is the most important tool which ascertains the inclusion phenomena of the guest drug molecule inside the host CD molecule. Hence, further investigations of inclusion complexes were performed by ^{1}H-NMR analysis. Chemical shifts and the changes of the chemical shifts of protons in MP: CD complex compared with the pure compounds of MP and CDs (**Figure VIII.8** and **VIII.9**).

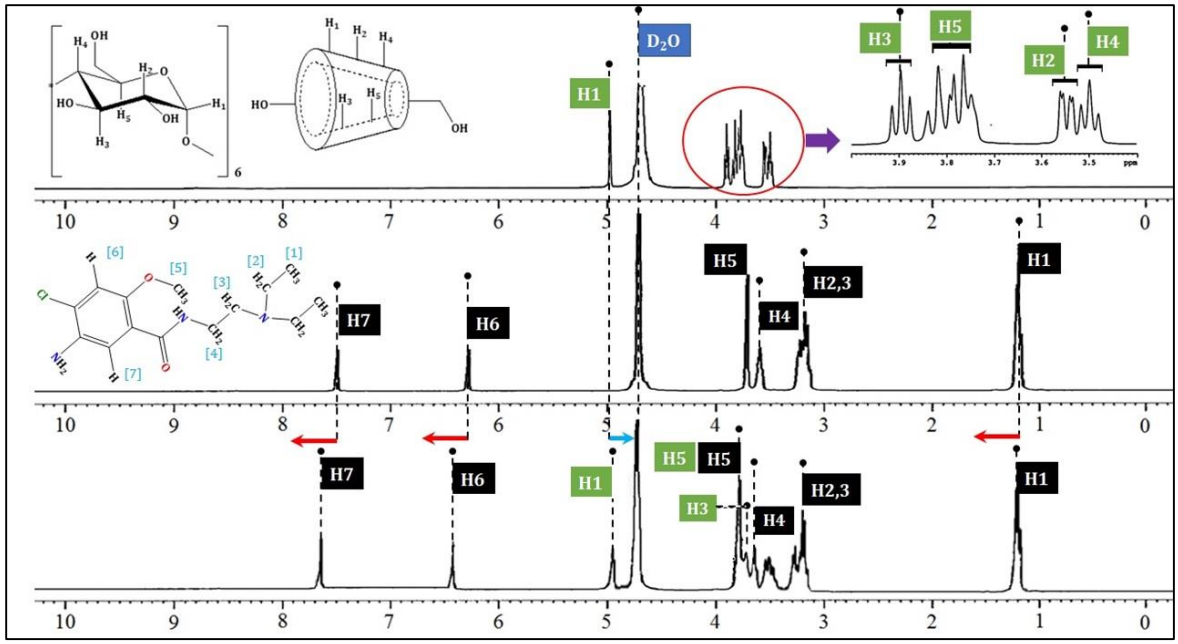


Figure VIII.8: ^{1}H NMR spectra of (a) α-CD, (b) MP and (c) 1:1 M ratio of α-CD & MP in D_2O at 298.15 K.

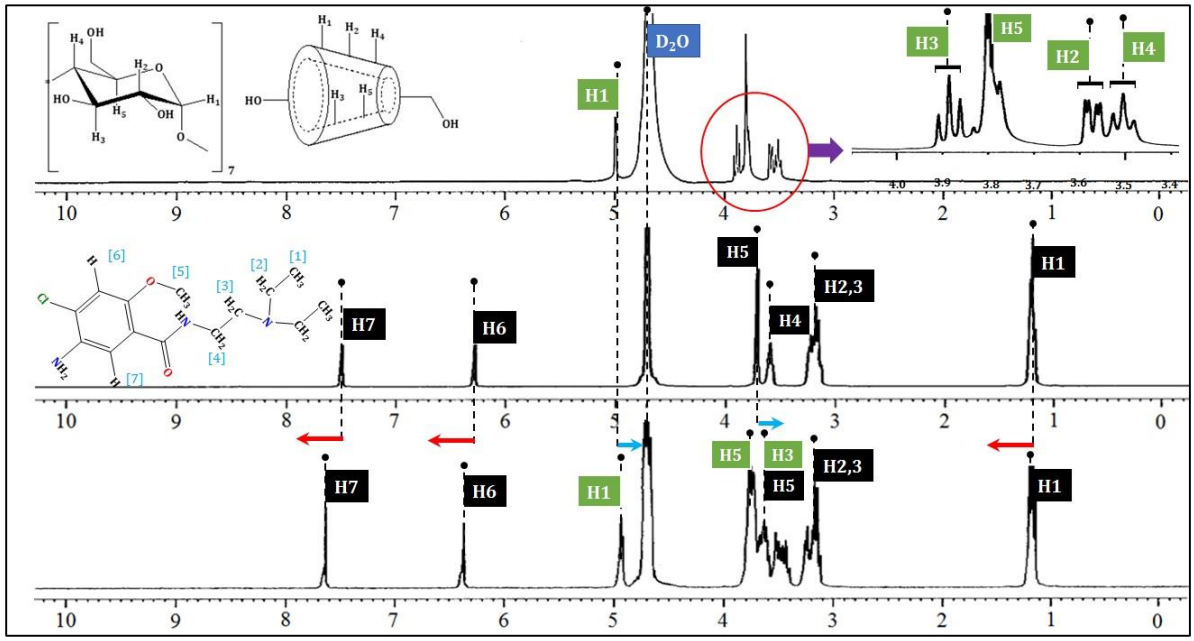
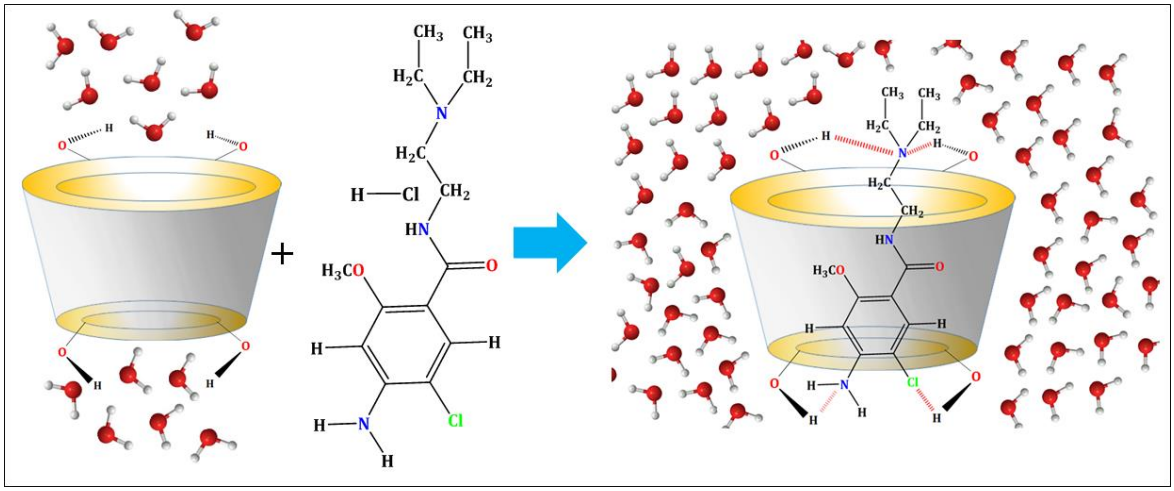


Figure VIII.9: ^{1}H NMR spectra of (a) β-CD, (b) MP and (c) 1:1 M ratio of β-CD & MP in D_2O at 298.15 K.

In the structure of CD it may be observed that the H3 and H5 hydrogens are located inside the conical cavity, particularly, the H3 are placed near the wider rim while H5 are placed near the narrower rim and the other H1, H2 and H4 hydrogens are located at the exterior of the CD molecule (**Scheme VIII.1**) [28,52]. The observable changes in the chemical shifts of CD protons were detected for H3, H5 and H6 protons which is in accordance with the involvement of these protons in the formation of inclusion complex [20,31,53,54]. The highest proton shifts of CD in the complex have been observed for the H3 protons, and slightly lower for the H5 and H6 protons. The signals of the included MP are shifted by complexation to a variable extent. The spectral changes that can be observed in case of encapsulation of aromatic guest molecules are due to the interactions of interacting protons of CD by the aromatic moiety of the guest [54]. The chemical shift difference for the protons belonging to the phenyl ring are higher than that for the other group protons i.e. the H6 and H7 protons of the aromatic ring were more involved in the interaction with CD. Conversely, the protons of three ethyl group bonded with N atom experience very little perturbation. Thus from the above discussion we can say that the changes in chemical shifts of protons H-5, H-3, and H-6 of both CD (**Figure VIII.8** and **VIII.9**) and protons H6, H7 and H5 of the MP (Fig. 5), which show the most marked variations. Upfield chemical shift is also observed for the exterior protons of the CD but to lesser extent. The shifts of the interacting protons illustrates the mechanism of insertion as depicted in **Scheme VIII.2**.



Scheme VIII.2: Plausible schematic presentation of mechanism for formation of 1:1 inclusion complex between metoclopramide hydrochloride and cyclodextrin.

Based on the NMR data, the plausible mode of interaction of MP-α-CD and MP-β-CD complexes were proposed, which suggested that lipophilic aromatic ring of the MP entered into the cavity of CDs from the wider side, with the amide (-CONH) and methoxy (-OMe) residues inside the CD cavity, and the $-N(CH_2CH_3)_2$ group was close to the wider rim and exposed outside the cavity. Also the $-NH_2$ and –Cl moieties exposed outside the cavity near the narrower rim.

Selected ^1H NMR data

Metoclopramide Hydrochloride: ^1H NMR (D_2O, 298.15 K): δ/ppm 1.154-1.202 (6H, m), 3.144-3.237 (6H, m), 3.584-3.602 (2H, m), 3.697-3.706(3H, m), 6.262-6.271 (1H, s), 7.474-7.484 (1H, s).

α- Cyclodextrin (α-CD): ^1H NMR (D_2O, 298.15 K): δ/ppm 3.480-3.517 (6H, t, *J*= 9.00 Hz), 3.534-3.560 (6H, dd, *J*= 10.00, 3.00 Hz), 3.749-3.839 (18H, m), 3.877-3.914 (6H, t, *J* = 9 Hz), 4.965-4.971 (6H, d, *J* = 3 Hz).

β- Cyclodextrin (β-CD): ^1H NMR (D_2O, 298.15 K): δ/ppm 3.497-3.543 (7H, t, *J* = 9.2 Hz), 3.570-3.603 (7H, dd, *J* = 9.6, 3.2 Hz), 3.790-3.848 (21H, m), 3.878-3.925 (7H, t, *J* = 9.2 Hz), 5.003-5.012 (7H, d, *J* = 3.6 Hz)

MP-α-CD (1:1 molar ratio): ^1H NMR (D_2O, 298.15 K): δ/ppm 1.162-1.212 (6H, m), 3.149-3.245 (6H, m), 3.427-3.531 (12H, m), 3.607-3.646 (2H, m), 6.404 (1H, s), 7.617 (1H, s), 3.717-3.760 (6H, m), 3.805 (21H, m), 4.923-4.934 (6H, m).

MP-β-CD (1:1 molar ratio) ^1H NMR (D_2O, 298.15 K): δ/ppm 1.157-1.208 (6H, m), 3.180-3.281 (6H, m), 3.428-3.553 (12H, m), 3.612-3.696 (2H+6H, m), 6.392-6.406 (1H, s), 7.622-7.667 (1H, s), 3.747-3.819 (21H+3H, m), 4.936-4.947 (6H, m).

VIII.3.8 FT-IR Spectra of solid inclusion complexes

The solid inclusion complex formation is analyzed by FT-IR spectroscopy. FT-IR spectrum is used to confirm the formation of the solid inclusion complex by considering the deviation of peak shape position and intensity [55-58]. The characteristic IR frequencies of MP, α-CD, β-CD and their solid ICs are listed in **Table VIII.12** with the chemical bonds responsible for the corresponding stretching frequencies and the spectra

are shown in **Figure VIII.10** and **VIII.11**. In the IR spectra, symmetric stretching of vibrations of amine N–H is observed at 3316 cm^{-1} [59]. Owing to the result of inclusion the stretching of N-H vibration got slightly shifted. The peaks observed at 3369 cm^{-1} and 3201 cm^{-1} were assigned to amide group N–H asymmetric and symmetric stretching vibrations. The carbonyl stretching vibration of C=O group is appeared at 1640 cm^{-1} for amides [60]. The IR spectrum of the MP (**Figure VIII.10** and **VIII.11**) is also characterized by absorption peaks at 2939 cm^{-1} (for C-H stretching vibration in aromatic ring), 1590 cm^{-1} (for N-H bending vibration), 1213 cm^{-1} and 1026 cm^{-1} (for asymmetric and symmetric C-O stretching vibration of $-OCH_3$ group), 1309 cm^{-1} (for aromatic -C-N stretching vibration), 593.41 cm^{-1} (for C-Cl stretching vibration).

However, several peaks of the MP are either absent or shifted which is due to the change in environment of the guest molecule after inclusion in the cavity of CDs. The $(N-H)_{amide}$ and $(C-H)_{aromatic}$ band of the MP are almost completely masked by very intense and broad CDs bands. The $(>C=O)_{amide}$ stretching signal was at 1640.81 cm^{-1} for MP, which was shifted at 1624 cm^{-1} in case of α-CD and at 1619 cm^{-1} in case of β-CD IC may be as a result of encapsulation into the CD cavity.

Broad characteristic peaks of –OH at about 3363 cm^{-1} and 3372 cm^{-1} are present in the spectrum for α and β-CD. However, the peaks are shifted at 3398.66 cm^{-1} and 3397 cm^{-1} respectively in the solid inclusion complex. The –O-H stretching of both α and β-CD is shifted in the spectrum of both ICs possibly due to involvement of the –O-H groups of the host molecules in hydrogen bonding with the guest molecules. The stretching frequencies of the other characteristic peaks, which are shifted in solid inclusion complex due to various interactions, are given in the table 9. According to the above FT-IR analysis of inclusion complexes, we might recommend that the aromatic ring of the guest molecule is encapsulated in the hydrophobic cavity of α and β-CD. Hence, the FT-IR study provides significant indications of formation of ICs in the solid form, supporting the outcomes of the other above studies.

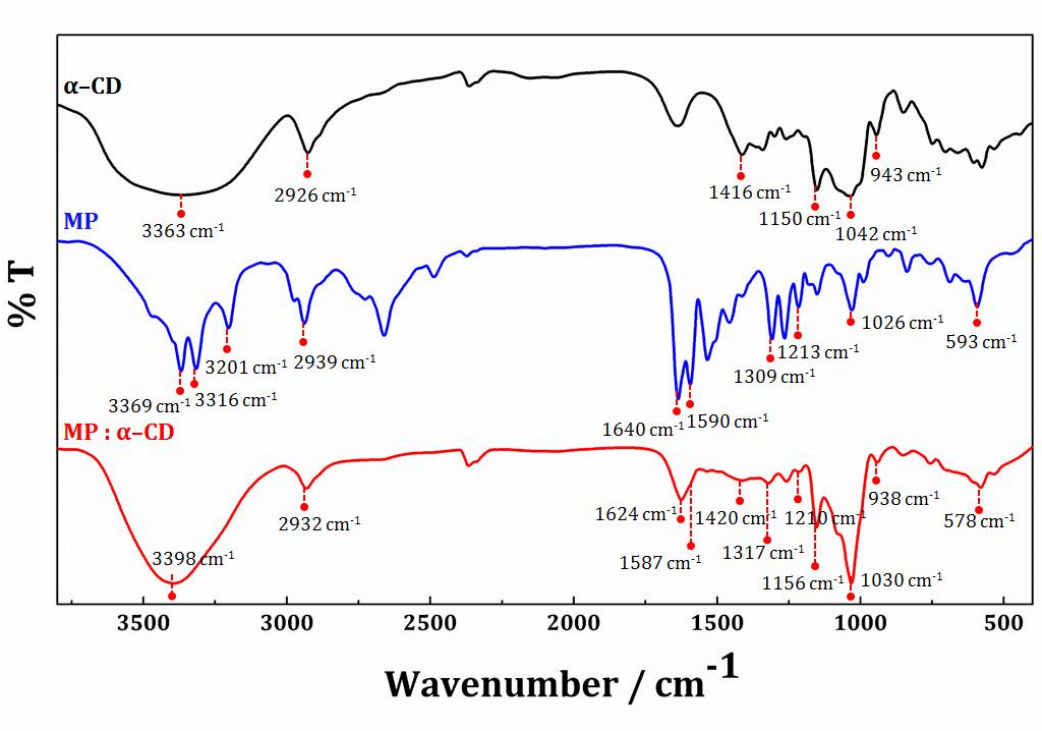


Figure VIII.10: FTIR spectra of free α-CD, MP and their 1:1 inclusion complex (MP:α-CD).

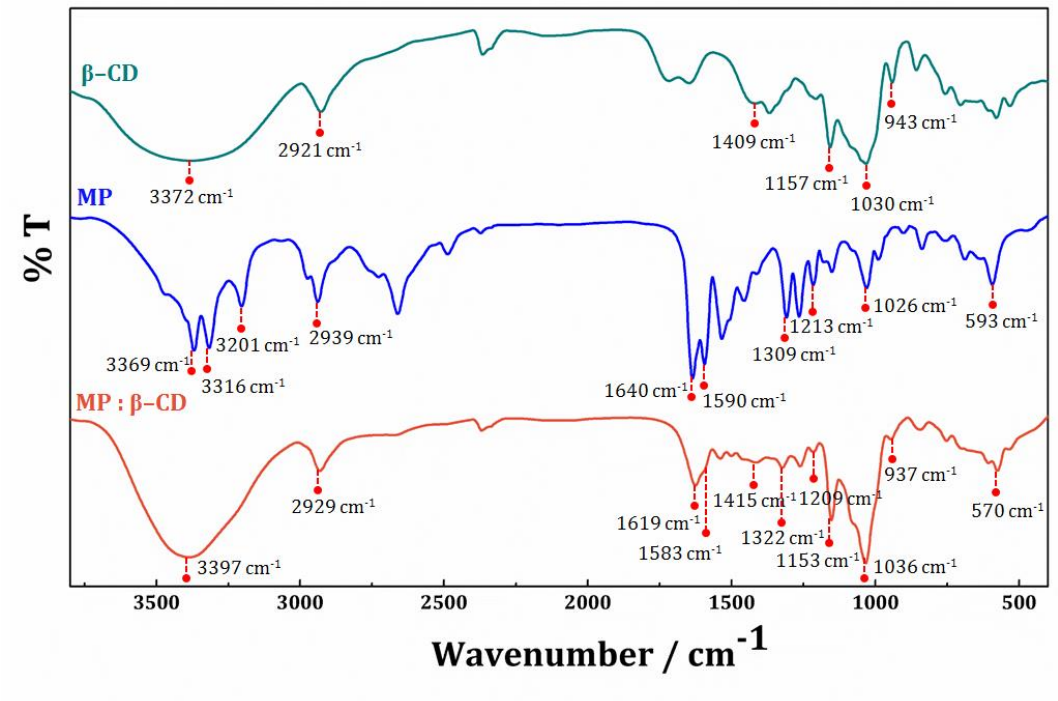


Figure VIII.11: FTIR spectra of free β-CD, MP and their 1:1 inclusion complex (MP:β-CD).

Table VIII.12: Comparison between the Frequencies change (cm^{-1}) of different functional group of free compound and their solid complexes

MP			
Functional Group	**Wavenumber (cm^{-1})**	**Functional Group**	**Wavenumber (cm^{-1})**
N-H asymmetric stretching of amide	3369	N-H bending	1590
N-H symmetric stretching of amide	3201	(aromatic)-C-N stretching	1309
N-H stretching of amine	3316	–C-O asymmetric stretching of $-OCH_3$	1213
>C=O stretching of amide	1640	–C-O symmetric stretching of $-OCH_3$	1026
Aromatic –C-H streching	2939	–C-Cl stretching	593
α-Cyclodextrin		**β-Cyclodextrin**	
Functional Group	**wave number/ cm^{-1}**	**Functional Group**	**wave number/ cm^{-1}**
stretching of O-H	3363	stretching of O-H	3372
stretching of –C-H from $-CH_2$	2926	stretching of –C-H from $-CH_2$	2921
bending of –C-H from $-CH_2$ and bending of O-H	1416	bending of –C-H from $-CH_2$ and bending of O-H	1409
bending of C-O-C	1150	bending of C-O-C	1157
stretching of C-C-O	1042	stretching of C-C-O	1030
Skeletal vibration involving α-1,4 linkage	943	skeletal vibration involving α-1,4 linkage	943

MP-α-CD inclusion complex		MP-β-CD inclusion complex	
Functional Group	**wave number/ cm^{-1}**	**Functional Group**	**wave number/ cm^{-1}**
stretching of O-H of α-CD	3398	stretching of O-H of β-CD	3397
stretching of –C-H from $–CH_2$ of α-CD	2932	stretching of –C-H from $–CH_2$ of β-CD	2929
bending of –C-H from $–CH_2$ and bending of O-H of α-CD	1420	bending of–C-H from $–CH_2$ and bending of O-H of β-CD	1415
bending of C-O-C of α-CD	1156	bending of C-O-C β-CD	1153
Stretching C-C-O	1030	Stretching C-C-O	1036
skeletal vibration involving α-1,4 linkage	938	skeletal vibration involving β-1,4 linkage	937
>C=O stretching of amide	1624	>C=O stretching of amide	1619
(aromatic)-C-N stretching	1317	(aromatic)-C-N stretching	1322
–C-Cl stretching	578	–C-Cl stretching	570
N-H bending	1587	N-H bending	1583
–C-O asymmetric stretching of $–OCH_3$	1210	–C-O asymmetric stretching of $–OCH_3$	1209

VIII.3.9 Driving force of the inclusion complex formation

The formation of host-guest ICs between the MP drug and CDs not only depends upon the size of the guest molecules but also on the cavity diameter of host. The cavity diameter of α and β-CD are 4.7-5.3 Å and 6.0-6.5Å respectively. Considering the size of MP, it is found that β-CD is more suitable for forming ICs due to the size of its cavity is more appropriate to encapsulate MP molecules which is in agreement with spectroscopic and physicochemical observations. Another structural suitability of the CD molecule for the hydrophobic guest molecule has been explained by Shekaari and his co-worker [61] where, polar water molecules inside into the hydrophobic CD molecule are bound by polar-apolar interaction which is however not so strong and as a consequence the relatively more hydrophobic drug molecules form inclusion complex with relatively stronger apolar-apolar interaction removing the water molecules from the cavity It results in a more stable lower energy state of the system and also reduces the ring strain of CD moiety. The stiochiometry of the host guest IC is 1:1 probably because of difficulty for the second molecule of MP to be trapped by the cavity after inclusion of one. The N atoms of the MP form H-bonds with the –OH groups at the rim of CD, thus stabilizing the whole IC.

VIII.4. CONCLUSION

The results obtained from UV-Visible, Fluorescence, NMR, FT-IR spectra and Mass analysis serve as a proof for the formation of inclusion complex of MP with both α and β-CD and MP. Taking all the parameters and results in account the plausible mechanism of the inclusion was depicted. The association constants of the complexes, calculated from uv and fluorescence data, discovered that the capability of β-CD to form an inclusion complex with MP is higher than that of α-CD, probably due to its larger cavity size. ^{1}H NMR data demonstrated that the hydrophobic aromatic ring with the amide (-CONH) and methoxy (-OMe) residues of MP were embedded inside the cavity of CDs, leaving the other residue exposed outside the cavity, while surface tension, conductivity and Job's measurement suggest 1:1 stoichiometry. Therefore, complexes of MP with CDs would certainly show advantage over the free drug usage in the field of medicine. As the CDs are prepared from starch by enzymic conversion, their safety profile is also assured and this formulation would open new vistas in the field of drug delivery.