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Changes in the conformational structure, microscopic and macroscopic pK_as of meloxicam on complexation with natural and modified cyclodextrins

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Spectroscopic and phase solubility techniques have been used to study the complexation of neutral meloxicam (Mel) with α-, β-, γ- and HP-β-cyclodextrins (CDs). The results indicate that neutral Mel has two conformational structures, enol and zwitterions, with the latter more dominant in water. The two pK_as of Mel were found to change in the presence of β-CD, where a blue shift in λ_{max} was observed but not in the presence of α, HP-β- and γ-CD. Rigorous analysis of phase solubility diagrams indicate that β- and HP-β-CD form 1 : 2 Mel/β-CD type complexes with Mel while α- and γ-CD form only 1 : 1 complexes. The fact that the overall 1 : 2 Mel/CD complex formation constant (β_{12}) was found significantly higher for β-CD than for HP-β-CD, combined with further spectroscopic studies, indicate that β-CD favors inclusion of the neutral enol form over the zwitterion. Unlike α-, HP-β- and γ-CDs, the hydrophobic microenvironment of a tight 1 : 2 Mel/β-CD complex was found to mimic those of organic solvents, thus favoring inclusion of the enol rather than the zwitterion, and hence shifting the tautomerization equilibrium towards the enol conformer.

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of α-1,4-D-glucose units. CDs have hydrophobic cavities at the center of the molecule allowing formation of inclusion complexes with a range of organic compounds (Duchene 1987). They are used to improve solubility, bioavailability and stability (Uekama et al. 1998). They are also used to reduce irritation of some irritant compounds *in vivo* (Lin et al. 1989).

Meloxicam (Mel) is a non-steroidal anti-inflammatory drug belonging to the oxicam group (Noble and Balfour 1996). It has a limited solubility in water. Tsai et al. (1993) reported that Mel exhibits only one pK_a (4.08) corresponding to the enolic OH. In contrast, Luger et al. (1996) estimated two pK_a values for Mel in aqueous solution, at 1.09 and 4.18, corresponding to ionization of the protonated thiazole nitrogen and the enolic OH group, respectively. Thus, Mel exists as an anion at neutral pH and in weakly basic solutions, and is converted to cationic species at very low pH. Around its isoelectric pH (2.6), neutral Mel assumes two tautomeric conformational structures in water: zwitterion and enol.

β-CD and some of its derivatives were used to enhance the solubility and bioavailability of Mel in aqueous solutions (Struengmann et al. 1999). Further, the dissolution rate of Mel in water was enhanced in the presence of α-, β- and γ-CD (Naidu et al. 2004). Banerjee et al. (2004) investigated the Mel/β-CD inclusion complexation in aqueous solution (pH = 5.5) using steady state fluorescence measurements, and suggested that neutral Mel was incorporated into to the β-CD cavity with a 1 : 1 binding stoichiometry and a complex formation constant of $114\,M^{-1}$. In their work, they assumed the absence of zwitterions based on the work of Tsai et al. (1993). However, no further studies aimed at validating the form of neutral Mel encapsulated into β-CD were pursued.

The present work reports a spectroscopic investigation of which form of the neutral Mel (enol or zwitterion) is more favourably complexed with α-, β-, HP-β- and γ-CD. Combined with pH solubility profiles in the absence and presence of β-CD, it was possible to estimate both macroscopic and microscopic pK_as of Mel, and determine which way complexation with CD affects the Mel pK_as and the zwitterions-to-enol tautomerization equilibrium. Phase solubility techniques were also used to estimate the Mel/CD complex formation constants for α-, β-, HP-β- and γ-CD as well as soluble host-guest complex stoichiometry.

2. Investigations, results and discussion

Estimates of the macroscopic Mel pK_a values were obtained through nonlinear regression of the aqueous pH-solubility profiles depicted in Fig. 1, which were measured in the absence and presence of 7.6 mM β-CD in 0.05 M phosphate buffer at 30 °C. In the absence of β-CD, two macroscopic pK_a's were obtained within the pH range 0

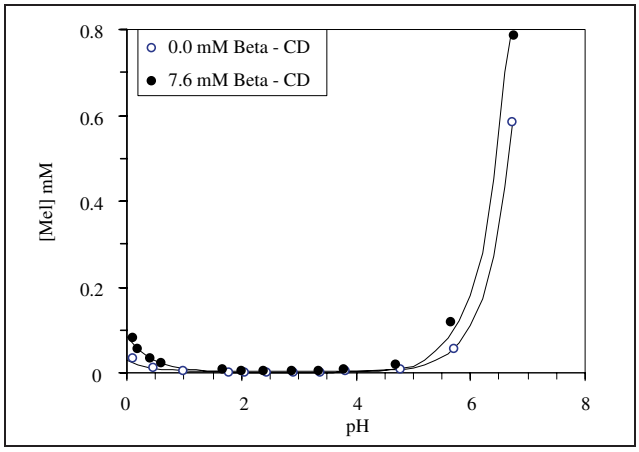


Fig. 1: pH solubility profiles of Mel in the absence and presence of 7.6 mM β-CD using 0.05 M phosphate buffer at fixed ionic strength ($\mu = 0.2$) and 30 °C

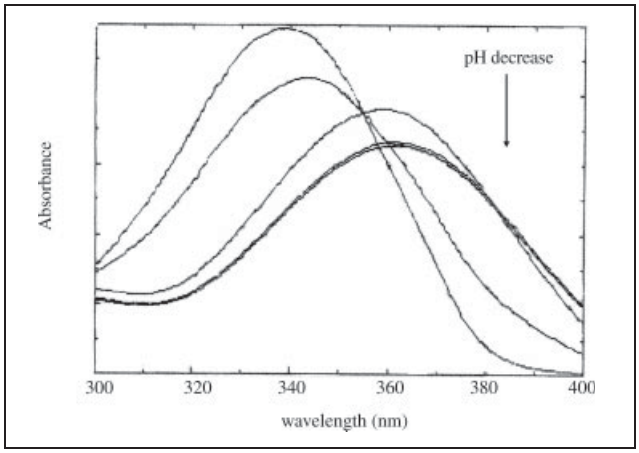


Fig. 2: Variation of the UV/Visible absorption spectrum of Mel (5.69 μM) in 0.05 M phosphate buffer ($\mu = 0.2$) at different pHs (in arrow direction, pH: 5.5, 2.8, 2.0, 1.0, 0.0)

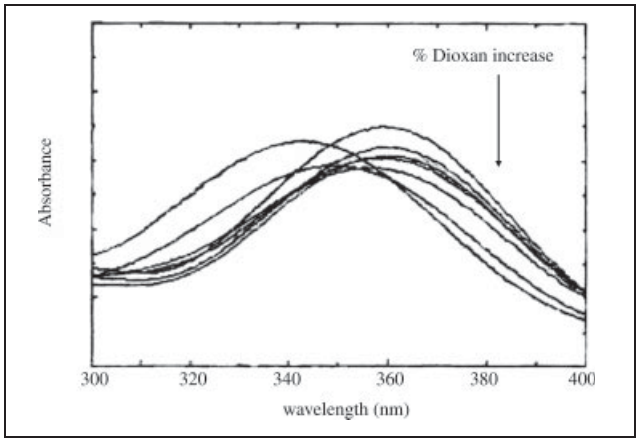


Fig. 3: Variation of the UV/Visible absorption spectrum of Mel (5.69 μM) with the concentration of dioxane in a water-dioxane solvent mixture (in arrow direction, % dioxane increases from 0 to 10, 20, 40, 60, 80 and 100% v/v)

to 8: $pK_{a1} = 1.10$ corresponding to deprotonation of the enolic group of cation (1) to yield an equilibrium mixture of the zwitterion (2) plus the neutral enol (3) species, and $pK_{a2} = 4.20$ which corresponds to deprotonation of the equilibrium zwitterion (2) plus enol (3) mixture to yield the anion (4) (Scheme). The pK_{a1} and pK_{a2} values obtained for Mel at 30 °C are in close agreement with those reported in the literature for buffered aqueous solutions of Mel at 23 °C (Luger et al. 1996).

Referring to the following microscopic ionization scheme involving cation **1**, zwitterion **2**, enol **3** and anion **4**, and noting that $K_{a12}\,K_{a24} = K_{a13}\,K_{a34}$, the macroscopic ionization constants are related to the microscopic ionization constants according to (Takacs-Novak et al. 1995 and 2004):

$$K_{a1} = K_{a12} + K_{a13} = K_{a12}(1 + K_{23})$$

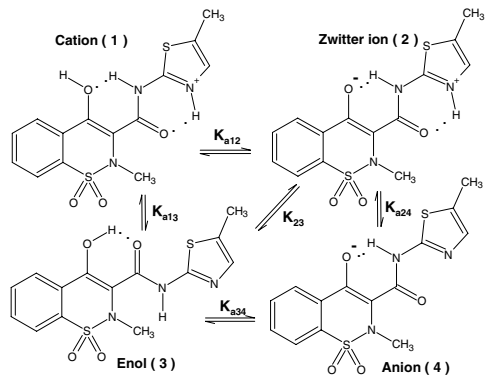
$$K_{a2} = K_{a12}K_{a24}/(K_{a12} + K_{a13}) = K_{a13}K_{a34}/(K_{a12} + K_{a13}) = K_{a24}K_{a34}/(K_{a24} + K_{a34})$$

where K_{23} is the zwitterion **2** to enol **3** tautomerization constant defined by

$$K_{23} = [\text{enol } \mathbf{3}]/[\text{zwitterion } \mathbf{2}] = K_{a13}/K_{a12} = K_{a24}/K_{a34}$$

Analysis of the variation of absorption spectra of Mel (5.69 μM) with pH (Fig. 2) and with % dioxane concentration in an aqueous dioxane-water mixtures (Fig. 3) yielded an estimate of the zwitterion-to-enol tautomerization constant K_{23} at 0.0162, which indicates that the zwitterion is highly stabilized in water over the enol form.

Scheme



Combining the variation of the inherent solubility of Mel (S_o) with pH (from pH solubility profiles) at pHs 1.10, 2.80 and 6.80 (0.0328, 0.00137 and 5.71 mM, respectively) with the macroscopic pK_a values in the absence of β-CD ($pK_{a1} = 1.10$, $pK_{a2} = 4.20$), the microscopic ionization constants were estimated at $pK_{a12} = 1.11$, $pK_{a13} = 2.90$, $pK_{a24} = 4.19$, $pK_{a34} = 2.40$ (Table 1). Thus it is apparent that the measured macroscopic pK_a values of Mel are essentially those of the zwitterion, which is predominant over the enol form in water ($K_{23} \ll 1$).

Table 1: Estimates of macroscopic pK_a values (pK_{a1} and pK_{a2}) for Mel in the absence and presence of 7.6 mM β-CD obtained from pH-solubility profiles at 30 °C where $\Delta pK_a = pK_{a\,\text{Complex}} - pK_{a\,\text{Mel}}$

	Mel	$Mel_{complex}$	ΔpK_a
pK_{a1}	1.10 (0.10)	2.3 (0.10)	1.20 (0.20)
pK_{a2}	4.20 (0.10)	3.90 (0.1)	−0.30 (0.20)
pK_{a12}	1.11		
pK_{a13}	2.90		
pK_{a24}	4.19		
pK_{a34}	2.40		
K_{23}	0.0162		

The microscopic pK_a values and the zwitterions-to-enol tautomerization constant (K_{23}) of Mel are also indicated (look up the microscopic ionization and tautomerization scheme). Numbers in brackets denote standard errors

In contrast, the corresponding macroscopic pK_a values obtained for Mel in the presence of β-CD (7.6 mM) were estimated at 2.30 and 3.90, respectively (Table 1). This indicates that on complexation of Mel with β-CD, the pK_{a1} value shifts from 1.10 to 2.30 ($\Delta pK_{a1} > 0$) while the pK_{a2} value shifts from 4.20 to 3.90 ($\Delta pK_{a2} < 0$), which also suggests that the neutral enol form of Mel is more favorably included into the hydrophobic β-CD cavity than the highly charged zwitterion.

Further studies were conducted by measuring the UV absorption spectrum of Mel (5.0 μM, 300 to 400 nm) at different β-CD concentrations (Fig. 4). The results indicate that the absorption maximum at 360 nm, which corresponds to the dominant zwitterion of Mel at pH 2.8, consistently shifts towards 338 nm as β-CD concentration increases. This behavior was found similar to the shift observed as the concentration of the organic solvent (dioxane) increases in aqueous Mel (5.69 μM) solution (Fig. 3). These findings indicate that the conformational structure of Mel shifts from the zwitterion to the enol form as the concentration of β-CD increases in aqueous solution. In other words, complexation with β-CD favors the inclusion of the neutral enol form of Mel into the hydrophobic β-CD cavity rather than the highly charged zwitterion.

It is interesting to note that no notable shift in the absorption spectrum (300 to 400 nm) of Mel (5.0 μM) was observed upon the addition of either α-, γ- or HP-β-CDs. This strongly indicates that neutral Mel does not shift to enol upon complexation except in the β-CD case. To have a better picture and to understand these observations, phase solubility diagrams of Mel with different CDs (α-, β-, HP-β- and γ-CD) were measured at pH 2.8 and 30 °C, where Mel essentially exists as a zwitterion with very little enol form ($K_{23} \ll 1$), and the results are depicted in Fig. 5. Rigorous analysis of the phase solubility diagrams, through linear and nonlinear regression procedures discussed elsewhere (Zughul and Badwan 1998) yielded estimates of the complex formation constants, which are listed in Table 2. Both β-CD and HP-β-CD form 1 : 1 and 1 : 2 Mel/CD type complexes in solution, while α- and γ-CD form only 1 : 1 type complexes. Formation of 1 : 1 type complexes at pH 2.8 and 30 °C follows the order α-CD < β-CD < HP-β-CD < γ-CD. This order indicates the lower affinity of the fairly large Mel molecule to include into the relatively small α-CD cavity, an affinity that increases with an increase in the size of the CD-cavity going from α-CD to γ-CD. It is also apparent that the presence of rather loose and peripheral hydroxypropyl groups in HP-β-CD allows better interaction of Mel with one HP-β-CD than with one β-CD. In contrast, the overall 1 : 2 complex formation constant ($\beta_{12} = K_{11}\ K_{12}$) for 1 : 2 Mel/CD complex is significantly higher for β-CD than HP-β-CD (Table 2). Thus two β-CD molecules tend to enclose one Mel molecule in their cavities more tightly than would one or two HP-β-CD molecules under same conditions of CD concentration (7.6 mM), pH (2.8) and temperature (30 °C). This is clearly evident in Fig. 6 which shows the variation of the concentration of 1 : 1 and 1 : 2 type Mel/CD complexes for β-CD and HP-β-CD against CD concentration at pH 2.8. The figure shows that the 1 : 2 Mel/β-CD complex predominates over the 1 : 1 com-

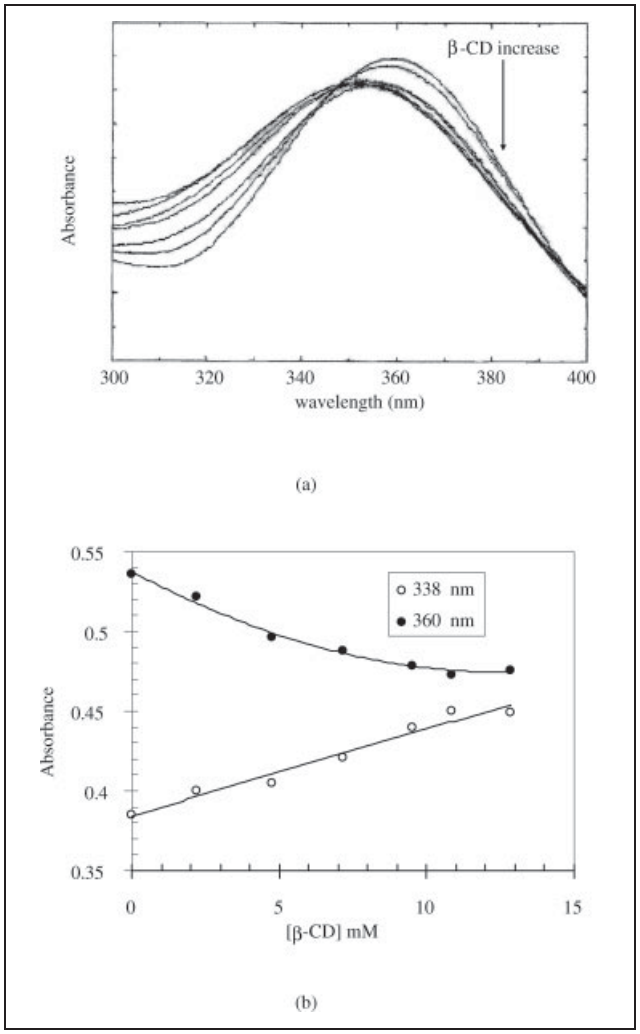


Fig. 4: Variation of the (a) UV/Visible absorption spectrum of Mel (5.0 μM) at different β-CD concentrations (b) absorbance of the zwitterion at 360 nm and of the enol form of Mel at 338 nm with β-CD concentration

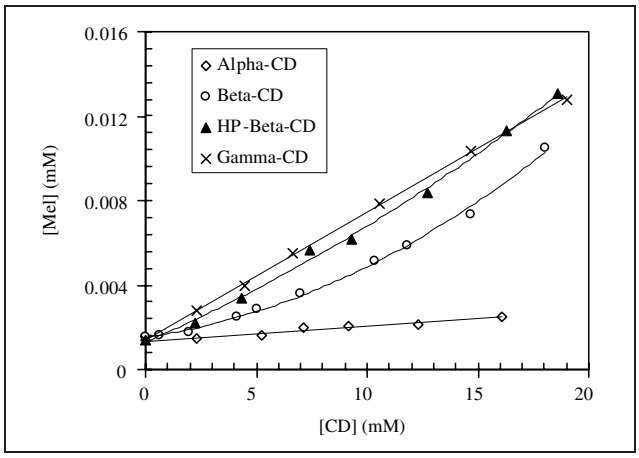


Fig. 5: Phase solubility diagrams of Mel in aqueous CD solutions of 0.05 M phosphate buffer (μ = 0.2) at pH 2.8 and 30 °C

Table 2: Estimates of complex formation constants for Mel with each of α-, β-, γ- and HP-β-CD obtained from phase solubility diagrams measured at pH around 2.8 and 30 °C

CD	K_{11} (M^{-1})	K_{12} (M^{-1})	β_{12} (M^{-2})
α-CD	52 (3)	–	–
β-CD	92 (4)	135 (7)	1.24 (0.12) × 10^4
HP-β-CD	321 (11)	23 (2)	7.38 (0.90) × 10^3
γ-CD	447 (16)	–	–

Numbers in bracket denote standard errors

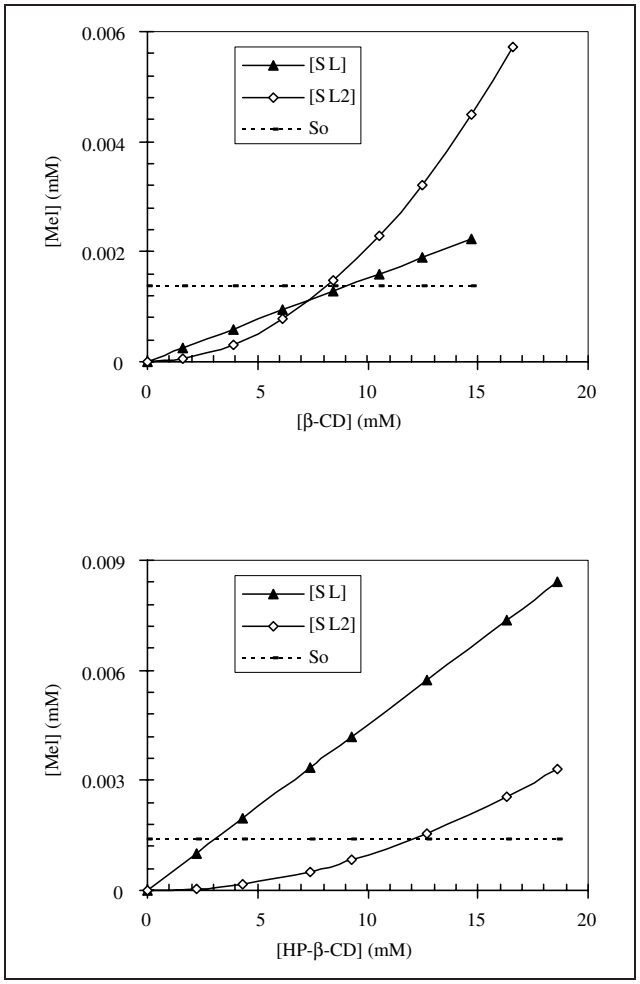


Fig. 6: Variation of 1 : 1 and 1 : 2 Mel/β-CD complex concentrations with (a) β-CD concentration and (b) HP-β-CD concentration, both in 0.05 M phosphate buffer ($\mu = 0.2$) at pH 2.8 and 30 °C

plex beyond 7.5 mM CD while the opposite is true for the Mel/HP-β-CD. This evidently corroborates the finding that the 1 : 2 Mel/β-CD complex favors inclusion of the neutral enol form of Mel more than the corresponding highly charged zwitterion, which also explains the variation of pK_as of Mel in the presence of β-CD from those in the absence of β-CD.

The results given in Table 2 do not agree with those reported earlier on Mel/CD complexes for α-, β- and γ-CD (Naidu et al. 2004). This is apparently due to the fact that Naidu et al. conducted their measurements using unbuffered water where Mel exists as a mixture of its anionic plus nonionic species, at 28 °C, while in this work the phase solubility diagrams were obtained in aqueous solutions buffered at pH 2.8 and 30 °C, where the neutral form is predominant. Moreover, the phase solubility diagrams of Mel/β-CD and Mel/HP-β-CD obtained in this work were clearly of A_P type thus indicating the formation of higher order complexes, unlike those of Naidu et al. where A_L type diagrams were obtained for unbuffered water. The value of K_{11} reported by Banerjee et al. (2004) for Mel/β-CD at pH 5.5 was $114 \pm 15\,M^{-1}$, which though appears close to 92 ± 4 obtained in this work, yet it was estimated at a higher pH and assuming only 1 : 1 complex formation. This strongly indicates that pH as well as medium effects influence both complex stoichiometry and stability in solution.

In conclusion, Mel forms soluble 1 : 1 complexes with CDs at pH 2.8 with the following order of complex stability: α-CD < β-CD < HP-β-CD < γ-CD. In addition, Mel forms soluble 1 : 2 type complexes only with β- and HP-β-CD at the same pH. The enol form of Mel is more favorably included into the hydrophobic cavities of a tight 1 : 2 Mel/β-CD complex configuration more than the zwitterion. The shifts in the macroscopic ionization constants of Mel towards those of the enol form in the presence of β-CD were evident from corresponding shifts in the UV absorption spectrum of Mel, which were observed as the concentration of β-CD or dioxane added to a fixed concentration of Mel in water increases. No similar shifts were observed in the presence of α-, HP-β or γ-CD.

3. Experimental

3.1. Materials

All chemicals were provided by The Jordanian Pharmaceutical Manufacturing Co. (JPM). Mel was of pharmaceutical grade obtained from Sun Pharmaceutical/India. β-CD and HP-β-CD were obtained from AVEBE/Netherlands while α-CD and γ-CD were obtained form Cerestar/Germany. Phosphoric acid, sodium hydroxide and sodium chloride were of analytical grade obtained from Surechem/UK. All other chemicals were of analytical grade, and water was double distilled and deionized (0.8 μS).

3.2. Instrumentation

Thermostatic water bath shaker (1083, GFL/Germany), UV/Visible spectrophoto-meter (Du 650i, Beckman/ USA) and pH meter (3030, Jenway/UK).

3.3. Methods

3.3.1. pH-Solubility profiles

Equal amounts of Mel in excess of its equilibrium solubility were added to 50 mL of 0.05 M phosphate buffer solutions ($\mu = 0.2$) at different pHs. These solutions were shaken at 200 pm for 48 h at 30 °C then let to settle and filtered through 0.45 μm, 25 mm diameter, cellulose acetate disposable syringe filters (MFS/USA). The pH of each solution was measured, and then suitably diluted with sodium hydroxide solution to pH 11 and the absorbance was measured at a fixed wavelength of 360 nm. The solubility in each solution was calculated from a predetermined calibration curve. The same procedure was repeated in the presence of 7.6 mM β-CD.

3.3.2. Phase solubility diagrams

Phase solubility studies were performed as described by Higuchi and Conners (1965). Equal amounts of Mel in excess of its equilibrium solubility were added to flasks containing 50 mL buffered aqueous CD solutions (0.05 M phosphate buffer, $\mu = 0.2$) at pH 2.8 of different CD concentrations. The flasks were left shaking for 48 h in the water bath shaker at 200 rpm to attain equilibrium at 30 °C. The solutions were filtered through 0.45 μm, 25 mm diameter, cellulose acetate disposable syringe filters (MFS/ USA). The pH of each solution was measured and the solubility determined as indicated above.

3.3.3. UV-Spectroscopy study

Effect of pH: A stock solution of Mel (0.285 mM) was prepared in 0.1 M NaOH and used to prepare solutions of Mel at a fixed concentration (5.69 μM) but different pHs using 0.05 M phosphate buffer (ionic strength $\mu = 0.2$).

Effect of CD: A stock solution of Mel (5.0 μM) was prepared in 0.05 M phosphate buffer at pH 2.8 $\mu = 0.2$). The solution was filtered and used to prepare different concentrations of CDs by the addition of different weights of solid CDs. Absorbance was also measured at different wavelengths (300–400 nm) using 5 cm UV cell.

Effect of water-dioxane composition: A stock solution of Mel (0.285 mM) was prepared in liquid dioxane. Solutions of Mel (5.69 μM each) were prepared in dioxane-water mixtures (0 to 100% v/v) and the absorbance measured at different wavelengths (300–400 nm).

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