CHAPTER VIII

PHYSICOCHEMICAL INVESTIGATIONS OF DIVERSE INTERACTIONS BETWEEN A SIGNIFICANT BIO-ACTIVE MOLECULE AND CYCLIC OLIGOSACCHARIDES IN VARIOUS PHASES

8.1. Introduction

Padimate O [2- ethylhexyl 4-(dimethylamino) benzoate] (PMO) is a UV- absorbing agent (drug) and is water insoluble oily-liquid and currently used as an ingredient in cosmetics and sunscreen formulations. It absorbs some of the sun's UV radiation and thereby, preventing direct DNA damage by UV-B and protect skin against sunburn [1]. UV radiation in the range of 290-320nm are known as UV-B. UV-B is responsible for most important biological effects on human body, as for skin, it causes short and long term harmful effects [2]. So, careful use of PMO can slow or momentarily prevent the skin problems such as wrinkles, sunburn, sagging skin etc. Sunscreen based on PMO also reduces the number of and delays the appearance of UV-induced skin tumors [3-5]. In this purpose cyclodextrin can play an important role as it acts as an efficient good and safe drug carrier in human body [6-9].

Cyclodextrins (cyds) are well known non-toxic truncated macrocyclic host molecules, consisting of (α-1, 4) linked by glucopyranose units [10]. Cyds are commercially accessible in the form of α, β and γ with varying number of glucose units namely six, seven and eight respectively. Cyd**s** are proficient enough to form water soluble inclusion complexes with many lipophilic water insoluble drugs (guest) and also improve the physicochemical properties of guest molecules [11-13]. The cyd**s** are also able to form complex with some cosmetic ingredients and their solubility, thermal-stability, bioavalibility, bad odour, skin delivery are deeply improved [14-16]. Cyd**s** do not absorb UV-Vis light, so they are able to protect a UV active guest molecule from oxidation and photo-degradation [17]. Hence, encapsulation of sunscreen-agents in the core of Cyds has developed into an interested field of study [18]. The solubility and photo-stability of sun-screen agents have been increased through complexation with cyds [19]. No covalent bonds are formed or broken during complex formation and in aqueous solution in aqueous solution, the complexes are readily dissociated and free guest molecules are in equilibrium with the molecules bound within the cyd cavity. This is a dynamic process whereby the guest molecule continuously associated and dissociated from the host cyd.

The aim of this present work is to form inclusion complexes of PMO with α-cyd and β-cyd respectively in aqueous as well as in solid phase (scheme S1) and controlling the release of the sunscreen agent without any chemical and biological modification. These complex formation have been investigated by the study of UV-Vis, FTIR, Mass spectroscopy and XRD methods.The results revealed that the complexes are formed sucessfully with 1:1 stoichiometry. So, the resultant inclusion complexes of PMO with cyds cane be potentially introduced in cosmetic delivery systems as new prospect.

8.2. Experimental Section

8.2.1. Chemicals

Padimate O, α-cyclodextrin and β-cyclodextrin of puriss grade are bought from SigmaAldrich, Germany and used as such. The mass fraction purity of Padimate O, α-cyclodextrin and β-cyclodextrin are ≥ 0.99, ≥ 0.99 and ≥ 0.98, respectively.

8.2.2. Apparatus

As Padimate O is insoluble in water, PMO solutions were prepared in aqueous ethanol. Solubilities of α and β- cyd have been tested in triply distilled and degassed water and found fair solubility of the cyclodextrins. All the stock solutions are prepared by mass (weighed by Mettler Toledo AG-285 with uncertainty ±0.0003g), the solutions are prepared by mass dilution at 298.15K. Solutions are prepared taking care to avoid weight loss due to evaporation.

UV–visible spectra were taken by JASCO V-530 UV/VIS Spectrophotometer, with uncertainty in wavelength as ±2 nm.

Fourier transform infrared (FT-IR) spectra were recorded on a Perkin Elmer FT-IR spectrometer according to the KBr disk technique. A manual press was used to form the pellets. The FTIR measurements were performed in the scanning range of 4000−400 cm^{-1} at room temperature.

HRMS analyses were executed with Q-TOF high resolution instrument by positive mode electro-spray ionization.

The powder XRD patterns of the compounds were recorded by using Cu-Kα radiation (Bruker D8 Discover; 40kV, 30 mA).

Thermogravimetric analysis (TGA) was carried out (Metler Toledo) in nitrogen atmosphere (flow rate = 50 mL min−1) in the temperature range of 20−900 °C.

8.2.3. Preparation of solid inclusion complex

The two solid inclusion complexes [PMO/α- cyd and PMO/β- cyd] have been prepared taking 1:1 molar ratio of both the drug and cyds. In each case 1.0 mmol cyd is dissolved in 20 mL water and 1.0 mmol of PMO in 20 mL ethanol separately. Then the ethanol solution of PMO is added to the aqueous cyd solution and shielded from light. The mixture is then allowed to stir for 72 hrs at room temperature. The solvent is evaporated under vacuum at 50^{o}C with a rotary evaporator. The resulting residue is filtered and a solid white powder is found, which is dried in vacuum desiccators for 4 days in presence of P_2O_5 as drying agent [20].

8.3. Result and Discussion

8.3.1. Job plot: demonstrates the stoichiometry of the host–guest inclusion complex

One of the important technique that was used to investigate the stoichiometry of ICs is Job's method [21], with the help of UV-Vis spectra by plotting ΔA×R versus R (where, ΔA= difference of absorbance of PMO in presence and absence of cyd and R= [PMO]/[PMO]+[cyd]). Absorbance values are monitored at 308nm at 298 K for a series of solution (Table 1and 2). The plots (Fig. 1) depict a maximum at ~0.5 of the 'R' fraction demonstrating one cyd molecule binds with one PMO monomer unit, i.e. 1:1 stoichiometry [22-25] and further support its determination by ESI-mass technique.

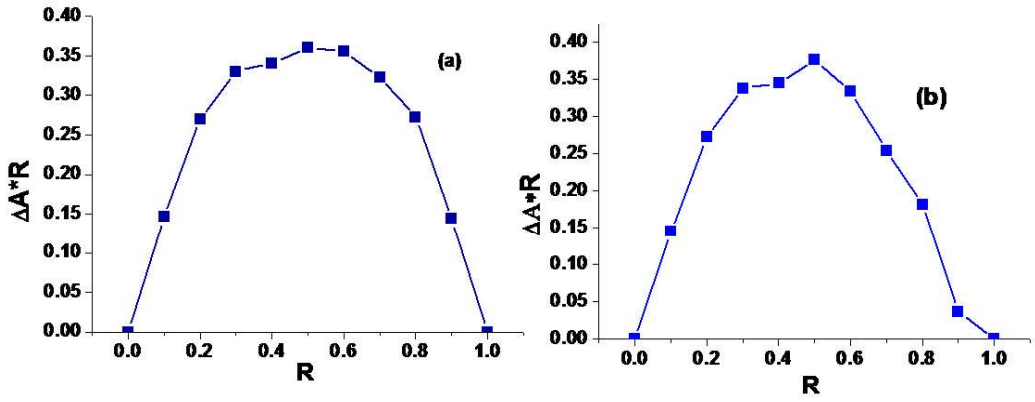


Figure 8.1. Job plot of (a) PMO/α- CD and (b) PMO/β-CD systems at 298.15K.

8.3.2. Association constant of complexes and thermodynamic parameters

The absorption spectra of the ICs of PMO/α- cyd and PMO/β- cyd systems at three different temperatures have been performed, where the concentration of guest PMO is kept constant (50 μM) while CDs concentrations are varied. In both cases, the association

constant (Ka) for the formation of PMO/CDs complexes have been estimated by considering the changes of intensity of absorption maxima at 308nm with the CD concentrations. It is observed that with increasing CDs concentrations the intensity of PMO increased gradually (Table 8.3-8.4). These facts confirm the encapsulation of PMO molecule into the hydrophobic core of Cyds molecule as a consequence of presence of non-covalent interactions [25-26]. From the changes of absorbance the association constant (1:1 stoichiometry ratio) can be calculated by using double reciprocal plots of Benesi-Hildebrand equation 1 [27-28](Table8.1)

$$\frac{1}{\Delta A} = \frac{1}{\Delta \varepsilon Ka[PMO]} \times \frac{1}{[Cyd]} + \frac{1}{\Delta \varepsilon [PMO]} \quad (1)$$

The above mentioned equation is a linear equation and *Ka* value is measured dividing the intercept by the slope from double-reciprocal plots (Fig. 8.1-8.2)[29].

Table 8.1: Association constant (*Ka*) and thermodynamic parameters of different PMO/cyclodextrin complex systems.

	Temp. (K[a])	*Ka* (M^{-1})[b]	ΔH (KJ/mol)	ΔS ($Jmol^{-1}K^{-1}$)	ΔG ($kJmol^{-1}$) (298.15 K)
PMO+α-cyd	298.15	152.00	-36.73	-81.51	-12.45
	303.15	115.76			
	308.15	94.00			
PMO+β- cyd	298.15	225.83	-24.68	-37.79	-13.43
	303.15	185.69			
	308.15	163.56			

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

Again, with the help of the van't Hoff equation 2, various thermodynamic parameters (ΔH°, ΔS°, ΔG°) in the case of both ICs phenomena can be calculated (Table 1) using the value of *Ka* [30]

$$\ln Ka = -\frac{\Delta H^0}{RT} + \frac{\Delta S}{R} \quad (2)$$

Plots of ln*Ka* Vs.1/T are linear for both PMO/α-CD and PMO/β-CD complexes (Fig. 8.3). The enthalpy (ΔH°) and entropy (ΔS°) of the complexation process have been determined

from the plots. Negative value of ΔH° confirms that the processes are exothermic and there are some stabilization interactions in the systems, negative ΔS° indicates the presence of ordered arrangements i.e. occurrence of complex formation between the PMO molecules and the cyds. ΔG= -RTln*Ka* is useful equation to calculate the values of free energy change (ΔG°), negative ΔG° value suggests that the complexation phenomena are spontaneous and thermodynamically stabilized.

8.3.3. FT-IR study

The resulting bands in ICs of the included part of the guest molecules are altered or somewhere disappeared or their intensities altered [23, 28, 31]. The alternations of the vibrational frequencies of bonds are due to molecular interactions such as hydrogen bonds, hydrophobic, van der Waals interactions [32]. The infrared spectrum of α-CD, β-CD and their corresponding complexes (PMO/α-CD and PMO/β-CD) with pure PMO are shown in figure 2and 3. FTIR spectroscopy is a useful method to confirm the inclusion complex in solid state because

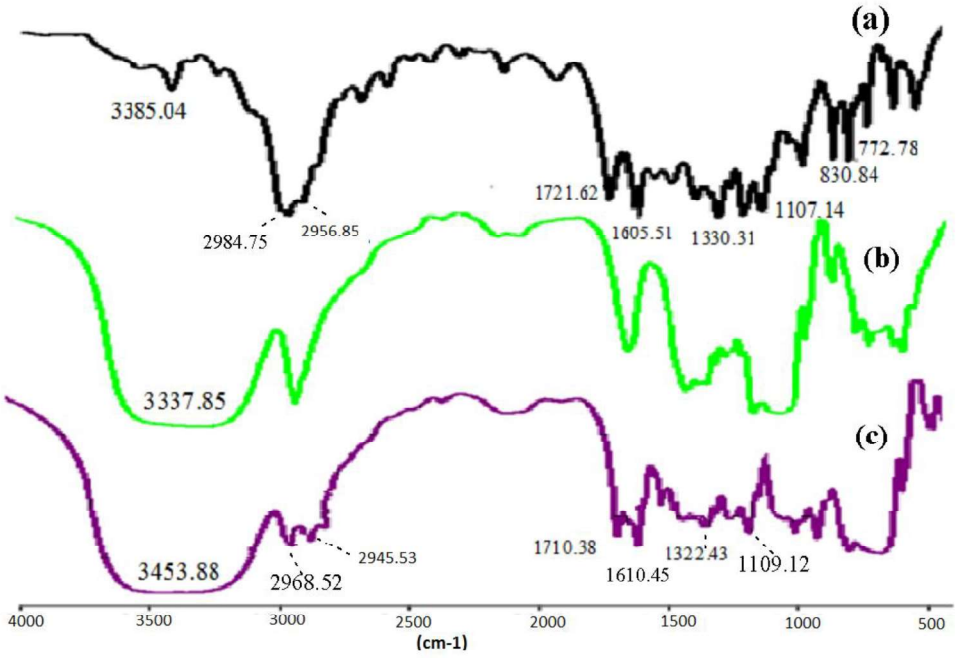


Figure 8.2. FTIR spectra of (a) PMO, (b) α-cyd and (c) PMO/α-cyd (1:1 molar ratio) solid inclusion complex in KBr.

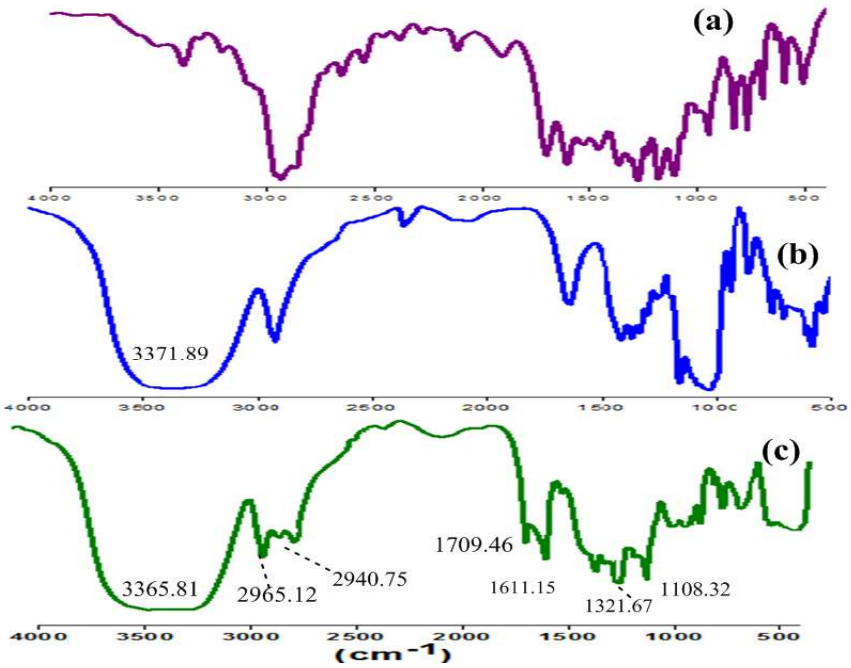
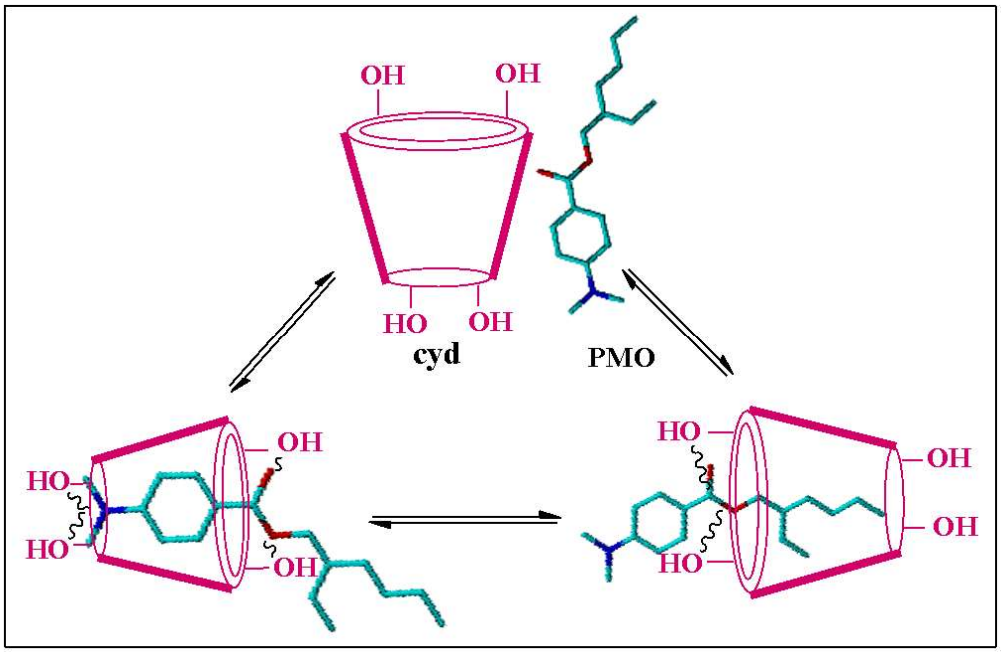


Figure 8.3. FTIR spectra of (a) PMO, (b) β-CD and (c) PMO/β-CD (1:1 molar ratio) solid inclusion complex in KBr.

The FTIR spectra of α-CD and β-CD are assigned by broad band at 3337.85 cm^{-1} and 3371.89 cm^{-1} due to primary and secondary —OH groups that are linked by H-bonds and the C-H stretching frequency of Cyds appeared at 2929.47 cm^{-1} and 2922 cm^{-1} respectively. The broad bands are shifted to higher frequency (α-CD: 3453.88 cm^{-1}, β-CD: 3365.81 cm^{-1}) and is broadened after formation of ICs and the C-H stretching frequency shifted to new frequency [33]. The characteristic peaks at 3385.04 cm^{-1} (aromatic C-H), 1721.62 cm^{-1} (>C=O group), 1107.14 cm^{-1} (C–O–), 1605.51 cm^{-1} (-C=C- benzene), 2956.85 cm^{-1} (sp^3 C-H) have been assigned for PMO in Figures 2(a) and 3(a) and the presence of these peaks in complexes Fig. 2 and 3(c) with significant reducing intensities and with new shifting values indicating the encapsulation of PMO molecule into the nano core of cyclodextrin. At the peaks around 772.78—830.84 cm^{-1} are appeared for di-substituted benzene. The characteristic peaks of -C—N- stretching vibration at 1330.31 cm^{-1} and for the -N—CH_3 stretching appears at 2984.75 cm^{-1} which were shifted to lower frequency at 1322.43/1321.67: α-CD/ β-CD cm^{-1} and 2968.52/2965.12: α-CD/β-CD cm^{-1} respectively and from this result we may conclude about the formation of inclusion complexes. These variations in the infrared spectrum of the ICs can be endorsed to intermolecular interactions of PMO with α-CD and β-CD respectively

and form these results we can predict the probable mechanism of interactions between the selected host and guest (1:1) molecule which is shown in scheme 8.1.



Scheme 8.1. The probable mechanism of the association phenomena of the complexation process for PMO with cyclodextrin with 1:1 stoichiometry.

8.3.4. Mass spectra

Electrospray ionization mass-spectrometry (ESI-MS) is very useful method to detect the stoichiometry in non-covalent complexes. Mass spectra method gives m/z ratios and thus provides direct information about the stoichiometry [34-36]. The positive ESI-mass spectrum of this work has been reported in Fig. 4 and the assigned peaks values have been listed in Table 2. Four intense peaks of singly charged ions at m/z ratios 1250.517, 1272.519, 1412.571 and 1434.575 representing the protonated and sodiated adduct of [PMO +α-CD+H]$^+$, [PMO +α-CD+Na]$^+$, [PMO +β-CD+H]$^+$ and [PMO + β -CD+Na]$^+$ respectively. These experimental results of the PMO/α-CD and PMO/β-CD complexes suggest the formation of ICs with 1:1 stoichiometry.

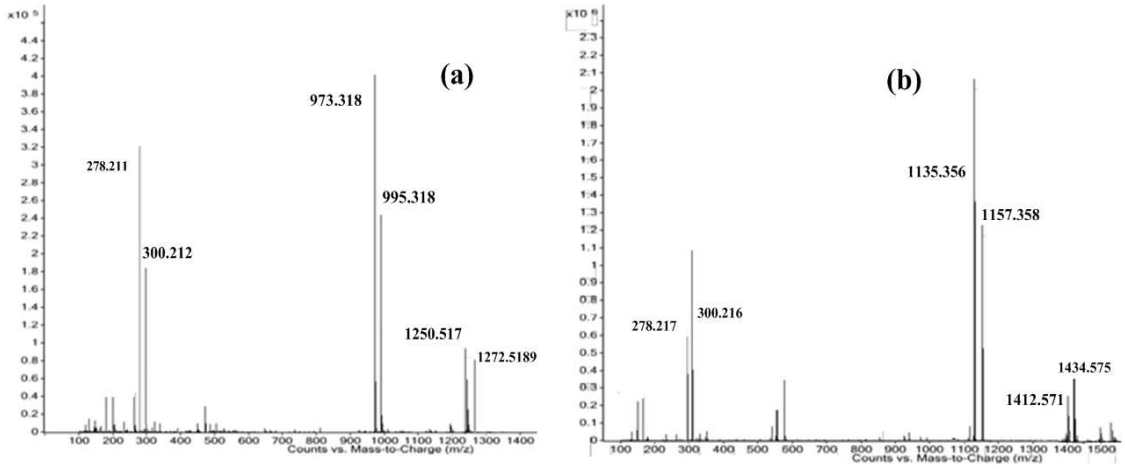


Figure 8.4. ESI mass spectra of (a) PMO/α-CD inclusion complex and (b) PMO/β-CD inclusion complex.

Table 8.2: The observed peaks at different m/z with corresponding ions for the solid inclusion complexes

PMO-α- cyd inclusion complex		**PMO -β- cyd inclusion complex**	
Ion	**m/z**	**Ion**	**m/z**
$[PMO+H]^+$	278.211	$[PMO+H]^+$	278.217
$[PMO+Na]^+$	300.212	$[PMO+Na]^+$	300.216
$[\alpha\text{- cyd} +H]^+$	973.318	$[\beta\text{- cyd} +H]^+$	1135.356
$[\alpha\text{- cyd} +Na]^+$	995.318	$[\beta\text{ - cyd} +Na]^+$	1157.358
$[PMO +\alpha\text{- cyd} +H]^+$	1250.517	$[PMO +\beta\text{- cyd} +H]^+$	1412.571
$[PMO +\alpha\text{- cyd} +Na]^+$	1272.519	$[PMO + \beta\text{ - cyd} +Na]^+$	1434.575

8.3.5. Powder X-ray diffraction (PXRD) pattern

Fig. 8.5 shows the X-ray diffraction pattern of α-CD, β-CD and their corresponding complexes with PMO. The intensive peaks of α-CD at 9.64, 12.05, 13.33, 14.31, 15.11, 15.79, 19.29, 19.92, 21.74 and of β-CD that is reported in our previous work [22] representing the crystalline nature of both Cyds that become reduced, disappeared and shifted in the solid complexes where the peaks indicate the amorphous nature of guest as well as cyclodextrins [37-38]. This suggests the assimilation of PMO molecule into the CDs cavity due to having channel-type packing structure of cyclodextrin [25, 39-43] and causing to enhance the solubility of PMO [44-45].

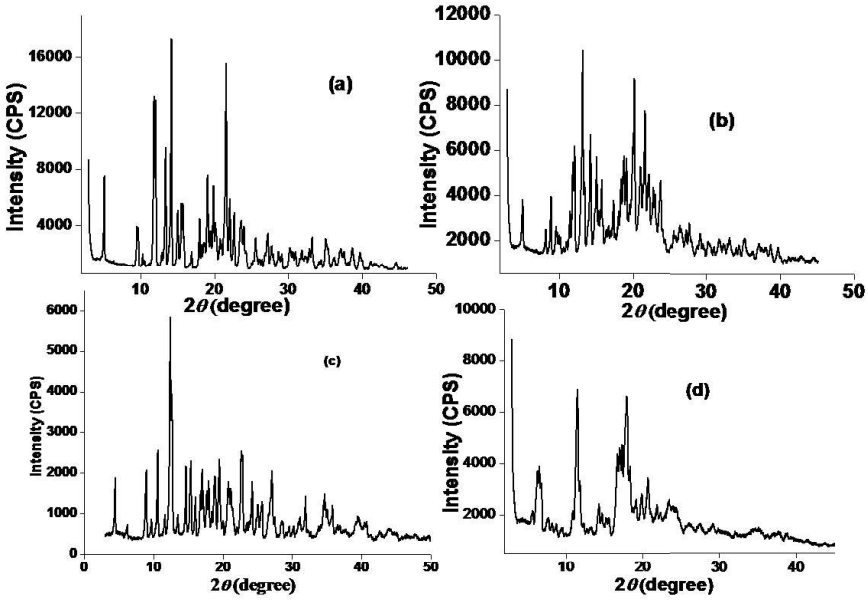


Figure 8.5. Powder X-ray diffraction pattern of (a) α-CD, (b) PMO/α-CD (1:1 molar ratio), (c) β-CD and (d) PMO/ β-CD (1:1 molar ratio) inclusion complex.

8.3.6. Thermo gravimetric Analysis (TGA)

Thermal degradation properties of PMO/α-CD and PMO/β-CD complexes have been studied by TGA method [11] where the weight loss of the samples measured as a function of temperature under nitrogen atmosphere at a heating rate of 3°C/min. The TGA profiles have been represented in Figure 6 where α-cyd and β-cyd exhibit two regions of major weight (wt.) loss. The first region at the temperature range at 32°C-120°C (α-cyd) and 33°C-125°C (β- cyd) demonstrated the loss of absorbed water from the samples and the second region

attributed to the decomposition of the samples at 236°C-298°C and 281°C-306°C respectively. On the other hand encapsulation forms of PMO/α-CD (32°C-143°C, wt. Loss: 5.47%; 143°C-238°C, wt. Loss: 24.14%; 238°C-350°C, 64.84%) and PMO/β-CD (33°C-150°C, wt. Loss: 7.14%; 150°C-245°C, wt. Loss: 14.87%; 245°C-375°C, wt. Loss: 71.71%) complexes show three steps of weight loss with increasing temperatures (Fig. 6(a)-6(b)) [46-47]. These changes in thermal analysis can be attributed to interactions of PMO with α-cyd and β- cyd and demonstrated to form stable inclusion complexes [45, 48].

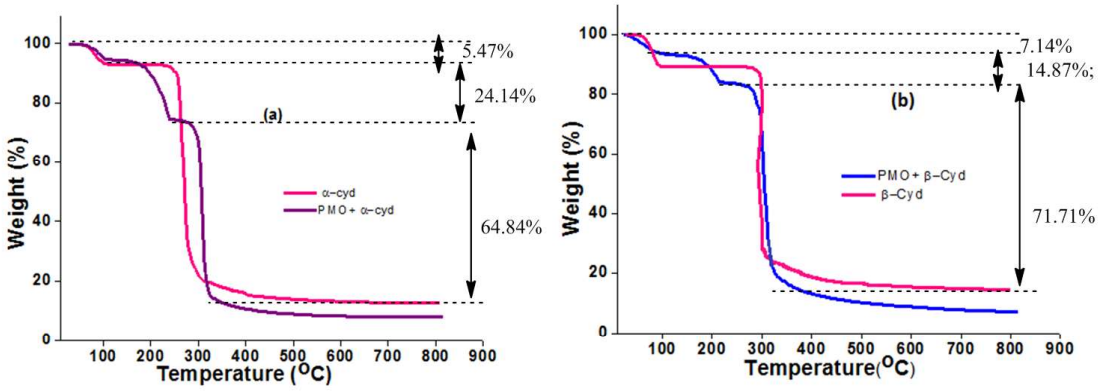


Figure 8.6. TGA profiles of (a) α-cyd ; PMO/α-cyd and (b) β-cyd; PMO/β-cyd inclusion complex systems.

8.4 Conclusion

From the results of our experiments, it has been concluded that the UV-B protector sunscreen agent padimate O can form an inclusion complex with α-CD and β-CD which can be used as regulatory releaser of this agent. The encapsulation of PMO molecule inside the hydrophobic core of cyclodextrins has been proved in aqueous medium by UV-Vis spectroscopy and in solid state by FTIR, Mass, XRD and TGA analysis. The stoichiometric behaviour with 1:1 ratio of the complexes has been confirmed through continuous variation job's method and ESI-MS experiments. The association constant values demonstrated PMO is somewhat in good agreement with β-cyd than α-cyd and the thermodynamic parameters making the overall process thermodynamically favourable. FTIR spectroscopy, PXRD and TGA support the complexation phenomena and according to the results of PXRD and TGA techniques, it can be said that newly obtained PMO/ α-cyd and PMO/ β-cyd complexes have different physicochemical properties compared to their free forms. Thus it can be concluded that our work may be regarded as an alternation way to protect our skin from sun damage and beneficial to medicinal science.