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PHYSICOCHEMICAL CHARACTERIZATION OF α – CYCLODEXTRIN COMPLEXES OF TAMOXIFEN CITRATE AND PHASE SOLUBILITY STUDIES

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ABSTRACT: The objective of the present investigation is to study the possibilities to improve the aqueous solubility and dissolution rate of Tamoxifen citrate (TC) – an oral anticancer drug, based on inclusion complexes with α– cyclodextrin (α –CD). The inclusion processes are discussed based on absorption, emission, FT-IR, ^{1}H-NMR, SEM and phase solubility study. The α –CD study shows that the drug form 1:1 inclusion complexes. The solubility and dissolution results revealed that there was a considerable increase in solubility and dissolution of all inclusion complexes. Scanning Electron Microscopy (SEM) to analyze physicochemical interaction between drug and carrier and evaluation of the crystal morphology and their approximate size. The inclusion complex appears as tiny aggrigates of amorphous pieces of irregular sizes. In both absorption and emission the intensity increase with increase in concentration of α –CD. The aromatic ring is encapsulated in the non-popular α –CD cavity. The formation constant value reveals a hydrogen bonding interaction is present between the drug and α –CD cavity. The presence of aliphatic chains has not significantly changed the absorption and emission spectral behaviour and inclusion process.

INTRODUCTION: Tamoxifen Citrate is an antagonist of the estrogen receptor in breast tissue via its active metabolite. In other tissues such as the endometrium, it characterized as a mixed agonist/ antagonist. Tamoxifen citrate is the usual endocrine (anti-estrogen) therapy for hormone receptor positive breast cancer in pre-menopausal women and is also a standard in post-menopausal women although aromators, inhibitors are also frequently used in that setting. [1]

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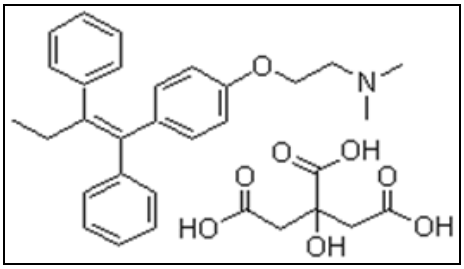

FIG.1: STRUCTURE OF TAMOXIFEN CITRATE

Some breast cancer cells require estrogen to grow. Estrogen binds to and activates the estrogen receptor in these cells. Tamoxifen citrate is metabolized into the estrogen receptor, but do not activate it. Because of this competitive antagonism, Tamoxifen citrate acts like a key broken off in the lock that prevents any other key from being inserted preventing estrogen from binding to its receptor. Hence breast cancer cell growth is

blocked.[2] Its effect is low, because of its low solubility in water. This can be enhanced by including it with α – cyclodextrin.

The rate of absorption and the extent bioavailability for such an insoluble hydrophobic drug are controlled by the rate of dissolution in gastro intestinal fluids. Therefore it is important to enhance the solubility and dissolution of the drug in order to improve its bioavailability [3].

One possible way of overcoming this problem is to alter the physical properties of the drug by forming a complexation with α – cyclodextrin in capable of trapping a variety of molecules within to produce inclusion compounds. Selective physicochemical determinations such as absorption, emission. FT-IR. SEM and ^{1}HNMR were used to characterized the complexes.

MATERIALS AND METHODS:

Tamoxifen citrate supplied by Area Lab Pvt. Ltd. Mumbai, α – cyclodextrin was purchased from Sigma Aldrich, and used without further purification. All materials used in this study comply with pharmaceutical and analytical standards.

Preparation of α – cyclodextrin – Tamoxifen citrate inclusion complex:

The stock solution of α – CD were prepared in triply distilled water. The highest concentration of α – CD was 1.0 X 10^{-2} mol dm^{-3}. The Tamoxifen citrate stock solution was added to the different concentration of α – CD solution. The mixed solution was diluted that is 0.2ml Tamoxifen citrate and different volumes of α – CD to 10 ml with distilled water and shaken thoroughly[4]. Then the absorption and fluorescence spectrum were taken. The absorption spectral measurements were carried out with a Systronics Double Beam spectrophotometer 2203 SMART and fluorescence measurements were made using a ELICO SL 170 spectrofluorometer.

Phase-Solubility Study:

Phase-solubility study was carried out according to the method described by Higuchi and corners. In brief, excess amount of Tamoxifen citrate (110 mg) was added to 10 ml of aqueous solution containing various concentrations of α – cyclodextrin and shaken for 48 hrs at room temperature in a shaker until equilibrium reached [5]. Afterward, samples were withdrawn, filtered through a 0.45 µm membrane filter. Drug concentration was analyzed with UV deflection at a wave length 278 nm. Experiments were performed in triplicate. The apparent stability constant (Kst) was calculated from the linear portion of the phase – solubility diagram, Kst = Slope/intercept (1-slope) where slope is obtained from the initial straight –line [6].

Preparation of Solid Inclusion Complexes:

Accurately weighed amount of drug was added to solution of methanol and temperature was maintained at 45^0C, the α-cyclodextrin solution was mixed with continuous stirring using magnetic stirrer. The constant stirring was performed for 48 hrs[7]. The inclusion complexes formed in the solution was filtered. The complexes were obtained in white powder form and that was used for the following characterization studies.

(i) Fourier Transform Infrared Spectroscopic Analysis:

The FT-IR spectra of pure drug, pure α –CD, and inclusion complexes were taken by using 7600 FT-IR spectrophotometer (Shimadzu).

(ii) ^{1}H-NMR:

^{1}H-NMR spectra were recorded for the drug, α – CD and the inclusion complexes in NIIST, Trivandrum.^{1}H-NMR spectra were recorded on AV 500 NMR spectrometer (Bruker) at 500 MHz. Chemical shifts are reported in delta (ppm) relative to TMS as internal standard.

(iii) Scanning Electron Microscopy:

The drug Tamoxifen citrate, α –CD and inclusion complex were morphologically analyzed with STIC, Cochin. JEOL – JSM – 6390 LV Scanning Electron Microscope.

RESULTS AND DISCUSSION:

Effect of α – cyclodextrin:

Table 1 and **Fig. 2** and **3** depict the absorption and fluorescence maxima and spectrum for the drug Tamoxifen citrate containing various concentrations of α-CD. The absorption maxima of Tamoxifen citrate appear at 274.5nm. Upon

increasing the α-CD concentration the absorbance increased. The absorption maxima shows a blue shift. That is the absorption maxima moves from 274.5 to 266.8nm. The above results are due to the drug transferred from more protic environment to less protic α -CD cavity environment. Dissolution of the guest molecule through the hydrophobic interaction between the guest and non-polar cavity of the α -CD.

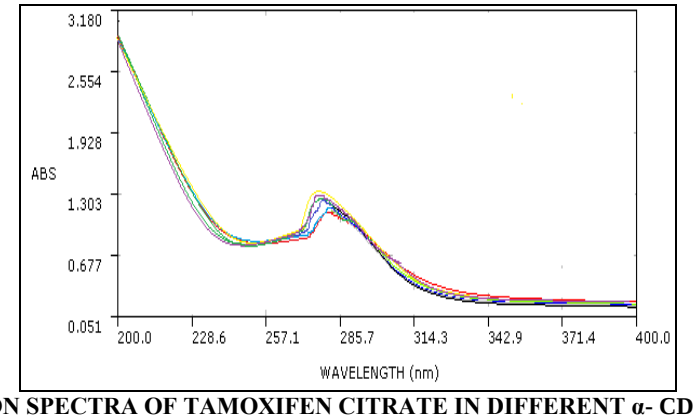

FIG 2 : ABSORPTION SPECTRA OF TAMOXIFEN CITRATE IN DIFFERENT α- CD CONCENTRATION

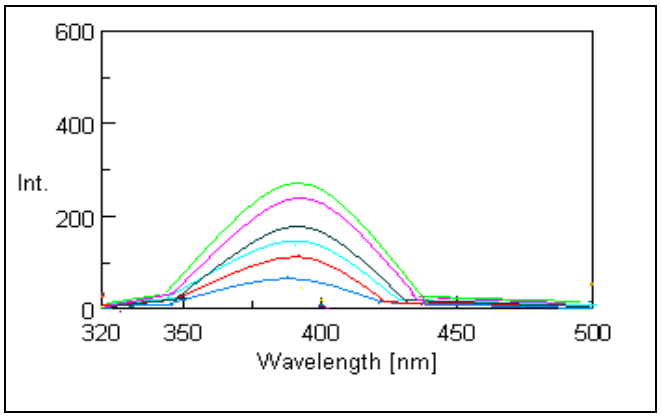

FIG.3: FLUORESCENCE SPECTRA OF TAMOXIFEN CITRATE IN DIFFERENT α- CD CONCENTRATION

TABLE 1: ABSORPTION MAXIMA (nm) AND FLUORESCENCE MAXIMA (nm) OF TAMOXIFEN CITRATE IN DIFFERENT CONCENTRATION OF α-CD.

S.No	α -CD concentration	Absorption		$\frac{1}{A-A_0}$	Emission		$\frac{1}{I-I_0}$	$\frac{1}{[\alpha-CD]}$	$\frac{1}{[\alpha-CD]^2}$
		λ max (nm)	Absorbance		λ max (nm)	Intensity			
1	0	274.5	1.193		386	97.720			
2	0.002	272.0	1.259	15.152	388	117.264	0.051	500	2,50,000
3	0.004	271.0	1.287	10.638	389	139.25	0.024	250	6,25,000
4	0.006	270.4	1.295	9.804	392	191.88	0.011	166.667	27,777.7
5	0.008	268.0	1.300	9.346	392	234.5	0.0073	125	15,625
6	0.01	266.8	1.347	6.494	393	273.616	0.0057	100	10,000

The flouorescence characteristics of the drug seen to undergo drastic changes in the presence of α -CD. In the presence of α -CD two important changes are observed in the S_1 state. (i) emission intensity increases with α -CD concentration and (ii) a structural emission spectrum is observed along with a large red shift from 386 to 393nm. The vanderwaals forces and hydrophobic interactions are ruptured in the α -CD cavity. This is because the size of the drug is greater than α -CD cavity. The geometrical limitations of the α -CD cavity would restrict the free rotation of the aliphatic chain [7].

In order to determine the stoichiometry of the inclusion complex, the absorbance and fluorescence dependence behaviour of the drug molecule on α -CD were analysed using the Benesi – Hildebrand equation 1:1 complexes equation.

$$\frac{1}{A-A_0} = \frac{1}{A'-A_0} + \frac{1}{K(A-A_0)[\alpha-CD]}$$

where K is the formation constant, A_0/I_0 is the initial absorption /fluorescence intensity of free guest A′/I′ the absorption/ fluorescence intensity of α -CD inclusion complexes and A/I is the observed absorption/ fluorescence intensity. A plot of $\frac{1}{I-Io}$ verses $\frac{1}{[\alpha-CD]}$ both for absorption and fluorescence gives linear line as showing **Fig (4** and **5).** This shows that an 1:1 inclusion complex is formed between Tamoxifen citrate and α -CD. This analysis reflects the formation of Tamoxifen citrate with α -CD[8]. The free energy change was calculated from the formation constant (K) ΔG = - RT ln k.

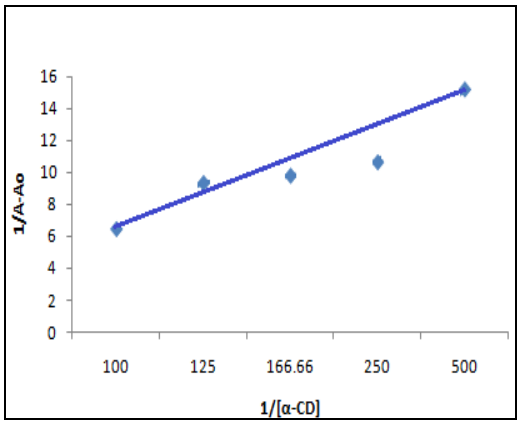

FIG. 4: PLOT OF $\frac{1}{A-A_0}$ **Vs** $\frac{1}{[\alpha-CD]}$

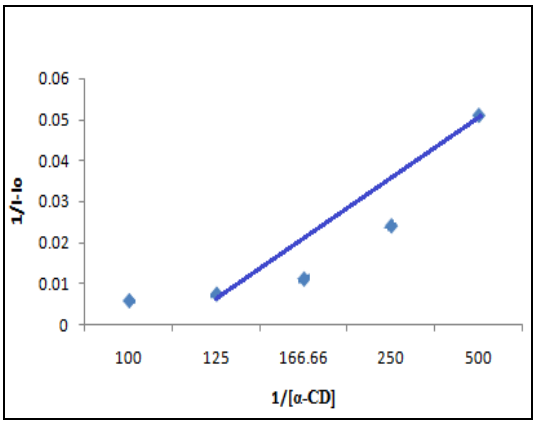

FIG. 5: PLOT OF $\frac{1}{I-I_0}$ **Vs** $\frac{1}{[\alpha-CD]}$

The value of thermodynamic parameter ΔG is negative, which suggests that the inclusion proceeded at 303 k. The experimental results indicate that the inclusion reactions of the α -CD with the drugs are exothermic processes the hydrogen bonding interaction, van der waals interaction and breaking of the water cluster around this polar guest compound are the driving force for the inclusion complex formation. The strength of interaction dependent on the size of the α-CD cavity and size of the substituent in the complex [9]. The CDs are truncated cone–shaped molecule with height approximately 7.9 A^0 and internal diameter 4.9A^0. The interaction of the phenyl ring with α-CD may achieve a maximum contact area with the internal surface of the cavity of the of the α-CD. Therefore the drug partially embedded in the α -CD cavity. The increase of absorbance and fluorescence intensity by the addition of α -CD solution suggested that the aromatic ring is incapsulated in the non-polar part of the α -CD cavity. The association constant 'K' value 272 M^{-1}, is a reasonable measure of hydrogen bonding interactions, it is caused only by the α -CD concentration. 'K' values are proportional of the hydrophobic and hydrogen bonding interactions.

Phase Solubility Study:

The phase solubility diagram for the complex formation between Tamoxifen citrate and α – cyclodextrin in present in (**Fig. 6**). This plot shows that the aqueous solubility of the drug increases linearly as a function of α – cyclodextrin concentration. It is clearly observed that the solubility diagram of Tamoxifen citrate in the presence of α – cyclodextrin can be classified as the A_L type [10]. The linear host guest correlation with slope of less than 1 suggested the formation of a 1:1 complex (Tamoxifen citrate: α – cyclodextrin) with respect to α –CD concentrations. The apparent stability constant K_{st} obtained from the slope of the linear portion. Phase solubility value Kst was found to be 509 M^{-1} which indicates that the Tamoxifen citrate and α – cyclodextrin forms a soluble and complexes at1: 1 ratios.

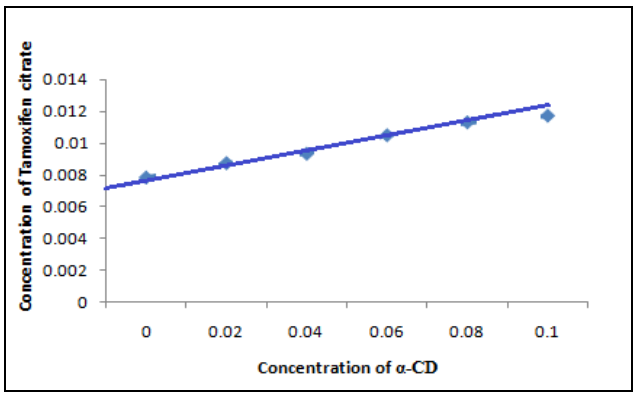

FIG.6: PHASE SOLUBILITY DIAGRAM FOR TAMOXIFEN CITRATE – α – CD COMPLEX

Structure Analysis of Inclusion Complexes:

(1) FT-IR Study:

Host-guest interaction in an inclusion complex is mediated by weak forces between molecules such as hydrogen bonds and hydrophobic interactions [11]. FI-TR absorption spectra for Tamoxifen citrate, α – CD, inclusion complex (TC and α –CD) are given in **Fig. (7-9**).

The FI-TR spectra of Tomoxifen citrate and the solid inclusion complex were studied. The N-H stretching frequency at 3379. 29cm^{-1} became broad in the complex. The C-H stretching at 3278.90cm^{-1} was shifted in the complex. The C-H bending vibration at 879.59cm^{-1} is shifted to 756.00 cm^{-1} in the complex. The C=O bending vibrations at 1604 cm^{-1} and 1658 cm^{-1} are moved in the inclusion complex to 1651 cm^{-1}. The C-N frequency found at 1203 cm^{-1} is moved in the inclusion complex to 1257 cm^{-1}. The CH_2 deformation frequency appeared at 1435 cm^{-1} is moved in the inclusion complex to 1419 cm^{-1}. The aromatic C-H bending frequency at 771 cm^{-1} is shifted in the inclusion complex to 756 cm^{-1}. The above results confirms that Tamoxifen citrate form inclusion complex with α-CD.

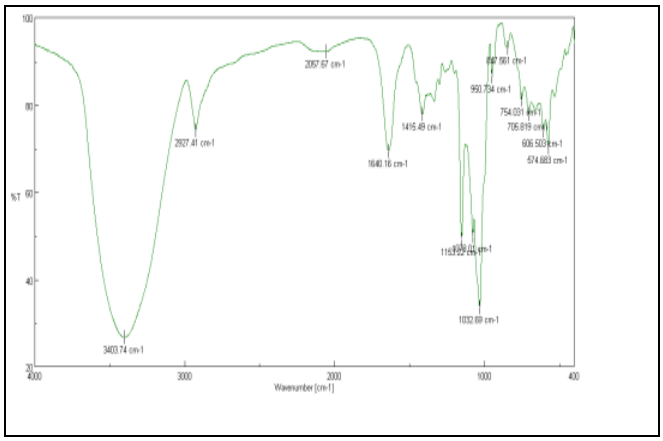

FIG. 7: FT-IR SPECTRUM OF α -CD

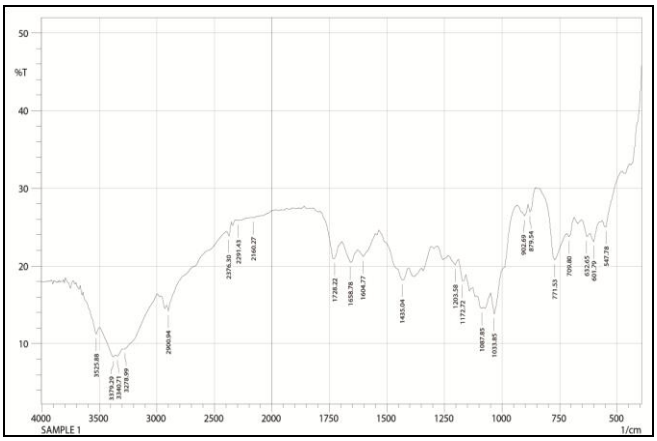

FIG.8: FT-IR SPECTRUM OF TAMOXIFEN CITRATE

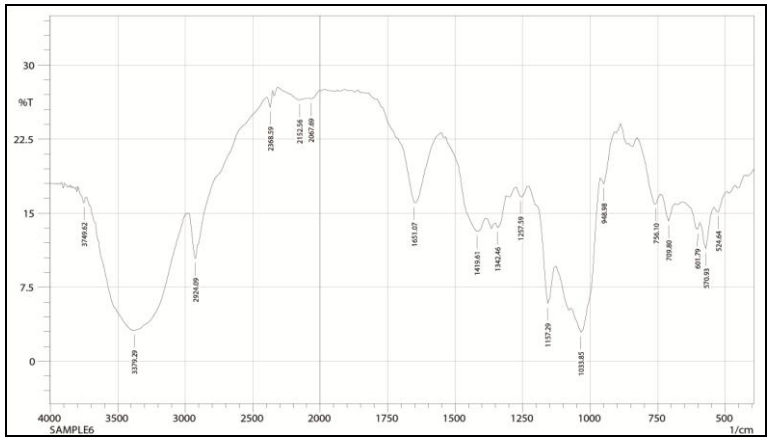

FIG.9: FT-IR SPECTRUM OF INCLUSION COMPLEX (α-CD WITH TC)

^{1}H-NMR Study of the Complex:

^{1}H-NMR can be directly used to observe the inclusion of Tamoxifen citrate into α -CD cavity. **Fig. (10)** shows ^{1}H-NMR spectrum of (a) α -CD, (b) Tamoxifen citrate (c) The inclusion complex of α -CD and Tamoxifen citrate. As the drug included

in to the α-CD cavity the H-3 and H-5 protons are seriously affected, and show more prominent changes chemical shift in ^{1}H-NMR Spectra than the other protons of α -CD. The H_3 and H_5 protons of α -CD which are located on the interior of the cavity showed more up-field shifts from 3.501 to 3.599 ppm and 3.487 to 3.557 ppm. respectively. The high upfield shift changes in the α -CD cavity protons was due to the penetration of the Tamoxifen citrate in the α -CD cavity resulting in the formation of α -CD –TC inclusion complexes. On other hand, H-1, H – 2 and H – 4 protons which are located on the exterior of the cavity showed only minimal up-field shifts. These results suggest that the inclusion process involves mostly the hydrophobic part of Tamoxifen citrate and the hydrophobic cavities of α-cyclodextrin. A strong inclusion between the aromatic ring of the drug molecule and the interior wall of α -CD cavity.

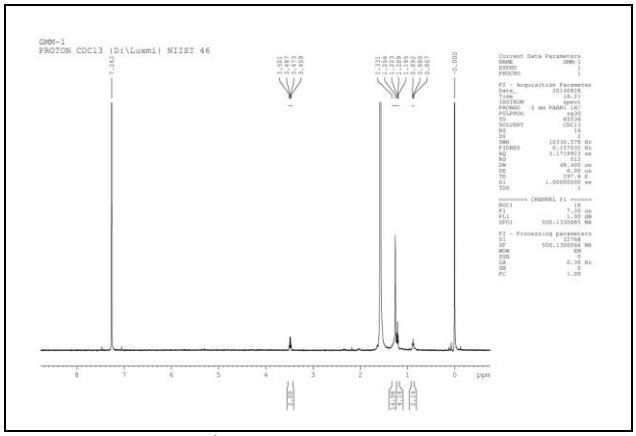

FIG. 10: ^{1}H-NMR SPECTRA OF α-CD

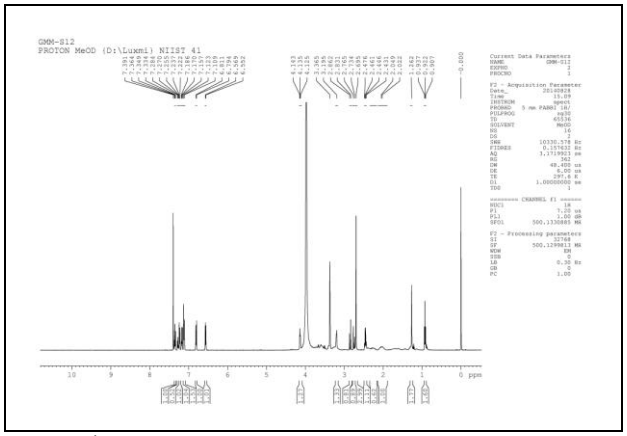

FIG.11: ^{1}H-NMR SPECTRA OF TAMOXIFEN CITRATE

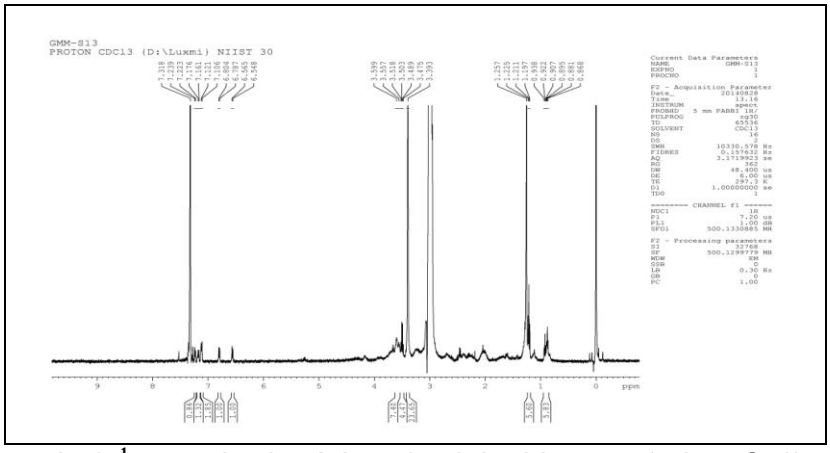

FIG. 12: ^{1}H-NMR SPECTRA OF INCLUSION COMPLEX (α-CD and TC)

TABLE 2: CHEMICAL SHIFTS (ppm) FOR THE PROTONS OF α -CD AND THE INCLUSION COMPLEX

	H_1	H_2	H_3	H_4	H_5
α -CD	7.262	3.473	3.501	3.459	3.487
Inclusion complex	7.318	3.475	3.599	3.393	3.557
Δδ(ppm)	0.056	0.002	0.098	0.066	0.07

Scanning Electron Microscopic Studies:

The surface morphology of drug, α –CD and inclusion complex (α –CD + drug) was determined by the scanning electron microscopy which are shown in **Fig (13 to 15**). Tamoxifen citrate and α-CD exists as solid particles and pieces of spherical particles. The solid dispersions appeared in the form of irregular particular in which the original morphology of (α –CD, Tamoxifen citrate) both components disappeared and tiny aggregates of amorphous pieces of irregular size was appeared. Here the reduced particle size, increased surface area and the close contact between α –CD the carriers and Tamoxifen citrate might be responsible for the enhanced drug solubility.

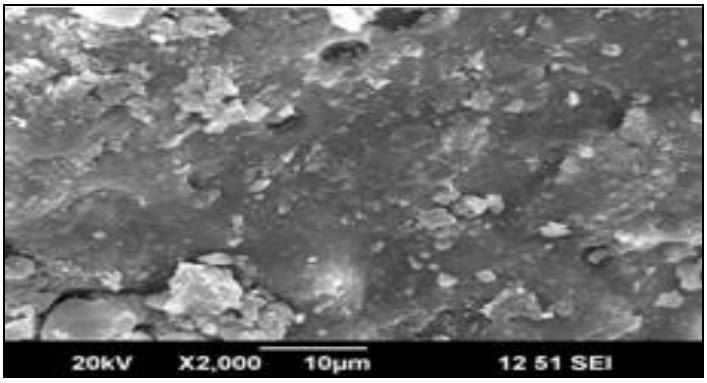

FIG.13: SEM IMAGE OF TAMOXIFEN CITRATE

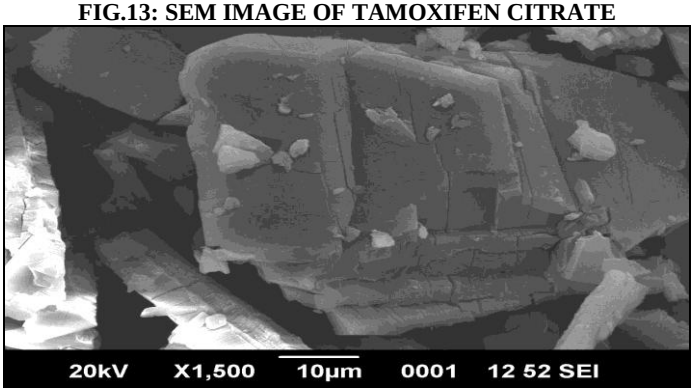

FIG.14: SEM IMAGE OF α-CD

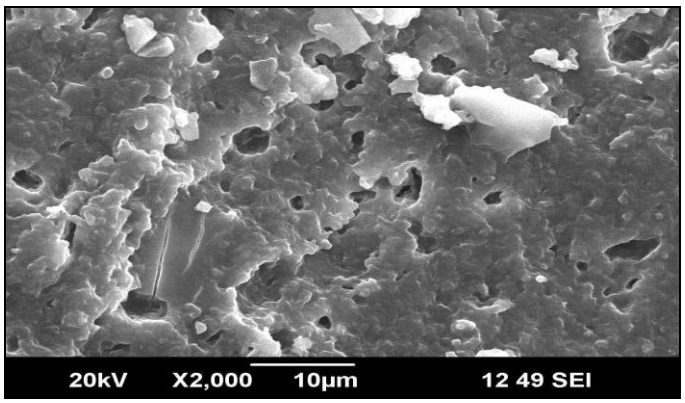

FIG.15: SEM IMAGE OF INCLUSION COMPLEX BETWEEN TAMOXIFEN CITRATE AND α-CD

CONCLUSION: The characterization of inclusion complexes were successfully carried out by using Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy, FT-IR spectroscopy and Scanning Electron Microscopy (SEM). These three techniques confirmed the anticancer drug Tamoxifen citrate form an inclusion complex with α-cyclodextrin in the ratio 1:1. Tamoxifen citrate entered into the hydrophobic cavity of α-CD which makes the solubility of Tamoxifen citrate in water increase. The phase solubility diagram showed an A_L type and the aqueous solubility of the drug increases linearly as a function of α - cyclodextrin concentration. The formation constant 'K' value implies the presence of hydrogen bonding interaction. Absorption Spectra shows blue shift, and emission spectra shows and red shift with increase in α-CD concentration. After inclusion the bioavaiability of drug is increased. The thermal and chemical stability of the drug is improved significantly, inclusion complexation retards the photo - oxidization of the drug. All these changes in property greatly improve the pharmaceutical actions of the drug.

REFERENCES:

1. Jordan.V.C.: Tamoxifen as a targeted therapy to treat and prevent breast cancer: British Journal of Pharmacology, 2006; 147(1); S269-276.
2. Criscitiello. C, Fumagali. D, Saini. K.S, Loi. S: Tamoxifen in early-stage estrogen receptor-positive breast cancer:

3. Overview of clinical use and molecular biomarkers for patient selection: Onco Targets Terapy: 2011; 4; 1-11.
4. Trand Firescu. C, Gyeresi. A, Kala. M, Eupure. C, Szabadai.Z: Study of influence of citric acid on Albendozole complexation with randam methylated-β-cyclodextrin: Farmacia; 2008; 4(1), 24-29.
5. Cristina Tablet, Liliana Minea, Luigi Dumitrache, Mihaela Hillebrand: Experimental and theoretical study of the inclusion complexes of 3-Carboxy Coumarin acid with β- and 2-Hydroxypropyl-β-cyclodextrins: Spectrochimica Acta Par A; 2012; 92, 56-63.
6. Mukesh Chandra Sharma, Smita Sharma: Preparation, physicochemical characterization, dissolution, formulation and spectroscopic studies of β-Cyclodextrins Inclution complexes: International Journal of Chem Tech Research; 2011; 3(1), 104-111.
7. Camelia Nicolescu, Corina Arama, Angela Nedelcu, Crina-Maria Monciu: Phase solubility studies of the Inclusion complexes of Repaglinide with β-Cyclodextrin and β-Cyclodextrin derivatives: Farmacia: 2010; 58 (5); 620-628.
8. Sevukarajan.M, Thanuja Bahala, Rahul Nair, Novel Inclusion complexes of Oseltamivir Phosphate with β-cyclodextrin: Physico-chemical characterization: Journal of Pharmaceutical sciences and Research; 2010; 2(9), 583-589.
9. Bakkia Lakshmi. S and Menaka. T: Study on the solid inclusion complex of Coumarin-1 with β-cyclodextrin; International Journal Pharmacy and Pharmaceutical Sciences; 2012; 4(4), 63-68.
10. Abd-Elgawad Radi and Shimaa Eissa: Electrochemistry of Cyclodextrin Inclusion complexes of pharmaceutical compounds; The open chemical and Biochemical methods, 2010; 3; 74-85.
11. Nitin Maski, Arul kumaran, Kundlik Girhepunje, Prashant Ghode, Suraj kumar, Randive and Ranju Pal: Studies on the preparation characterization and solubility of β-cyclodextrin-Diacerein inclusion complexes: International Journal of Pharmacy and Pharmaceutical sciences; 2009; 1(2), 121-13.
12. Nikhil. K. Sachan, Seema Pushkar, Solanki. S.S., and Degendra.S, Bhatere: Enhancement of solubility of Acyclovir by solid dispersion and Inclusion complexation methods: World Applied Science Journal; 2010; 11 (7); 857-864.

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