

## Novel Inclusion Complexs Of Oseltamivir Phosphate-With $\beta$ Cyclodextrin: Physico-Chemical Characterization.

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### ABSTRACT

The purpose of this research was to mask the intensely bitter taste of Oseltamivir Phosphate (OP) Taste masking was done using beta-cyclodextrin (CD). Prepared complexes were characterized using several techniques, namely phase solubility diagrams, differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FT-IR), X-ray powder diffractometry (XRPD), scanning-electron microscopy (SEM) to analyse any physicochemical interaction between drug and carrier. In vitro drug release studies for freeze dried complexes(FDC) were performed at pH, 1.2 and 6.8. In order to evaluate the efficiency of taste masking by complexation a taste perception study (TPS) was designed and followed Bitterness score was evaluated using gustatory sensation test. The FTIR, DSC and XRPD studies indicated inclusion complexation in FDC. In addition, prepared FDC exhibited better drug release at pH 1.2 and less at pH 6.8. The results of TPS indicate effective taste masking as compared pure drug. In conclusion, the study confirmed that CD can be utilized as an alternative approach for effective taste masking of OP.

**Key Words :** Oseltamivir Phosphate, Beta-cyclodextrin, Physico-Chemical Characterization.

### 1. INTRODUCTION:

Oseltamivir phosphate [compound name: (-)-ethyl (3R,4R,5S)-4-acetamido-5-amino-3-(1-ethylpropoxy)-cyclohex-1-ene-1-carboxylate monophosphate] has a potent inhibitory activity against neuraminidase of influenza virus, and has been used as an active ingredient of Tamiflu<sup>(R)</sup> a prophylactic and therapeutic agent treatment of influenza[1]. Masking the bitter taste of Oseltamivir phosphate (OP) is a prerequisite to improve the patient acceptance especially in the pediatric and geriatric population as this drug has been approved to use in persons of age of above 1 year. Cyclodextrins (CD) are cyclic oligosaccharides made up of six to twelve  $\alpha$ -D-glucopyranose monomers connected at 1 and 4 carbon atoms. CD with 6–8  $\alpha$ -D-glucopyranose units are denoted  $\alpha$ -,  $\beta$ -, and  $\gamma$ -CD, respectively. They have the property of forming inclusion complex with various guest molecules with suitable polarity and dimension because of their special molecular structure with hydrophobic internal cavity and hydrophilic external surface [2]. It has been demonstrated that several weak forces, including van der Waals, hydrophobic, dipole–dipole, and hydrogen bonding interactions, cooperatively determine the inclusion

complex behavior of the cyclodextrin host. This complex forming ability of CDs have been widely exploited in the pharmaceutical field for various applications; taste masking of bitter drugs is one such application[3,4]. Use of cyclodextrin as taste masking agent is widely reported [5]. Recently, novel formulations containing Ibuprofen, tromethamine and CD have been developed in order to eliminate Ibuprofen unacceptable taste and to produce a deliverable oral form.(6) In case of taste masking the general aim is minimum free drug release in the oral cavity. It is therefore important to find methods of forming complex with higher stability constants which would release negligible amount of drug at the site of minimal dilution (i.e. oral cavity) and give a rapid and complete release at comparatively higher dilutions (i.e. gastric lumen).(7,8,9) Present investigation was undertaken to mask the bitter taste of Oseltamivir phosphate by using  $\beta$  cyclodextrin ( $\beta$  CD).

### 2.EXPERIMENTAL

#### 2.1.MATERIALS AND METHODS

Oseltamivir phosphate was obtained from Cipla Laboratories Pvt. Ltd. $\beta$ -Cyclodextrin was purchased from Hi media chemicals, All other chemicals and solvents used were of pharmaceutical and analytical

grade. Double distilled water was used throughout the study for experimental work.

### 2.2. Phase solubility studies:

Phase-solubility studies were carried out with double distilled water according to the method described by Higuchi and Connors (10). Excess amount of Oseltamivir phosphate (1g) was added to 1ml stock solution with varying concentrations (3, 6,9,12 and 15 mM) of  $\beta$ -CD. The suspensions were then shaken at  $25.0 \pm 0.5$  °C for 48 hours to allow attainment of equilibrium. The samples were filtered and the drug content of was determined spectrophotometrically on a UV-spectrophotometer (UV-1700(Shimadzu, Japan) at 220 nm. The stability constant of the complex with uncharged Oseltamivir phosphate was calculated from the phase-solubility diagram with the assumption of 1:1 stoichiometry according to the following equation as described by Higuchi and Connors:

$$K_{1-CD} = \frac{tg\alpha}{CI_0(1-tg\alpha)}$$

Where  $cI_0$  is the intrinsic solubility of the most basic form of drug in the absence of  $\beta$ -CD.

### 2.3. Preparation of inclusion complexes

Inclusion complexes of Oseltamivir phosphate with  $\beta$  cyclodextrin was prepared by the freeze drying (FD) method. The required amount of cyclodextrin was dissolved in double distilled water to get a clear solution. The drug was then dispersed into this aqueous solution of  $\beta$  CD in molar ratio (1:1) followed by continuous stirring for 24 hrs at room temperature. The suspension obtained was then subjected to FD. All samples were stored in dessicator until further use.

### 2.4 .Differential Scanning Calorimetry (DSC) Studies:

The thermal behaviors of drug, CD freeze-dried complex(FDC), were examined

using differential scanning calorimetry (Perkin Elmer, pyris 4 series DSC equipment (Massachusetts, USA). An accurately weighed sample of each solid, equivalent to 5mg, was heated in a sealed aluminum pan, using an empty pan sealed as reference, over the temperature range from 0 to 350 °C, at a rate of 5 °C/min. An indium standard was used for temperature calibration.

### 2.5. Fourier Transform Infrared (FT-IR) Studies:

Fourier transform infrared (FT-IR) spectra of the samples were obtained in the range of 400 to 4,000  $\text{cm}^{-1}$  using a (Thermo IR 200 spectrophotometer ) by the KBr disc method.

### 2.6. X-Ray Diffraction Studies

The study was carried out using a X-Ray Diffractometer using  $\text{Cu } \alpha$  radiation. The tube operated at 45 kV, 9mA and data was collected over an angular range from 0 to  $50^\circ$   $2\theta$  of the diffraction angle in continuous scan mode using a step size of  $0.02^\circ$   $2\theta$  and a time of 0.4 s.

### 2.7. Scanning Electron Microscopy

SEM analysis was performed for the pure drug by using ZEISS Electron Microscope, EVO MA 15 to study about the shape and surface characteristics. The particles were coated with gold-palladium to render them electrically conductive and then observed under Electron microscope.

### 2.8. Invitro dissolution studies

Dissolution studies of samples weight equivalent to 30 mg were performed using USP XXII apparatus at the stirring speed of 50 rpm and temperature maintained at  $37 \pm 0.5^\circ\text{C}$  with buffer 0.1N HCl as dissolution medium. The samples of 5ml were withdrawn at regular intervals of 5,15,30,45,60 min. every time the samples are replaced with 5ml of the fresh dissolution medium. The filtrates of the samples were analyzed at 220 nm. Percentage drug release and other parameters of the samples were calculated by using PCP Disso V3 software.

## 2.9. Taste Perception Studies Of $\beta$ Cyclodextrin Inclusion Complexes Of Oseltamivir Phosphate:

A protocol for conducting taste assessment by taste panel studies was submitted to the Institutional Ethical Committee. Taste assessment studies were conducted according to the approved protocol on six healthy human volunteers. Pure drug (2 mg),  $\beta$  CD inclusion complexes of the drug (equivalent to 2 mg Oseltamivir Phosphate), were provided randomly to volunteers divided into three different groups, each of two volunteers. The sequence of the taste testing was different for each group to avoid any bias pure drug (taste score 4) and placebo (taste score 1) were used as positive and negative controls, respectively. Subjects scored the intensity of bitterness by placing the given amount of sample on the tongue, tasting it for one minute, and thoroughly rinsing their mouths with water after each sample evaluation. Each volunteer judged the taste of the sample using a score involving a five point scale which ranged from 0 to 4 (0 = pleasant, 1 = tasteless, 2 = slightly bitter, 3 = moderately bitter and 4 = extremely bitter).

## 3.RESULTS AND DISCUSSION

### 3.1. Phase solubility studies:

The aqueous solubility of Oseltamivir phosphate at various concentrations of  $\beta$  CD was studied. It was found that the solubility of drug increased with the increasing concentration of  $\beta$  CD up to an optimal concentration of 9mM, decreasing thereafter, suggesting no further increase in the solubility with further addition of  $\beta$  CD. Phase solubility curve for the system was found to be of Higuchi's  $B_s$  type phase solubility curve was presented in the fig.1.

Values of stability constant of Drug- CD complex was found to be  $746.7 \text{ M}^{-1}$ . The stability constant could be attributed to the increase in the complexing ability of cyclodextrin towards the drug by

establishing interactions such as hydrophobic bonds, Van der Waals dispersion forces, or hydrogen bonds and/or promoting the release of high-energy water molecules present in the cyclodextrin cavity. This higher stability constant indicates the greater stability of the complex ensuring the improvement of taste effectively.

### 3.2. Differential Scanning Calorimetry (DSC) Studies:

DSC studies were performed on the individual components and on the freshly prepared freeze dried product of Oseltamivir phosphate with  $\beta$  cyclodextrin. Oseltamivir phosphate exhibited one endothermic peak at  $218.76^\circ\text{C}$ . DSC studies of prepared formulation showed one endothermic peak at  $201.11^\circ\text{C}$ . This clearly indicates that there is possible interaction between Oseltamivir phosphate and  $\beta$  cyclodextrin. Reduction in the endothermic peak value from  $218.76^\circ\text{C}$  to  $201.11^\circ\text{C}$  suggests that decreased crystallinity of the Oseltamivir phosphate in the complexes. Comparison of DSC study of Oseltamivir Phosphate,  $\beta$  Cyclodextrin and lyophilized product of Oseltamivir phosphate with  $\beta$  cyclodextrin was given in the Fig. 3.

### 3.3. X-Ray Diffraction Studies

XRD analysis was carried out to confirm formation of a new solid state. The crystallinity of Oseltamivir phosphate was indicated by the presence of multiple sharp peaks. Comparative X-Ray Diffraction pattern was given in Table No.1 X-Ray diffraction pattern of Oseltamivir phosphate- $\beta$  cyclodextrin complexes indicated the characteristic peaks of Oseltamivir phosphate but with reduced intensity. (Fig.3) This indicates the presence of Oseltamivir phosphate in the complexes and reduction of intensity indicates the reduced crystalline nature of the formulation which can also be confirmed by the reduction in the value of

2 θ	OP	β CD	OP β CD
13.4	1001	271	308
13.45	1143	294	327
18.15	1004	185	200
19.5	169	314	653
21.05	1135	136	186
21.1	1141	132	203
21.15	919	124	235

Table 1: Comparison of intensities of X-Ray Diffraction pattern for drug, excipient and formulation.

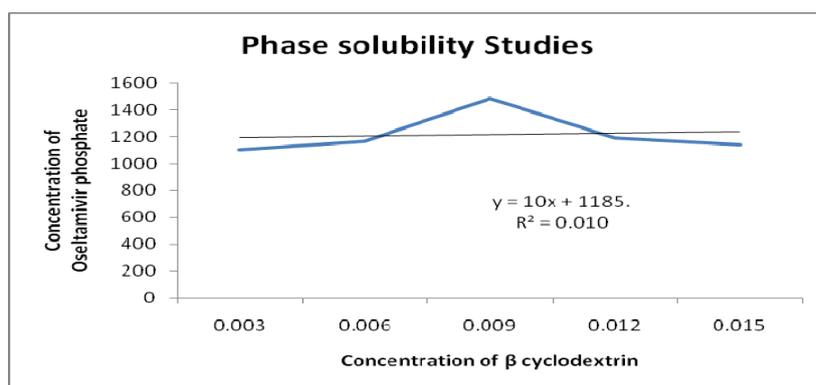


Fig. 1: Phase Solubility Studies of Oseltamivir Phosphate

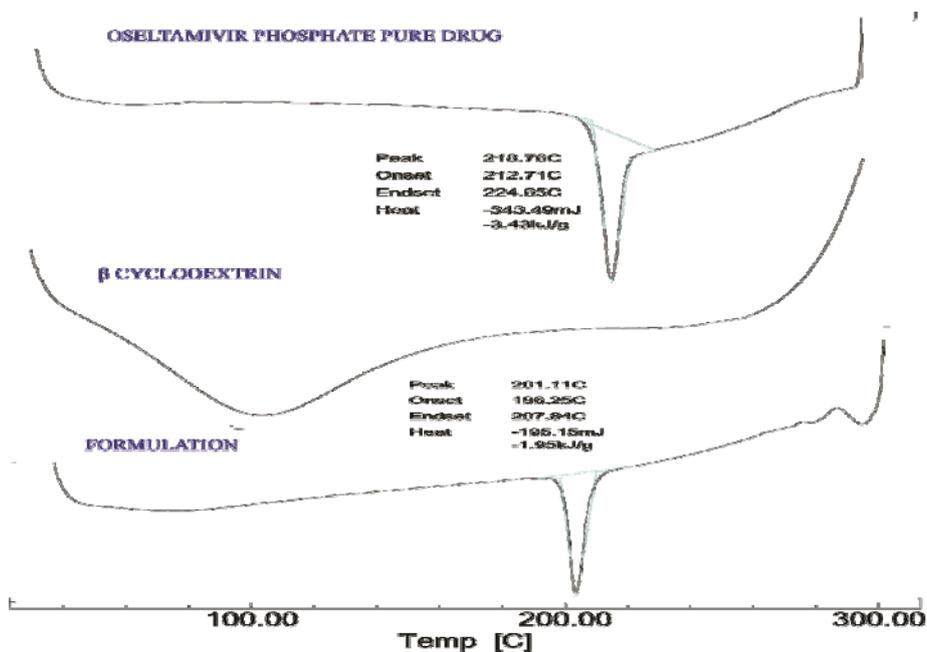


Fig. 2: DSC curves for pure drug, β CD and formulation.

endothermic peak in Differential Scanning Calorimetric Studies.

**3.4 Fourier Transform Infrared (FT-IR) Spectroscopy of Oseltamivir phosphate-β cyclodextrin complexes:**

The FT-IR studies showed that the significant peaks of Oseltamivir Phosphate are at 3352.39, 3177.61, 2877.83, 1723.45,

1551.82 which can be assigned for NH<sub>2</sub> – amines, Aromatic ring, C-H stretching of aldehyde, C=O Stretching, C-C Stretching, C-C stretching for aromatic ring, C-N Stretching for aromatic amines respectively. The absence of peaks at 3352.39, 3177.61, 2877.83, 1723.45, and 1551.82 in the formulation confirms the

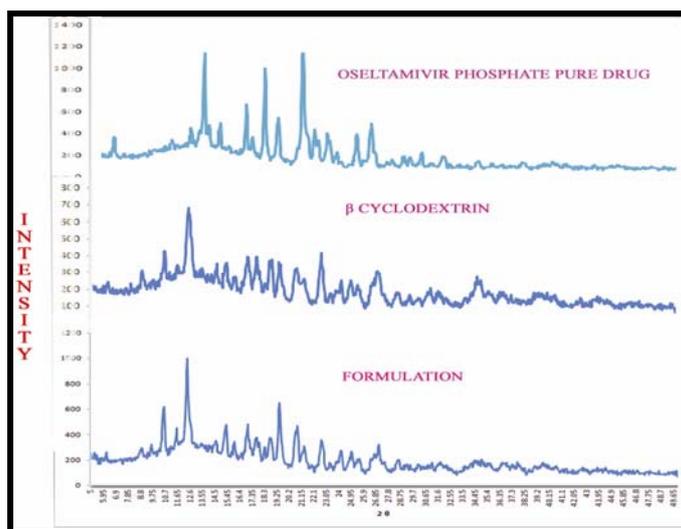


Fig. 3: Comparative X-Ray Diffraction pattern for drug, excipient and formulation.

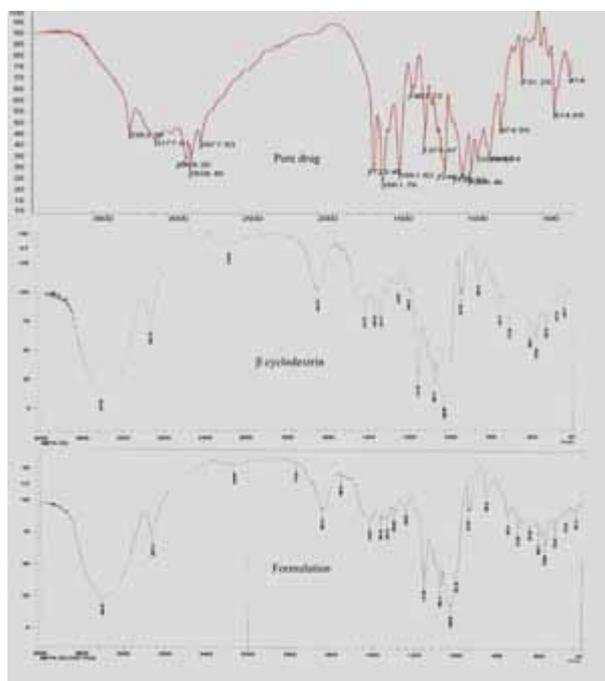


Fig.4: FT-IR spectrum for Pure drug, Cyclodextrin and formulation

formation of inclusion complex with  $\beta$ -cyclodextrin.[Table No.2 ] Figure No. 4 gives the comparative FT-IR spectrum of Oseltamivir phosphate- $\beta$  Cyclodextrin complexes.

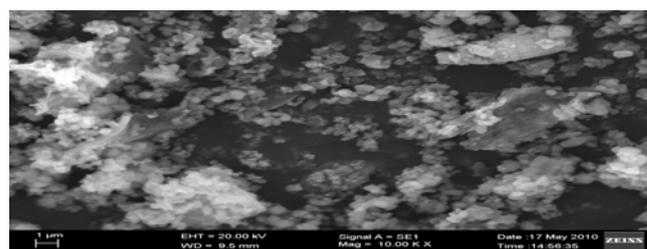
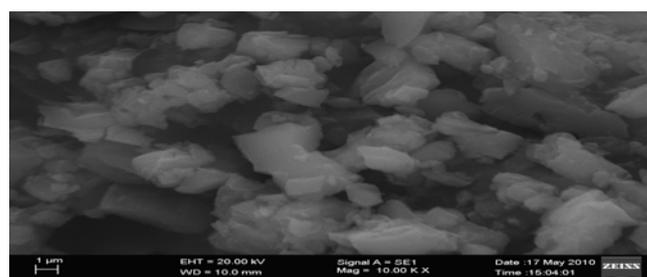
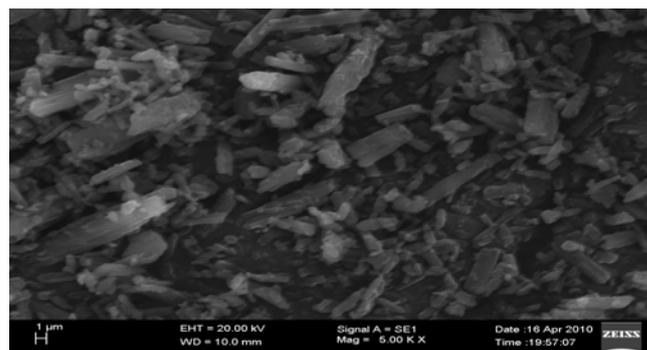
### 3.5. Scanning Electron Microscopy (SEM) Studies:

To study about the shape and surface characteristics SEM studies were performed for the individual components and Oseltamivir phosphate-  $\beta$  cyclodextrin complexes. Drug and  $\beta$ -CD inclusion complexes of drug showed significant difference in the microscopic structure. The reduction in the crystallinity was observed in the inclusion complexes. Comparative SEM photographs for

Oseltamivir phosphate and Complexes are given in Figure No. 4. Therefore, SEM results, along with those of DSC, FT-IR and XRPD, confirm the presence of inclusion complex in the solid state.

### 3.6. Invitro Dissolution Studies:

The results from dissolution studies carried in 0.1 N HCl and also with pH 6.8 phosphate buffer showed that there is minimal drug release from formulation in both the media. The dissolution studies in 0.1N HCl revealed that maximum drug release for pure drug as well as drug complex was seen at 30 min. In pH6.8 buffer pure drug showed complete release in 60 min whereas the formulation exhibited only 88% of drug release at the end of 60 min. The decreased release of the drug can be attributed to the high stability constant of the inclusion complex formed. In vitro dissolution profile for both pure drug and formulation along with standard deviations was given in (fig no 6) .From the Results of dissolution studies, when compared to pure drug minimal drug release has been observed for OP from inclusion complexes in pH 6.8 phosphate buffer, the pH which resembles the pH in

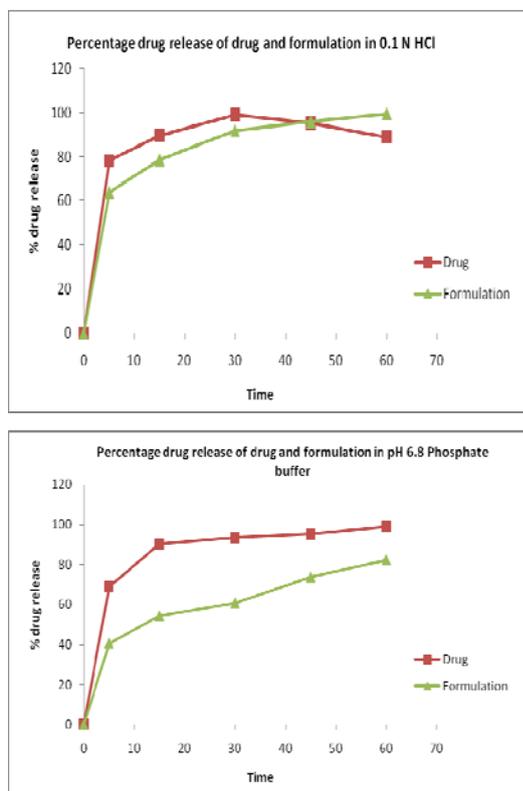


A)Oseltamivir phosphate, B.  $\beta$  CD, C. Oseltamivir phosphate+  $\beta$  CD  
**Fig 5: SEM photographs of Oseltamivir Phosphate, inclusion complexes of Oseltamivir Phosphate.**

oral cavity. So in oral cavity,  $\beta$  cyclodextrin may impede the interaction of drug with taste buds and results in improved palatability of extremely bitter tasting OP.

### 3.7. Taste Perception Studies of $\beta$ CD Inclusion Complexes of Oseltamivir Phosphate.

In order to evaluate the efficiency of taste masking by complexation a taste perception study was designed and followed. The rating of pure drug is 4 which indicate the bitter taste of the drug. The rating of the formulation is 2 which



**Fig. 6: Drug release profile for drug and formulation in 0.1 N HCl and pH 6.8 phosphate**

indicate the slightly bitter taste of formulation. Based on the average ratings it can be concluded that  $\beta$  CD was effective at improving the bitter taste of the drug.

#### 4. CONCLUSION

Oseltamivir phosphate- $\beta$  cyclodextrin complexes were prepared and characterized by SEM, FT-IR, XRD and

DSC studies. The improved palatability was confirmed by taste perception studies in healthy human volunteers. From this research work it can be concluded that  $\beta$  CD has potential of masking the bitter taste of Oseltamivir Phosphate.

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