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**A Glory Punitha**
Department of Chemistry and Research Centre, Scott Christian College (Autonomous), Nagercoil-629003, India

**J Prema Kumari**
Department of Chemistry and Research Centre, Scott Christian College (Autonomous), Nagercoil-629003, India

**Correspondence**
**A Glory Punitha**
Department of Chemistry and Research Centre, Scott Christian College (Autonomous), Nagercoil-629003, India

# Preparation and characterization inclusion complexes of fluorouracil α –CD / HP α – CD

**A Glory Punitha and J Prema Kumari**

**Abstract**
Solubility of poorly water soluble drugs is an important aspect of formulation and development. Fluorouracil is an anticancer drug with a small molecular weight. The absorbance and fluorescence spectra were record. Absorption maxima of Fluorouracil is blue shifted by the addition of α-cyclodextrin and Hydroxy propyl α- cyclodextrin. The fluorescence maxima are red shifted for both α – CD and HP α - CD. The association constant K is higher which confirms that the drug molecule completely include in to the α-Cyclodextrin cavity. The phase solubility study implies the formation of AL type diagram. The solubility of the drug increases with increase in concentration of both CDs. Thin layer chromatography studies were carried out. The drug fluorouracil is highly soluble in ethyl acetate-water mixture. The high Rf value proves that the drug is included strongly in the HPα-CD cavity. The obtained inclusion complex is the type with equimolar ratio1:1.

**Keywords:** Fluorouracil, α- cyclodextrin (α-CD), hydroxy propyl α-CD (HPα-CD), UV-visible spectroscopy, fluorescence spectroscopy

## 1. Introduction
Fluorouracil is a fluorinated pyrimidine belonging to the category of anti – metabolites. Fluorouracil (FU) is one of the most widely used agents in cancer therapy. Since its active form inhibits DNA synthesis by inhibiting the normal production of thymidine. It has antineoplastic action against several solid tumors including breast and colon cancers [1-5]. The chemical name of fluorouracil is 5 – fluoro -1H, 3 H pyrimidine 2,4 dione. Molecular formula of is $C_4 H_3 F N_2 O_2$. It is sparingly soluble in water. The molecular mass of the drug is 130.077g / mol. Cyclodextrin (CD) molecules are cyclic oligosaccharides made up of six to twelve α – CD glucopyranose monomers, which are connected at 1 and 4 carbon atoms. CDs have unique ability which they can form non – covalent, Host – Guest inclusion complexes with a variety of molecules including food additives [6-8].

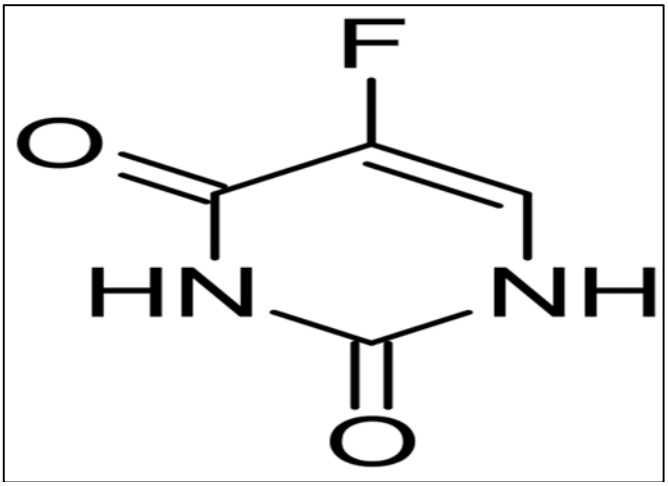


**Fig 1:** Structure of fluorouracil

## 2. Results and Discussion

**Table 1:** Absorption and Fluorescence maxima of Fluorouracil at different concentration of α – Cyclodextrin and Hydroxy propyl α – Cyclodextrin

| Alpha Cyclodextrin | | | | | Hydroxy Propyl alpha Cyclodextrin | | | |
|---|---|---|---|---|---|---|---|---|
| α -CD/HP α-CD Concentration | λabs | Absorbance | λflu | Intensity | λabs | Absorbance | λflu | Intensity |
| 0 | 265.6 | 1.480 | 390 | 32.450 | 265.6 | 1.480 | 390 | 32.450 |
| 0.002 | 263.8 | 1.812 | 400 | 37.200 | 262.8 | 1.790 | 404 | 36.890 |
| 0.004 | 260.2 | 1.870 | 402 | 39.590 | 260.4 | 1.850 | 407 | 38.510 |
| 0.006 | 252.0 | 1.955 | 406 | 42.437 | 259.2 | 2.165 | 409 | 39.827 |
| 0.008 | 249.6 | 1.975 | 408 | 42.958 | 255.4 | 2.540 | 411 | 41.347 |
| 0.01 | 246.4 | 2.350 | 413 | 45.897 | 253.6 | 2.650 | 415 | 43.574 |

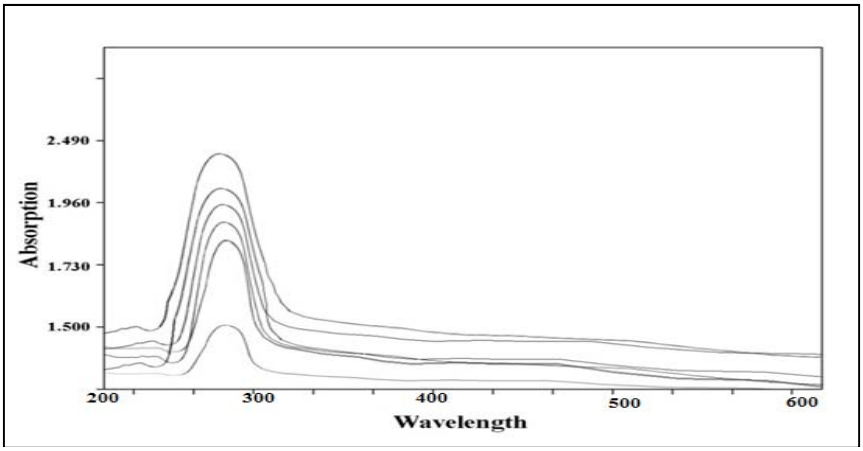


**Fig 1.1:** Absorption spectra of fluorouracil at different concentration of α – CD

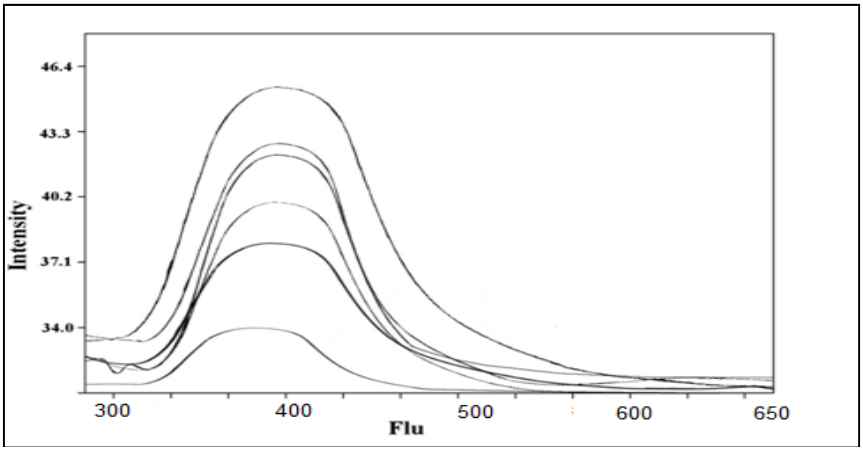


**Fig 1.2:** Fluorescence spectra of fluorouracil at different concentration of α – CD

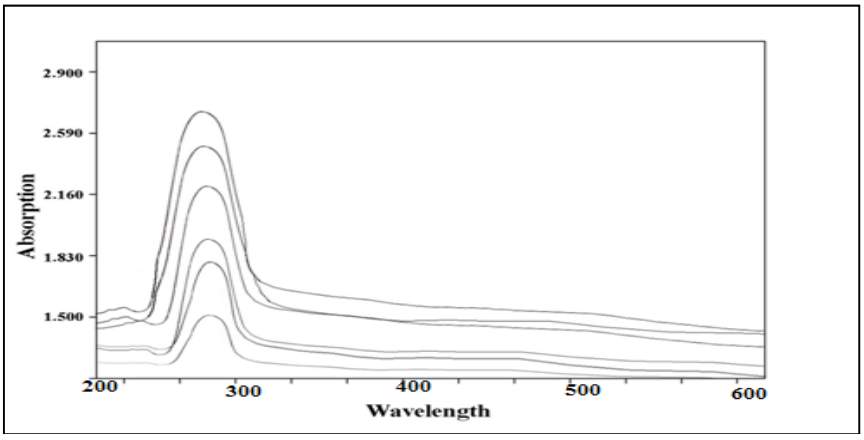


**Fig 1.3:** Absorption spectra of fluorouracil at different concentration of HP α – CD

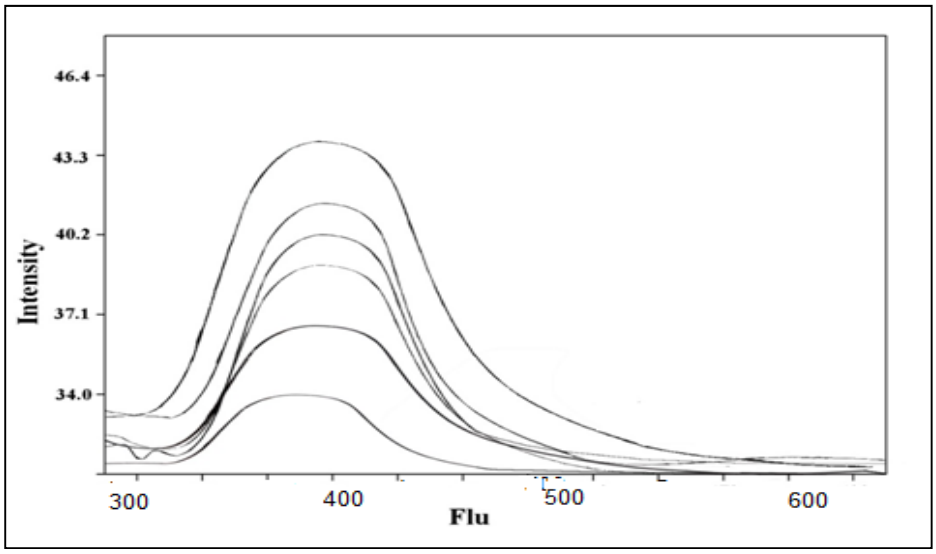


**Fig 1.4:** Fluorescence spectra of fluorouracil at different concentration of HP α – CD

Table (1) fig (1.1-1.4) shows the absorption and fluorescence maxima of fluorouracil solution containing various concentration of α–cyclodextrin and hydroxypropyl α–cyclodextrin. The absorption maxima of fluorouracil appear at 265.6 nm. Upon increasing the concentrations of both α–cyclodextrin and hydroxy propyl α–cyclodextrin the absorption maximas are blue shifted. This may be due to the interaction of the lone pair of the amino nitrogen with the $\pi$-cloud of the parent hydrocarbon. This interaction increases if an electron withdrawing group is attached in the *para* position. Thus, the nature of the electron withdrawing group in the *para* position decides the percentage charge transfer character in the $\pi$–$\pi$ transition as well as the value of the band maximum. In the fluorescence spectra it is red shifted by the addition of both α-cyclodextrin and hydroxypropyl α-cyclodextrin (i.e) 390 - 413nm and 390 - 415nm respectively.

The association constant (K) for the formation of an inclusion complex has been determined by analyzing the changes in the absorption and fluorescence maxima with the α-cyclodextrin concentration. The association constant and stoichiometric ratios of the inclusion complex of fluorouracil with α-cyclodextrin and Hydroxy propyl α -cyclodextrin can be determined by using the Benesi – Hilde brand relation [9]. The equations for 1:1 complexes are given below

Absorption

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

Fluorescence

$1 / I – I_0 = 1/ I – I_0 + 1 / K (I – I_0) [\alpha – CD]$

In the above equation,

$A_0 / I_0$ = intensity of absorption / fluorescence of drug without α-CD / HP α-CD

A/ I = Absorption / fluorescence intensity with a particular concentration of α-CD / HP α-CD

A' / I '= Absorption / Fluorescence intensity at the maximum concentration of α-CD / HP α-CD used

K = Association constant

Linearity is obtained in the plot of $1 / A – A_0$ or $1/ I - I_0$ verses 1 / [α –CD] / [HP α-CD] for 1: 1 complex. The association constant K was calculated from the slope of Benesi – Hilde brand plot using the equation

$K = 1 / slpoe (A' – A_0)$ for absorbance

$K = 1 / slope (I – I_0)$ for fluorescence

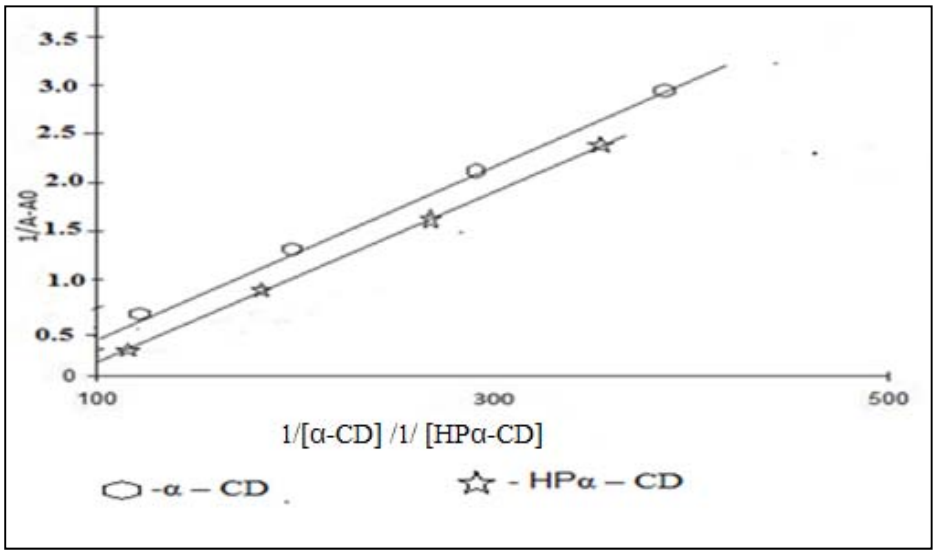


**Fig 2.1:** Benesi – Hilde brand plot of $1/A\text{-}A_0$ verus 1/[α–CD] /1/[HPα-CD] with fluorouracil

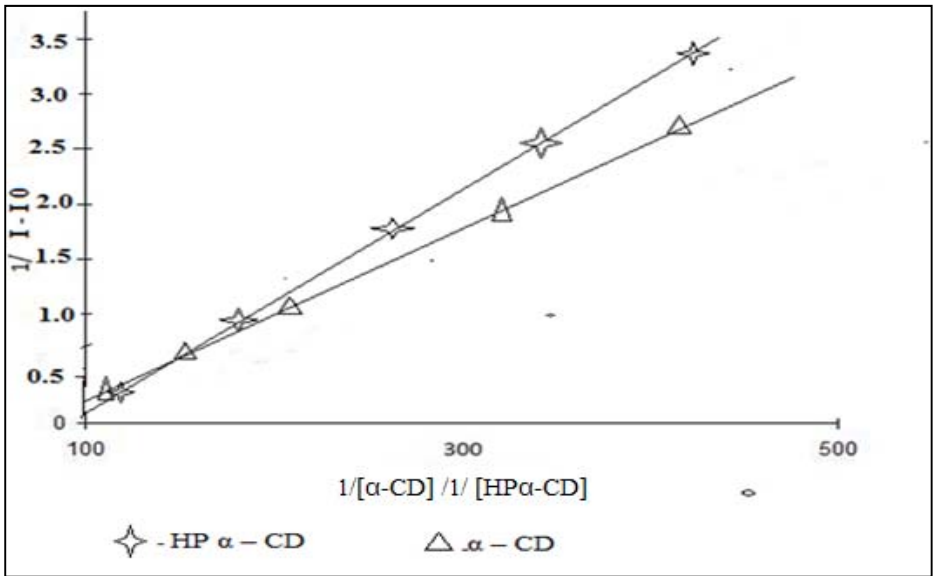


**Fig 2.2:** Benesi – Hilde brand plot of 1 / I – $I_0$ verus 1 / [α –CD] /1/[HPα-CD] with fluorouracil

Good linear correlations were obtained confirming the formation of 1 : 1 inclusion complexes. K = 79.872 $M^{-1}$ for absorbance 153.33 $M^{-1}$ for fluorescence in α-CD and K=166.61$M^{-1}$ for absorbance 183.85$M^{-1}$ for fluorescence in HPα-CD for fluorouracil. The higher association constant for absorbance shows that the drug molecule completely include in to the α-Cyclodextrin cavity.

**2.1 Phase solubility study**

The apparent stability constant of the complex was calculated from the phase solubility diagrams using the following equation,

$K_{1:1}$ = slope / $S_0$ (1 – Slope)

The slope is obtained from the initial straight line portion of the plot of concentration of fluorouracil against α – Cyclodextrin concentration. AL type solubility diagram was obtained as the solubility of fluorouracil increased linearly as the concentration of α-CD/ HPα-CD increases. The shape of the solubility curve indicate that a 1: 1 molar ratio is most probable for the inclusion complex formed. The stability constant of fluorouracil is 183.85 M-1 for α-cyclodextrin and 124.20 for Hydroxypropyl α-cyclodextrin. This result implies that both α-CD and HPα-CD are used as drug carrier for argumenting bioavailability of fluorouracil. So α-CD and HPα-CD are used to increase the solubility of fluorouracil instead of using organic solvents. If organic solvents are used they may cause liver and target organs. By using CDs the side effects and damage of inner organs can be prevented.

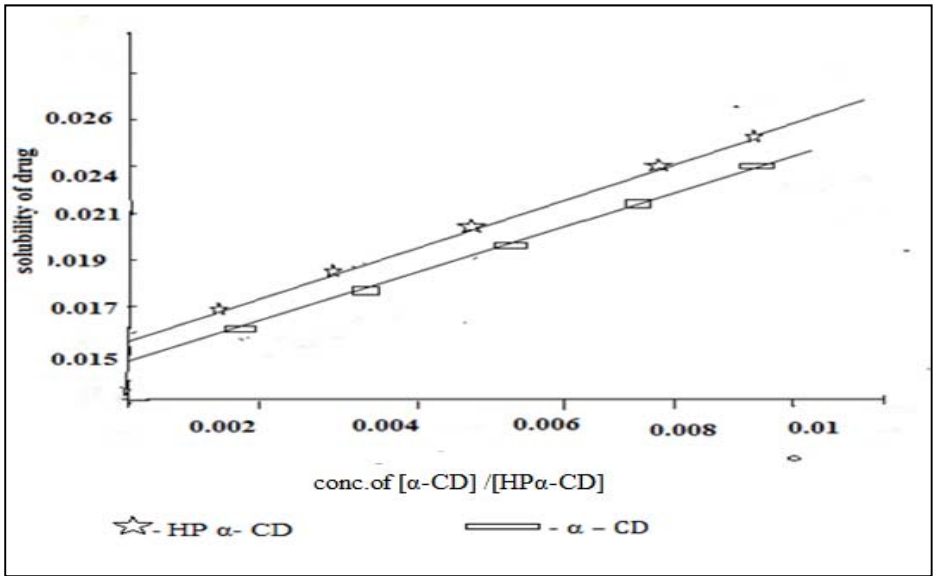


**Fig 3:** Phase solubility of fluorouracil at different concentration of α -CD& HP α –CD

**2.2 Thin layer chromatographic study**

Chromatographic investigations were performed at room temperature by the method of ascendant TLC on silica gel. The plates were spotted with 5µl aliquots of freshly prepared solution of drug and inclusion complex. The Rf values of the examined substances were determined. The migration distance was measured from the start line. Detection was performed by exposing the plates to iodine vapour.

**Table 3:** The composition of the mobile phases

| Solvents | Rf values | | |
|---|---|---|---|
| Composition | Drug | α-CD Inclusion | HP α- CD inclusion |
| Ethanol : Water ( 5 : 5 ) | 0.75 | 0.73 | 0.74 |
| Ethanol : Water ( 7 : 3 ) | 0.76 | 0.77 | 0.78 |
| Ethanol : Water ( 8 : 2 ) | 0.79 | 0.78 | 0.80 |
| Ethyl Acetate : Water ( 5 : 5 ) | 0.82 | 0.80 | 0.81 |
| Ethyl Acetate : Water ( 6: 4 ) | 0.84 | 0.83 | 0.83 |
| Ethyl Acetate : Water ( 9 : 1) | 0.86 | 0.83 | 0.85 |

According to the datas shown in Table (3) the compounds characterized by high lipophilicity hydroxypropyl α-cyclodextrin present a higher mobility compared to α-cyclodextrin. It is known that, the retention of the investigated substances, under the condition of planar chromatography on silica gel, is the result of the hydrogen bond formed with the silanol groups of the sorbent, dipole-dipole and other electrostatic interaction [10]. From the Rf value it may conclude that ethyl acetate water solvent mixture is the best solvent, As the concentration of the solvent increases the solubility of fluorouracil increases. The solubility of fluorouracil increases the Rf value. High Rf values indicates the strong inclusion of fluorouracil with CDs. Out of α-CD and HPα-CD the drug is strongly included in the HPα-CD cavity than α-CD cavity.

## 3. Conclusion

Absorption maxima of Fluorouracil is blue shifted by the addition of α-cyclodextrin and Hydroxy propyl α-cyclodextrin. The fluorescence maxima are red shifted for both α-CD and HPα-CD. The association constant K is higher for HPα-CD which shows absorbance that the drug molecule completely include in to the HPα-CD cavity than α-CD. From the phase solubility studies AL type diagram was obtained which indicate that a 1:1 molar ratio is most probable for the inclusion complex. The stability constant is greater for fluorouracil in both α-cyclodextrin and hydroxyl propyl α-cyclodextrin. From the TLC studies hydroxyl propyl α-cyclodextrin implies a higher mobility compared to α-cyclodextrin.

## 4. Acknowledgement

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