EVALUATION OF SOLUBILITY OF FLUTAMIDE BY USING SUPRAMOLECULAR TECHNIQUE


ABSTRACT

The poor aqueous solubility of flutamide gives rise to difficulties in pharmaceutical formulations for oral delivery, which may lead to poor bioavailability. To overcome this drawback, increasing the aqueous solubility of flutamide is an important goal. The present investigation deals with the approach of enhancement of flutamide by using a conformer tartaric acid and nicotinic acid utilizing a supramolecular technique. Moreover the study also determines the effect of solvent and the method influence the solubility and dissolution competence of flutamide. The solubility of the prepared co crystals enhanced the solubility in distilled water and pH6.8 phosphate buffer when compared to pure drug. From Invitro dissolution studies it shows increase in its dissolution profile of flutamide in water and pH 6.8 phosphate buffer compared to pure drug. From this study it can be concluded that by using supramolecular technique it is possible to enhance the solubility of the poorly soluble drugs.

Keywords: Flutamide, Supramolecular technique, Invitro dissolution studies.

INTRODUCTION

The oral route remains the preferred route of drug administration due to its convenience, good patient compliance and low medicine production cost. In order for a drug to be absorbed into the systemic circulation following oral administration, the drug must be dissolved in gastric fluids. The active pharmaceutical ingredients in solid dosage forms must undergo dissolution before it is available for absorption from the gastro intestinal tract. Drug molecule with limited aqueous solubility are becoming increasingly prevalent in research and development molecule of the type can provide a number of challenges in pharmaceutical development and may potentially lead to slow dissolution in biological fluid, insufficient and inconsistent systemic exposure and consequent sub optimal efficacy in patients, particularly when delivered via the oral route of administration. Advances in the pharmaceutical sciences have led to the establishment of a number of approaches for addressing the issues of low aqueous solubility.

The poor aqueous solubility of flutamide gives rise to difficulties in pharmaceutical formulations for oral delivery, which may lead to poor bioavailability. To overcome this drawback, increasing the aqueous solubility of flutamide is an important goal.

Present investigation deals with the approach of enhancement of flutamide by using a conformer tartaric acid and nicotinic acid utilizing a supramolecular technique. Moreover the study also determines the effect of solvent and the method influence the solubility and dissolution competence of flutamide [1].

MATERIALS AND EQUIPMENTS

The best possible Pharma grade materials available were used for this research as supplied by manufacturer. All other reagents and chemicals used are of analytical grade.

Following materials were used for the present study,
1. Flutamide (Matrix Lab, Hyderabad)
2. Cinnamic acid (Loba Chemie, Mumbai)

Following Equipments were used for the present study,
1. Electronic Analytical Balance (Shimadzu, Japan),
2. UV-Visible spectrophotometer UV-1700 (Shimadzu, Japan),
3. Dissolution apparatus (Electro Lab)
4. Hot plate (Sigma Instruments)
5. Scanning Electron Microscope (ZEISS Electron Microscope, EVO MA 15)
6. Single Crystal X-Ray Diffractometer (Enraf Nonius CAD4-MV31)

ANALYTICAL METHOD OPTIMIZATION
Number of analytical methods is available for estimation of flutamide such as ultra-violet spectroscopy, reverse phase HPLC with UV detection, gas chromatography, mass spectroscopy and spectrofluorimetric method. The following method was used for further studies [2].

UV-Visible Spectroscopy

Standard curve of flutamide in phosphate buffer (6.8pH) in 2% SLS
5mg of flutamide dissolved in 100ml mixture of phosphate buffer with SLS solution system by proper ultrasonication for 5-10 mins and further dilutions were made by using phosphate buffer in 2% SLS solution to obtain concentrations ranging 2,4,6,8 and 10µg/ml. The absorbance of solution was measured at 293nm using UV Visible Spectrophotometer [3].

Standard curve of flutamide in distilled water
5mg of flutamide dissolved in 100ml water with SLS solution system by proper ultrasonication for 5-10 min and further dilutions were made by using phosphate buffer in 2% SLS solution to obtain concentrations ranging 2,4,6,8 and 10µg/ml. The absorbance of solution was measured at 293nm using UV Visible Spectrophotometer. The readings obtained are tabulated in table and the graph was given in Fig 2.

PREFORMULATION STUDIES

The solid state properties of flutamide, and cinnamic acid were determined by FTIR, XRD etc.

Identification of drug
5mg of flutamide dissolved in 100ml mixture of phosphate buffer with SLS solution system by proper ultra-sonication for 5-10 min and further dilutions were made by using phosphate buffer in 2% SLS solution to obtain concentrations ranging 2,4,6,8 and 10µg/ml. The absorbance of solution was measured at 302 nm using UV Visible Spectrophotometer.

Solubility studies

The solubilities of pure compounds and co-crystal were determined using a 24-hour shake flask method (used previously for many compounds) [Wermuth, 2008]. To 1ml of the solvent excess amount of drug is added and it is kept for stirring for 24 hr in orbital shaker. After 24 hrs the samples with sufficient dilutions was analysed spectrophotometrically [4,5].

Characterization of pure drug

Fourier transform infrared (FT-IR) studies
For the pure drug, Fourier transform infrared (FT-IR) spectra were obtained. The spectra were recorded in a thermo-IR 200 FTIR spectrophotometer. Potassium bromide pellet method was employed and background spectrum was collected under identical conditions. Each spectrum was derived from 16 single average scans collected in the range of 400-4000 1/cm at the spectral resolution of 20 1/cm [6].

Differential scanning calorimetry (DSC)
Thermal analysis of pure curcumin, were recorded on a DSC (NETZSCH DSC 204). The temperature axis and cell constant of DSC were previously calibrated with indium. A heating rate of 10 C/min was employed with nitrogen pursing. Powder samples (15-30 mg) was weighed into an aluminum pan and analyzed as sealed with pin holes and an empty aluminum pan was used as reference [7].

X-Ray Powder Diffraction (XRPD)
Powder X-ray diffraction is a fingerprint characterization method for solid phases, such as co-crystals and salts. Every crystalline solid phase has a unique XRPD pattern, which can form the basis for its identification. The study was carried out using X-Ray Diffractometer using Cu ka radiation. The tube operated at 45 kV, 9mA and data was collected over an angular range from 0 to 500 20 of the diffraction angle in continuous scan mode using a step size of 0.050 20 and a time of 0.1 s. If the resulting XRPD of the solid product after grinding the pure solid compounds (the API and the coformer) is different from that of the reactants, then it may be inferred that a new solid phase has formed. The unexpected formation of a new polymorph for one of the components during grinding and or due to the presence of a co-former as an additive is another possibility [8].

RESULTS

UV-VIS spectrum: flutamide
UV-VIS spectrum of flutamide in 2% SLS phosphate buffer pH 6.8 was determined and spectrum was shown in Fig 3. It gave a peak at 302nm, the lambda max which is similar to the obtained reference.

FTIR (Fourier transform infra-red spectroscopy)
study of flutamide
In FT-IR analysis, the spectrum of pure flutamide showed an intense and well-defined bands characteristic to flutamide at 3354 1/cm (N-H stretching), 1710 1/cm (C=O stretching), 1539 1/cm (NO2 stretching) 1604 Aromatic C=C stretching. 1342 Cx3 group 861.898 Tri substituted benzene 1240 Amide C-N, this FTIR analysis shows that flutamide is pure. Interpretation of IR spectra of flutamide has shown fig 4.

X-ray powder diffraction (XRPD) is a powerful technique for the identification of the crystalline solid phases. Every crystalline solid phase has a unique XRPD pattern, which can form the basis for its identification. The X-ray powder diffraction (XRD) spectra of flutamide shows characteristic peak at 25.176° (100%), 9.739°, 15.018°, 18.28550° and 27.084° indicates pure flutamide.

In FT-IR analysis, the spectrum of pure cinnamic acid showed an intense and well-defined bands characteristic to cinnamic acid at 3641 1/cm (OH stretching vibration), 1669.1 1/cm (C=O stretching), 1622.7 1/cm (Aromatic C=C). this FTIR analysis shows that cinnamic acid is pure.

X-ray powder diffraction (XRPD) is a powerful technique for the identification of the crystalline solid phases. Every crystalline solid phase has a unique XRPD pattern, which can form the basis for its identification. The X-ray powder diffraction (XRD) spectra of flutamide shows characteristic peak at 25.176° (100%), 9.739°, 15.018°, 18.28550°, 20.546°, 27.084°, 29.249° and 31.710° indicates pure Cinnamic acid.

Solubility studies of pure drug
The solubility study of pure drug is performed in phosphate buffer pH6.8.

The solubility studies have been performed for the pure drug in water and pH 6.8 buffers. The pure drug showed a solubility 0.04 and 0.0432 mg/ml respectively.

SUPRAMOLECULAR SYNTHESIS
Solution crystallization (S.C) method
Flutamide and cinnamic acid in 1:1 ratio were dissolved in ethanol by heating to make a supersaturated solution and on cooling evaporation of solvent leads to crystallization. Crystals were collected by filtration and preserved.

Liquid assisted grinding (L.A.G) method
Flutamide and cinnamic acid in 1:1 ratio was grinded in a mortar and pestle using small quantity of ethanol of ethanol by liquid assisted grinding method. The crystals formed were collected separately and preserved.

Co-crystal by co-grinding method
Flutamide and cinnamic acid (co-crystal former) were taken in glass mortar and pestle and grounded up to 1 hr. and keep it for drying.

ASSAY OF CRYSTALS
1mg of flutamide crystals prepared by solution crystallisation liquid assisted grinding method and co-grinding method were taken and dissolved in Phosphate buffer pH6.8. From that 1ml was taken and diluted to 10ml. the absorbance of solution was measured at 431nm using ELICO UV-Visible spectrophotometer and drug content was calculated [9].

IN-VITRO DISSOLUTION STUDIES
Dissolution studies of samples weight equivalent to 50 mg were performed using USP XXII apparatus at the stirring speed of 50 rpm and temperature maintained at 37± 0.5°C with Phosphate buffer pH6.8 as a dissolution medium. The samples of 5ml were withdrawn at regular intervals of 5, 15,30,45,60 min and filtered. Every time the samples are replaced with 5ml of the fresh dissolution medium. The filtered samples were suitably diluted and analyzed for flutamide at 302nm. Percentage drug release from the samples was calculated by using disso software PCP disso V3 software [8].

RESULTS
FTIR studies
Crystal form I: flutamide-cinnamic acid (1:1) co-crystal (solvent evaporation): FTIR Studies
The IR spectra and interpretation for isoniazid, para amino salicylic acid and crystal form I were presented in figure no. 7.

In FT-IR analysis, the spectrum of co-crystal 1, the intense and well-defined bands characteristic to flutamide at 3354 1/cm (N-H stretching) was absent, 1710 1/cm (C=O stretching) was shifted to 1741/cm , 15391/cm (NO2 stretching) was absent, 1604 Aromatic C=C stretching was shifted to 1659 cm/1. The spectrum of cinnamic acid at 3641 1/cm (OH-stretching vibration) was found to be absent, 1669.1 1/cm (C=O stretching vibration) was found to be absent, 1622.7 1/cm (Aromatic C=C). From this result it is indicated that the formation of co-crystal of flutamide and cinnamic acid by solvent evaporation method.

Crystal form I: flutamide-cinnamic acid (1:1) co-crystal (solvent evaporation): Powder XRD
The X-ray powder diffraction (XRD) spectra of flutamide shown characteristic peaks at 25.176° (100%), 9.739°, 15.018°, 18.28550° and 27.084°. also spectra of cinnamic acid shows characteristic peak at 25.176° (100%), 9.739°, 15.01°, 18.850°, 20.546°, 27.084°, 29.249° and 31.710°. The X-ray powder diffraction (XRD) spectra of flutamide and cinnamic acid co-crystal shows characteristic peak at 29.063° which is 100% relative intensity which is different from individual components of flutamide and cinnamic acid also at 16.191°, 24.987°, 39.928° new peaks were appeared. This indicates formation of new crystalline phase.
Crystal form II: flutamide-cinnamic acid (1:1) co-crystal (kneading method):

FTIR Studies:
The IR spectra and interpretation for isoniazid, para amino salicylic acid and crystal form I were presented in figure no. 9.

In FT-IR analysis, the spectrum of co-crystal 1, the intense and well-defined bands characteristic to flutamide at 3354 1/cm (N-H stretching) was absent, 1710 1/cm (C=O stretching) was shifted to 1712/cm, 15391/cm (NO2 stretching) was absent, 1604 Aromatic C=C stretching was shifted to 1658 cm/1. The spectrum of cinnamic acid at 3641 1/cm (OH stretching) was absent, 1669.1 1/cm (C=O stretching) shifted to 1658 cm/1. The spectrum of cinnamic acid at 3641 1/cm (OH-stretching vibration) was found to be absent, 1669.1 1/cm (C=O stretching) shifted to 1605 1/cm. From this result it is indicated that the formation of co-crystal of flutamide and cinnamic acid by liquid assisted grinding method.

Crystal form II: flutamide-cinnamic acid (1:1) co-crystal (kneading method):
Powder XRD
The X-ray powder diffraction (XRD) spectra of flutamide shown characteristic peaks at 25.176° (100%), 9.739°, 15.018°, 18.2855° and 27.084°. Also spectra of cinnamic acid shows characteristic peak at 25.176° (100%), 9.739°, 15.01°, 18.850°, 20.546°, 27.084°, 29.249° and 31.710°. The X-ray powder diffraction (XRD) spectra of flutamide and cinnamic acid co-crystal shows characteristic peaks at 22.655° which is 100% relative intensity which is different from individual components of flutamide and cinnamic acid. Also at 8.541°, 21.220°, and 33.635° new peaks were appeared. This indicates formation of new crystalline phase.

Crystal form II: flutamide-cinnamic acid (1:1) co-crystal (Grinding method):

FTIR Studies:
The IR spectra and interpretation for isoniazid, para amino salicylic acid and crystal form I were presented in figure no. 27.

In FT-IR analysis, the spectrum of co-crystal 1, the intense and well-defined bands characteristic to flutamide at 3354 1/cm (N-H stretching) was absent, 1710 1/cm (C=O stretching) was shifted to 1711 1/cm, 15391/cm (NO2 stretching) was absent, 1604 Aromatic C=C stretching was shifted to 1660 cm/1. The spectrum of cinnamic acid at 3641 1/cm (OH-stretching vibration) was found to be absent, 1669.1 1/cm (C=O stretching) shifted to 1607 1/cm. From this result it is indicated that the formation of co-crystal of flutamide and cinnamic acid by co grinding method.

Crystal form III: flutamide-cinnamic acid (1:1) co-crystal (Co Grinding method):

Powder XRD
The X-ray powder diffraction (XRD) spectra of flutamide shown characteristic peaks at 25.176° (100%), 9.739°, 15.018°, 18.2855° and 27.084°. Also spectra of cinnamic acid shows characteristic peak at 25.176° (100%), 9.739°, 15.01°, 18.850°, 20.546°, 27.084°, 29.249° and 31.710°. The X-ray powder diffraction (XRD) spectra of flutamide and cinnamic acid co-crystal shows characteristic peaks at 22.658° which is 100% relative intensity which is different from individual components of flutamide and cinnamic acid. Also at 17.00°, 19.546°, and 43.990° new peaks were appeared. This indicates formation of new crystalline phase.

Drug content
Drug content of prepared crystal forms were determined in triplicate by spectrophotometrically. The practical yield was found satisfactory and ranged from 92.35% to 94.24% and 98.40 for those in pH 6.8 phosphate buffer. The values of prepared crystal forms were shown in table no 2.

Saturation Solubility
The solubility of pure compounds and co-crystal were determined using a 24-hour shake flask method (used previously for many compounds). To 5ml of the solvent excess amount of drug is added and it is kept for stirring for 24hr in orbital shaker. After 24 hrs the samples with sufficient dilutions was analysed spectrophotometrically.

The solubility studies of flutamide and prepared novel multicomponent crystal forms in distilled water and pH 6.8 phosphate buffer were shown in table no.3. This indicates that prepared crystal forms shows high solubility compare with pure drug. In the prepared crystal forms, Crystal forms 1 have least solubility in distilled water and pH 6.8 phosphate buffer.

In vitro dissolution studies
Invitro dissolution studies were done in triplicate for flutamide and prepared novel multicomponent crystal forms of flutamide in water and pH 6.8 phosphate buffer. The powder dissolution profiles for flutamide, Crystal form 1, 2, and 3 were shown in figure no.11. Dissolution data was shown in table no.4.

| Table 1. Solubility studies of pure drug |
|------------------|------------------|
| **SAMPLE** | **SOLUBILITY (mg/ml)** |
| Pure drug in water | 0.04±0.01 |
| Ph6.8pbs | 0.0432±0.02 |
Table 2. Drug content of prepared crystal forms

<table>
<thead>
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<th>Crystal form</th>
<th>10 mg of crystal form contains (% Yield) in pH 6.8 phosphate buffer</th>
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<tbody>
<tr>
<td>Crystal form 1</td>
<td>92.35</td>
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<tr>
<td>Crystal form 2</td>
<td>94.24</td>
</tr>
<tr>
<td>Crystal form 3</td>
<td>98.40</td>
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Table 3. Solubility data of flutamide and prepared crystal form

<table>
<thead>
<tr>
<th></th>
<th>gm/ml</th>
<th>gm/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>water</td>
<td>pH 6.8 Phosphate buffer</td>
</tr>
<tr>
<td>flutamide</td>
<td>0.04±0.01</td>
<td>0.0432±0.03</td>
</tr>
<tr>
<td>Crystal form 1</td>
<td>0.037±0.04</td>
<td>0.043±0.01</td>
</tr>
<tr>
<td>Crystal form 2</td>
<td>0.072±0.01</td>
<td>0.067±0.01</td>
</tr>
<tr>
<td>Crystal form 3</td>
<td>0.064±0.03</td>
<td>0.078±0.02</td>
</tr>
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</table>

Table 4. Dissolution data of flutamide and its crystal forms in pH 6.8 phosphate buffer

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>INH</th>
<th>Crystal 1</th>
<th>Crystal 2</th>
<th>Crystal 3</th>
</tr>
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<td>5</td>
<td>2.35</td>
<td>6.45</td>
<td>22.34</td>
<td>23.46</td>
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<td>10</td>
<td>7.55</td>
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<td>38.45</td>
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<td>19.56</td>
<td>44.22</td>
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<td>30</td>
<td>21.45</td>
<td>25.67</td>
<td>52.22</td>
<td>56.33</td>
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<td>45</td>
<td>28.54</td>
<td>29.56</td>
<td>60.45</td>
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</table>

Fig 1. Standard curve of flutamide

Fig 2. Standard curve of flutamide in distilled water
Fig 3. X-Ray Powder Diffraction (XRPD) study of flutamide

Fig 4. FTIR (Fourier transform infra-red spectroscopy) study of Cinnamic acid (F1)
Fig 5. X-Ray Powder Diffraction (XRPD) study of Cinnamic acid

Fig 6. FTIR (Fourier transform infra-red spectroscopy) study of Cinnamic acid (F2)
Fig 7. FT-IR study of flutamide-cinnamic acid (1:1) co-crystal (solvent evaporation)
Fig 8. Powder XRD of Crystal form I: flutamide-cinnamic acid (1:1) co-crystal (solvent evaporation)
Fig 8. Powder XRD of Crystal form II: flutamide-cinnamic acid (1:1) co-crystal (kneading method)
Fig 9. FT-IR analysis of Crystal form II: flutamide-cinnamic acid (1:1) co-crystal (Grinding method)
Fig 10. Powder XRD of Crystal form III: flutamide-cinnamic acid (1:1) co-crystal (Co Grinding method)

Figure 11. Dissolution profile of pure drug and prepared co-crystal forms in pH6.8phosphate buffer
DISCUSSION AND CONCLUSION
Preformulation studies
From the preformulation studies the solid-state characterization of flutamide by FTIR, and XRD studies has clearly demonstrated that flutamide is pure crystalline phase and cinnamic acid samples are pure and it was selected for the further investigations.

Crystal form 1: Flutamide-cinnamic acid (1:1) co-crystal (solvent evaporation method)
Crystal form I (1:1 molar ratio, Co-crystal) was prepared by solvent evaporation solution crystallization method. It characterized in terms of FTIR, and XRD subjected to Preliminary pharmaceutical characterization such as solubility, Invitro dissolution studies.

The crystal structure determination of Crystal form I reveals that the two molecules (Flutamide-cinnamic acid) are associated via a carboxylic acid-amide hydrogen bond heterosynthons.

IR and XRD support the formation of co-crystal between Flutamide-cinnamic acid at 1:1 stoichiometric ratio. It was showing increased solubility in distilled water and pH6.8 phosphate buffer when compared to pure drug.

From Invitro dissolution studies it shows no change in its dissolution profile, with 29.56% at the end of 60 min where pure drug has 28.54 drug release.

Crystal form II: Flutamide-cinnamic acid (1:1) co-crystal (Liquid assisted grinding method)
Crystal form II (1:1 molar ratio, Co-crystal) was prepared by Liquid assisted grinding method. It characterized in terms of FTIR, and XRD subjected to Preliminary pharmaceutical characterization such as solubility, Invitro dissolution studies.

The crystal structure determination of Crystal form II reveals that the two molecules (Flutamide-cinnamic acid) are associated via a carboxylic acid-amide hydrogen bond heterosynthons.

IR and XRD support the formation of co-crystal between Flutamide-cinnamic acid at 1:1 stoichiometric ratio. It was showing increased solubility in distilled water and pH6.8 phosphate buffer when compared to pure drug.

From Invitro dissolution studies it shows increase in its dissolution profile, with 60.45% at the end of 60 min where pure drug has 28.54 drug release.

Crystal form III: Flutamide-cinnamic acid (1:1) co-crystal (Cogrinding method)
Crystal form III (1:1 molar ratio, Co-crystal) was prepared by Cogrinding method. It characterized in terms of FTIR, and XRD subjected to Preliminary pharmaceutical characterization such as solubility, Invitro dissolution studies.

The crystal structure determination of Crystal form III reveals that the two molecules (Flutamide-cinnamic acid) are associated via a carboxylic acid-amide hydrogen bond heterosynthons.

IR and XRD support the formation of co-crystal between Flutamide-cinnamic acid at 1:1 stoichiometric ratio. It was showing increased solubility in distilled water and pH6.8 phosphate buffer when compared to pure drug.

From Invitro dissolution studies it shows increase in its dissolution profile, with 62.28% at the end of 60 min where pure drug has 28.54 drug releases.

This concept was applied to the co-crystallization of flutamide with carboxylic acids such as cinnamic acid by solvent evaporation, solvent drop and co-grinding assisted crystallization. The carboxylic acid-amide hydrogen bond has again been used to successfully create a new pharmaceutical co-crystal of flutamide, Crystal form I, II, III with cinnamic acid. The supramolecular interaction of flutamide (amide) with carboxylic acid of cinnamic acid resulted in genuine co-crystals. The crystallization of flutamide with cinnamic acid is in 1:1 ratio. The prepared co-crystal was characterized in terms of FTIR, and XRD subjected to Preliminary pharmaceutical characterization such as solubility, Invitro dissolution studies.

The crystal structure determination of Crystal form III reveals that the two molecules (Flutamide-cinnamic acid) are associated via a carboxylic acid-amide hydrogen bond heterosynthons. The solubility of the prepared co-crystals enhanced the solubility in distilled water and pH6.8 phosphate buffer when compared to pure drug. From Invitro dissolution studies it shows increase in its dissolution profile of flutamide in water and pH 6.8 phosphate buffer compared to pure drug. From this study it can be concluded that by using supramolecular technique it is possible to enhance the solubility of the poorly soluble drugs.

REFERENCES
1. Food and Drug Administration. Orphan designations pursuant to Section 526 of the Federal Food and Cosmetic Act as amended by the Orphan Drug Act (P.L. 97-414), Rockville, MD; 1996.