Capturing The Significance of X-Ray Crystallography in Pharmaceutical Field:

The Application to Characterize New Salt, Co-Crystal and Co-Amorphous

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Acyclovir

- Guanosine analogue antiviral drugs.
- The treatment of herpes simplex virus infections.
Outline

- **Screening and Characterization**
  - Screening methods and used additives
  - Selected cocrystals (PXRD, TG/DTA, DSC)

- **Application for oral dosage form**
  - Intrinsic dissolution of selected cocrystal
  - Crystal structure of selected cocrystal
  - Mechanism for solubility enhancement

- **Application for transdermal dosage form**
  - Transdermal adsorption property of selected complex
  - Solubility of amorphous complexes
  - Improvement of transdermal properties
2/3 Hydrate

*Commerciably available*

Recrystalized from ethanol solution

**P2₁/n**

- a 25.459(1) Å
- b 11.282(1) Å
- c 10.768(1) Å
- β 95.16(1)°

**Volume**

3080.342 Å³

**Z** 12

**R-factor** 5.3%

*Column structure*

*Stacking purine moiety*
Dihydrate

From ammonium solution

Channel structure
Stacking purine moiety

**Crystallographic Data**

- **Space Group:** P-1
- **Unit Cell Parameters:**
  - *a* = 6.8386(7) Å
  - *b* = 11.3679(14) Å
  - *c* = 14.942(2) Å
  - *α* = 82.845(4)°
  - *β* = 82.419(3)°
  - *γ* = 89.326(3)°
- **Volume:** 1142.5(2) Å³
- **Z:** 4
- **R-factor:** 7.71%
Dehydration of hydrates

Anhydrate2

$P2_1/c$

\[
\begin{align*}
a & = 10.9399(6) \text{ Å} \\
b & = 11.1837(6) \text{ Å} \\
c & = 8.1164(4) \text{ Å} \\
\beta & = 108.6277(34) \text{°} \\
\text{Volume} & = 941.009 \text{ Å}^3 \\
Z & = 4
\end{align*}
\]

Stacking purine moiety
Anhydrate2

Crystallization by Vapor-Diffusion. Procedure. (DMF+Acetonitrile)

P 2₁₂₁₂₁
a 4.5387(10) Å
b 15.0308(3) Å
c 28.3320(6) Å
Volume 1932.82 Å³
Z 8
R-factor 9.88 %

Different stacking mode
Dissolution properties for acyclovir polymorphs

Intrinsic dissolution rate
- area for disson: 0.2 cm²
- pH 6.8 Phosphate buffer
- 37°C
- 50 rpm

Similar dissolution rate

Transformation of anhydrate to hydrate was quick.
Anhydrates were useless

Hydration mechanism?
Hydration properties for acyclovir anhydrate2

Reversible

Anhydrate 2 ➔ Dihydrate ➔ 2/3 hydrate

2/3 hydrate

Anhydrate 2

Desorption

Dihydrate

Sorption

Change in mass/%

Target RH/%
Hydration properties for acyclovir anhydrate 1

Anhydrate 1 ↔ 2/3 hydrate

Irreversible

Anhydrate 2

25°C RH 95% 1 week

Anhydrate 1 ➔ 2/3 hydrate ➔ Dihydrate

Desorption
Comparison between transition behavior and stacking mode

**Similarity of stacking**
The phase transition mechanism is complicated.
XRPD patterns of acyclovir using HT capillary sample holder

- Anhydrate Form 2 (RT)
- Anhydrate Form 4 (210°C)
- 2/3 hydrate (RT)
Comparison from the view of stacking structure

2/3 hydrate
Anhydrate Form 4
Anhydrate Form 2
Anhydrate Form 1

antiparallel
parallel
Phase Transition of Acyclovir polymorphs

- **2/3 hydrate**
  - RH95% at 25°C → Anhydrate 1
  - RH0% at 25°C → Anhydrate 3
  - 120°C → Anhydrate 3
- **Anhydrate 1**
  - RH0% at 25°C → Anhydrate 2
  - 170°C → Anhydrate 4
- **Anhydrate 2**
  - 170°C
- **Anhydrate 3**
  - 120°C
- **Anhydrate 4**
  - 170°C
Conclusion

• There are two packing manners for purine moiety. Anhydrate 1, anhydrate 2, 2/3 hydrate and ACV dihydrate were packed in parallel, antiparallel, mixture of parallel—anti-parallel and parallel manners, respectively.

• Based on the packing manner of ACV, it can be seen why the phase transformation occurs with readily or with difficulty.
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dissolution properties

Sprangly soluble in water

Dissolution rate and solubility for water
Activity related to the solubility in base

Oral dosage form
Bioavailability: low

Transdermal
Transdermal Absorption: low

Improvement of solubility
Improvement of the physicochemical properties

Methods

- polymorphs
- Salt formation
- Solvate
- Cocrystal
- Package
- Solid dispersion
- gringing
- amorphous

Application for oral dosage form

Cocrystal → Improvement of dissolution properties

Application for transdermal dosage form

Amorphization → Increase the solubility in base → Increase the absorption
ACV has many functional groups, capable to make hydrogen bonding.

Generally recognized as safe compound.
Amorphization of Acyclovir

Conventional methods isn’t suitable for the amorphization

Preparation of Amorphous complex with additives
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Generally recognized as safe compounds used

- Citric acid
- Tartaric acid
- Malic acid
- Glycine
- Alanine
- Stearic acid
- Palmitic acid
- Docosanoic acid
- Lauric acid
- Aspartic acid
- Arginine
- Urea
- Nicotinamide
- Saccharin

Existence of the records as Oral or Transdermal application
PXRD patterns of samples

- Anhydrate1
- Anhydrate2
- 2/3 hydrate
- 2hydrate
- Citric acid
- Cocrystal
- Amorphous complex

2θ/degree
TG/DTA curve of samples

Anydrate1

Anhydrate2

2/3hydrate

2hydrate

Clear difference in curves
No possibility of the formation of solvate
DSC curves of amorphous complex

Glass transition temperature $T_g$ was higher than the room temperature. Confirmation of the physical stability of amorphous complex
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Crystal structure of Acyclovir - Citric acid Cocrystal

Single crystals weren’t obtained

Crystal structure analysis using powder diffraction data obtained by synchrotron radiation

Stoichiometry of Acyclovir : Citric acid was 1:1
Comparison of stacking structure of Acyclovir and its cocrystal

Stacking structure of Purine frame

Un-stabilize the stacking structure by intercalation of citric acid

Enhancement of the solubility
Distance in the purine frame in the crystals

π-π stacking distance (Å)

No difference

Change in the crystal structure by cocrystal

Improvement of dissolution property
Enhancement of solubility

Saturated Solubility of Cocrystal in various solvents

Physical mixture showed the similar solubility compare to the Cocrystal
ACV and Citric acid was interacted, even in the solution.
Initial dissolution profiles for ACV samples

Remarkable enhancement of solubility was observed.
Mechanism for improvement of dissolution property of ACV by Cocrystal formation

Expansion of Purine layer
Decrease in the lattice energy
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**In vitro** Transdermal test for ACV ointment

<Franz type diffusion cell>

- 15 mm Threaded cell top
- Cell ring
- Cell 15 mm × 7 mL
- Helix mixer 9 mm
- Water jacket
- Magnetic stirrer
- Sampling port
- Replace port with bubble trap

**Sample**

- Hairless mouse back skin
- pH 6.8 phosphate buffer

**Water** (37°C)

**Ointment base**: Macrogol
Permeability of ACV

- Low permeability of ACV
- 22 hours were necessary to permeate

Application of amorphous complex?
Saturated solubility of ACV in Macrogol base

- PEG400: Liquid
- PEG4000: Solid

Increased solubility for ACV-CA amorphous form.
Amorphous ACV complex was dissolved in super saturated states.
Permeability of ACV samples

Improvement of permeability of ACV was achieved.
Advantage of amorphization for transdermal application

Chemical, physical enhancement methods might affect the barrier function of skin.

Increase the concentration gradient may **not affect** the barrier function.

Safer method for transdermal application.