臺灣藥學聯合學術研討會

Solid Dispersion of Andrographolide: Enhanced Dissolution and Oral Bioavailability in Rats

穿心蓮內酯固體分散劑型：增加溶離度及大鼠口服生體可用率

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Date: 2018/01/13

Outline

- Introduction
- Aims of the study
- Materials and methods
- Results and discussion
- Conclusion

Introduction

Andrographolide (AG)

- Main active ingredient of *Andrographis paniculata*
- Diterpene lactone
- Molecular formula: $C_{20}H_{30}O_5$
- Molecular weight: 350.44 g/mol
- Potential effects: anti-inflammatory, anti-platelet, and anti-tumor

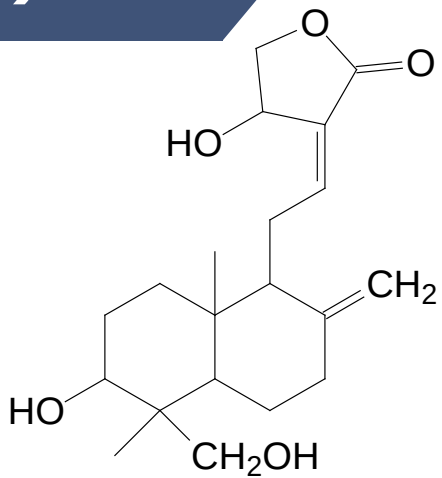


Fig. 1. The chemical structure of andrographolide.

Journal of Ethnopharmacology 135 (2011) 678-684.
Biochemical Pharmacology 84 (2012) 914-924.
Immunopharmacology and Immunotoxicology 29 (2007) 81-93.

Limitation of andrographolide

- **Biopharmaceutical classification (BCS) class IV**
  - Aqueous solubility: 46.26 ± 1.40 μg/mL
  - Log *P* value: 2.632 ± 0.135
- **P-glycoprotein efflux**

→ **Absolute bioavailability : 2.67%**

Phytomedicine 7 (2000) 351-364.
Journal of Pharmaceutical Sciences 100(11) (2011) 5007-5017.

Literature reviews

Table 1. The different formulations of andrographolide of previous studies with pharmacokinetic studies.

Formulations	Excipients	Animals	Relative bioavailability	Reference
Microcrystals	HPMC-E5 and microcrystalline cellulose	Beagle dogs	1.57-fold	*International Journal of Pharmaceutics 493 (2015) 214-223.*
Nanoparticles	Eudragit EPO and Pluronic F-68	Wistar rats	2.22-fold	*European Journal of Drug Metabolism and Pharmacokinetics 35 (2011) 123-129.*
Solid lipid nanoparticles	Compritol 888 ATO, GMS, lecithin and Tween 80	Wistar rats	2.42-fold	*Journal of Pharmaceutical Sciences 102 (2013) 4414-4425.*

Disadvantages of formulations

Microcrystals

- Manufacturing process was complicated
  → Wet milling & fluid bed
- Relative bioavailability increased less than 2-fold
  → 1.57-fold

Nanoparticles

- It might be unstable in suspension state
  → Stirring for 2 h at 40°C

Solid lipid nanoparticles

- Manufacturing process was complicated
  → 5 homogenization cycles & freeze drying

Develop the drug delivery system with simple and rapid manufacturing process

Solid dispersion

- Active ingredient dispersed in hydrophilic carriers to improve the dissolution rate and bioavailability

→ Transferring drugs to amorphous state, reducing particle size and increasing wettability and porosity

Drug Discovery Today 12 (2007) 1068-1075.

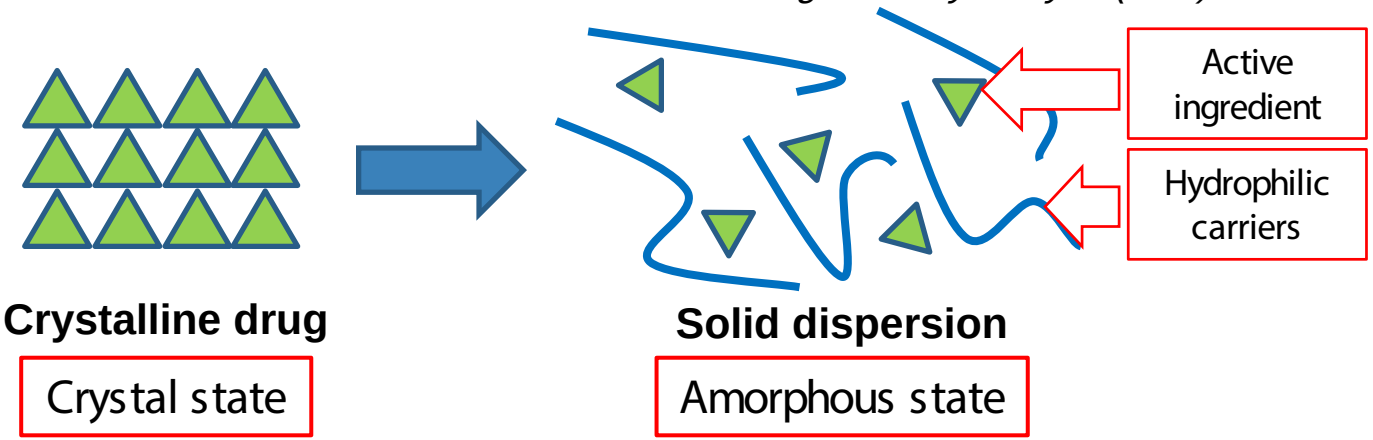


Fig. 2. The schematic diagram of solid dispersions.

Solid dispersion

Solid dispersion

- First generation
  Crystalline carriers
- Second generation
  Polymeric carriers
- Third generation
  Mixture of polymer and surfactant

Manufacturing processes

- **Solvent method**
- **Melting method**
- **Melting-solvent method**

Polymers

- Polyethylene glycol (PEG)
- Poloxamer
- Polyvinylpyrrolidone (PVP)

Generally regarded as safe (GRAS)

European Journal of Pharmaceutics and Biopharmaceutics 85 (2013) 799–813.

Table 2. Marketed tablet products using solid dispersions.

Product	Model drug	Carrier type
Gris-PEG®	Griseofulvin	PEG
Fenoglide®	Fenofibrate	PEG
Afeditab® CR	Nifedipine	Poloxamer/ PVP
Cesamet®	Nabilone	PVP

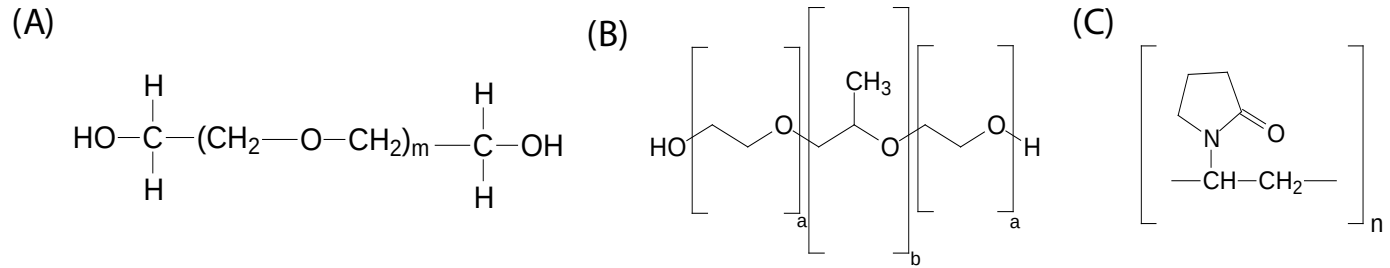


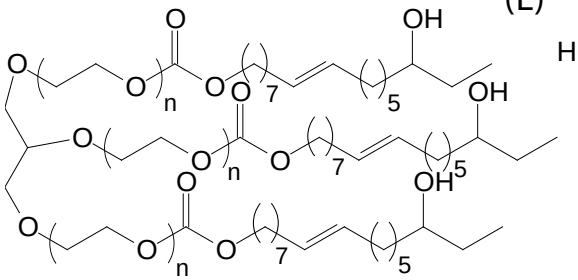
Fig. 3-1. The chemical structure of (A) polyethylene glycol , (B) poloxamer and (C) polyvinylpyrrolidone

Surfactants

- Cremophor EL (Polyoxyl 35 castor oil)
- Tween 80 (Polysorbate 80)
  - Nonionic surfactant
  - Stabilize the solid dispersion
  - P-glycoprotein inhibitor

Drug Discovery Today 12 (2007) 1068-075.
Journal of Pharmaceutical Sciences 93 (2004) 877-885.

(D)



(E)

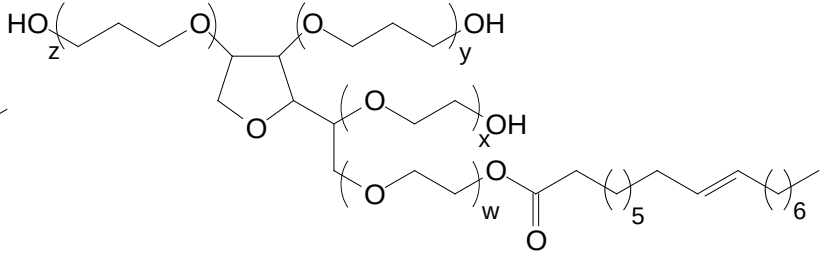


Fig. 3-2. The chemical structure of (D) Cremophor EL and (E) Tween 80.

Aims of study

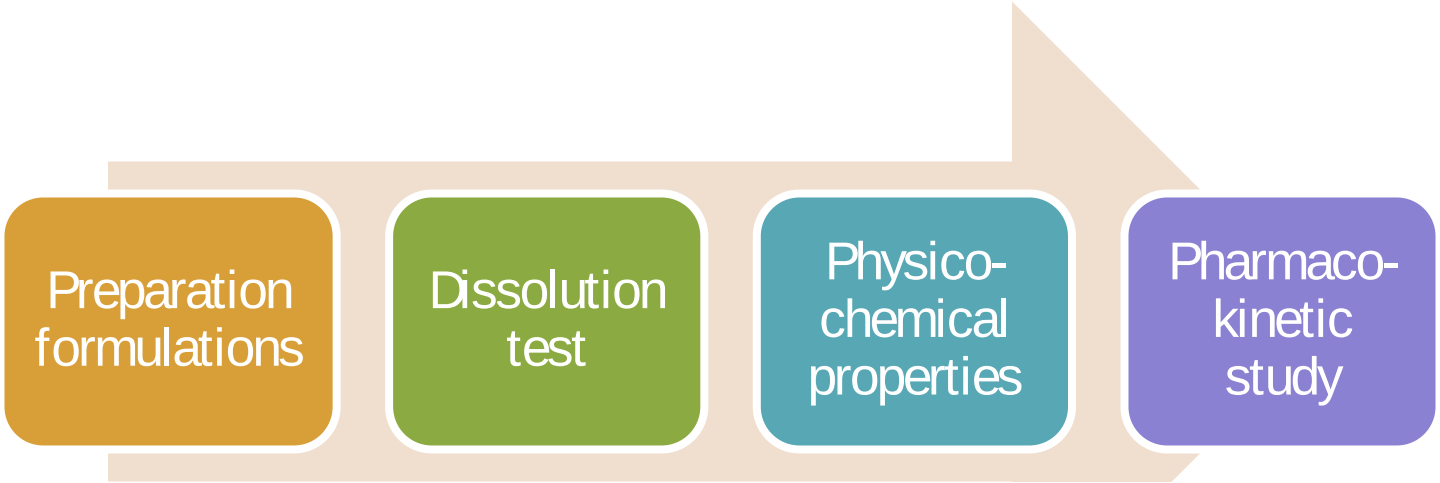
- **To develop solid dispersion system to improve the oral bioavailability of andrographolide**
  - *In vitro* studies
    - Dissolution test
    - Differential scanning calorimetry (DSC)
    - Fourier transform infrared spectroscopy (FTIR)
  - *In vivo* study
    - Pharmacokinetic study

Materials and methods

Materials

- **Active ingredient**
  - Andrographolide (≥98%)
- **Internal standard (IS)**
  - Fisetin (≥96%)
- **Solvent**
  - Ethanol (≥99.8%)
- **Carriers**
  - PEG 6000
  - Poloxamer 407
  - PVP K15
  - PVP K30
  - PVP K90
- **Surfactants**
  - Cremophor EL
  - Tween 80

Experiment process



- Different **carrier types**
- Different **molecular weight** of carrier
- Addition of **surfactants**

- Differential scanning calorimetry (DSC)
- Fourier transform infrared spectroscopy (FTIR)

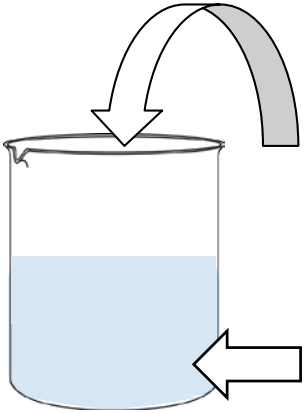
Formulations of solid dispersion

Table 2. Composition (w/w) of the solid dispersions of andrographolide.

Constituents (w/w)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20	F21	F22
AG	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PEG 6000	1	3	5	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Poloxamer 407	-	-	-	-	1	3	5	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PVP K30	-	-	-	-	-	-	-	-	1	3	5	7	-	-	-	-	-	-	-	-	7	7
PVP K15	-	-	-	-	-	-	-	-	-	-	-	-	1	3	5	7	-	-	-	-	-	-
PVP K90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	3	5	7	-	-
Cremophor EL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Tween 80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1

Preparation of solid dispersions

■ Solvent evaporation method



Add AG

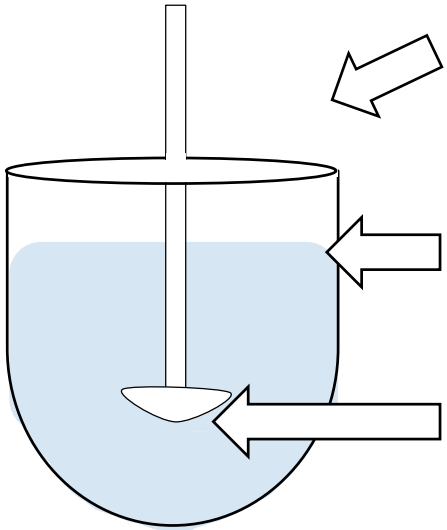
And add proper ratio weight of carriers and surfactants

EtOH

Sonicated → Rotary evaporation → Vacuum oven → Andrographolide solid dispersion

Dissolution test

■ USP Dissolution Apparatus 2 – Paddle method



Equivalent to content
50 mg AG

900 mL of
0.1 N HCl
37±0.5°C

Paddle apparatus
100 rpm

1 mL of the dissolution medium was sampled

Replenished by the addition of 1 mL of fresh dissolution medium

Collected 2 h
→ 0, 5, 10, 15, 30, 45, 60, 90 and 120 min

HPLC condition

- HPLC system
  - Shimadzu HPLC System with UV-Vis Detector
- Column
  - Luna C18 column (250 × 4.6 mm i.d.; 5 μm)
- Mobile phase
  - ACN:0.01M NaH_2PO_4 (28.6:71.4, v/v) pH 2.5
- Flow rate: 1.5 mL/min
- Injection volume: 20 μL
- Wavelength: 225 nm

Physicochemical properties

- **Thermal behavior**
  - Differential scanning calorimetry (DSC)
  - 2 mg sample in aluminum pans
  - Heating rate of 10°C/min
  - From 25°C to 300°C
- **Chemical bonding**
  - Fourier transform infrared spectroscopy (FTIR)
  - 15 mg sample in 500 mg KBr tablets
  - Resolution of 2 cm^{-1}
  - From 400 to 4000 cm^{-1}

Pharmacokinetic study

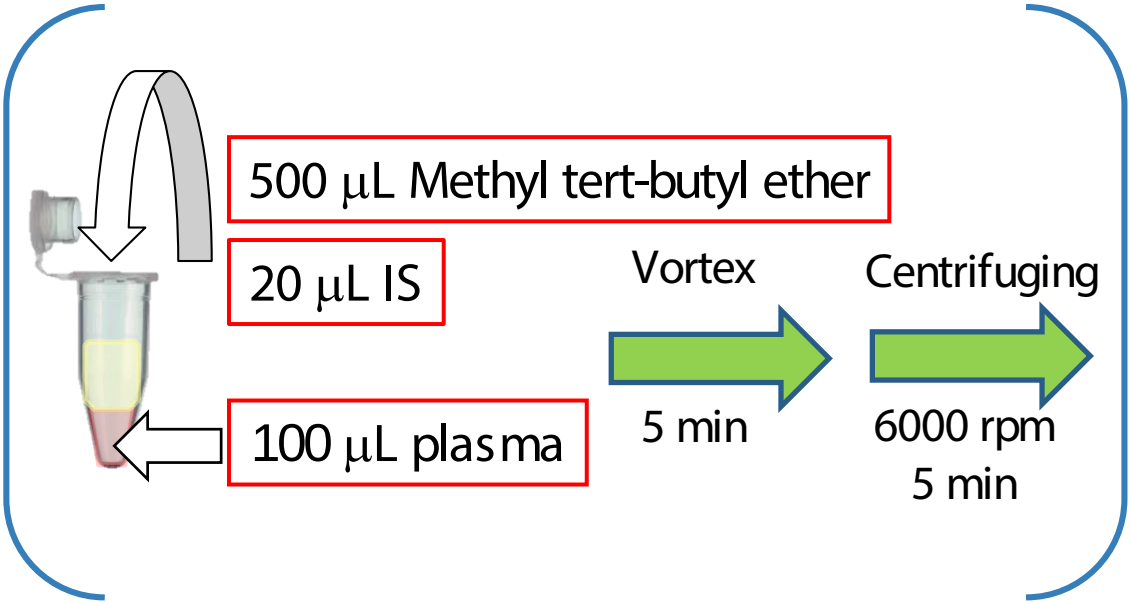
5 rats (Male Sprague-Dawley) in each groups
200 ± 20 g

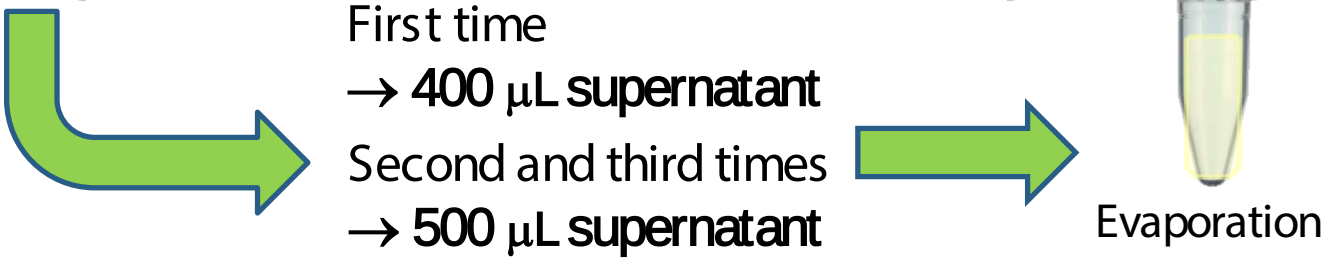
Group A
Oral administration (AG suspension 300 mg/kg)

Group B
Oral administration (AG solid dispersion 100 mg/kg)

Collected 8 h
→ 0, 5, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360 and 480 min

Plasma liquid-liquid extraction





HPLC condition

- HPLC system
  - Shimadzu HPLC System with UV-Vis Detector
- Column
  - Luna C18 column (250 × 4.6 mm i.d.; 5 μm)
- Mobile phase
  - ACN:0.01M NaH_2PO_4 (28.6:71.4, v/v) pH 2.5
- Flow rate: 1.0 mL/min
- Injection volume: 20 μL
- Wavelength: 225 nm

Results and discussion

Dissolution test – Different carriers

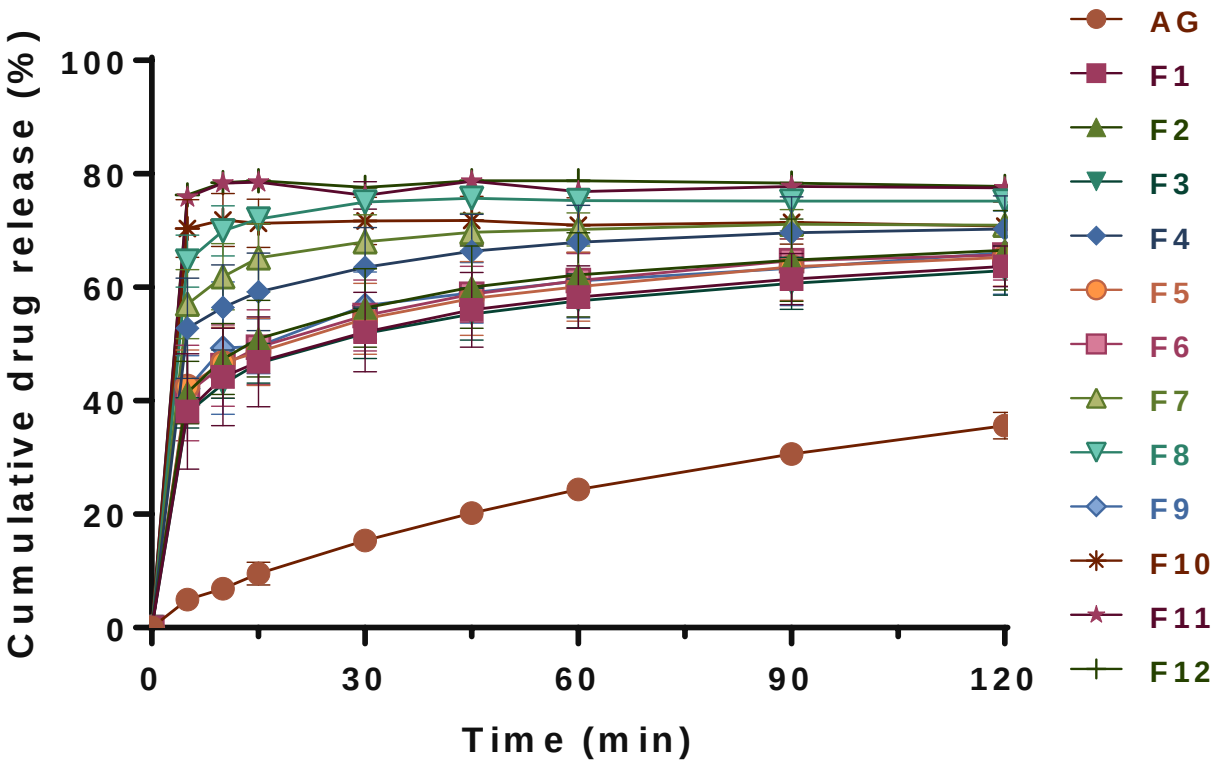


Fig. 4. *In vitro* dissolution profile of andrographolide and F1 to F12 in 0.1 N HCl medium. Data are expressed as the mean ± standard deviation for n=3 per group.

Dissolution test – Different carriers

Table 3. *In vitro* releasing values of andrographolide and F1 to F12.

Excipient	-	PEG 6000				Poloxamer 407				PVP K30			
Ratio (w/w)	-	1:1	1:3	1:5	1:7	1:1	1:3	1:5	1:7	1:1	1:3	1:5	1:7
Formulation	AG	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Q_{5min}	4.9 ± 1.8	38.2 ± 10.2*	37.9 ± 2.7*	41.5 ± 5.6*	52.8 ± 8.9*	42.5 ± 6.4*	41.4 ± 8.5*	57.0 ± 6.1*	64.7 ± 4.6*	42.1 ± 5.8*	70.4 ± 5.1*	75.9 ± 0.2*	76.3 ± 0.9*
Q_{120min}	35.6 ± 2.3	63.7 ± 3.6*	62.9 ± 4.3*	66.6 ± 7.0*	70.3 ± 5.9*	65.3 ± 5.1*	65.7 ± 4.4*	70.9 ± 1.4*	75.2 ± 1.7*	66.2 ± 7.3*	70.8 ± 4.3*	77.5 ± 0.1*	77.8 ± 1.4*
D.E.	22.4 ± 1.7	55.1 ± 5.8*	54.5 ± 4.2*	58.7 ± 6.9*	64.7 ± 6.5*	57.2 ± 5.8*	57.9 ± 4.9*	67.4 ± 3.2*	72.8 ± 1.3*	58.0 ± 6.2*	69.8 ± 4.2*	75.9 ± 0.7*	77.8 ± 1.7*
$t_{70\%}$(min)	-	-	-	-	120	-	-	60	15	-	5	5	5

Note: Q_{5min}, cumulative percentage release in 5 min; Q_{120min}, cumulative percentage release in 120 min ; D.E., dissolution efficiency; $t_{70\%}$, time required for 70% release,
Data are expressed as the mean ± standard deviation for n=3 per group, * $p < 0.05$, compared with AG group.

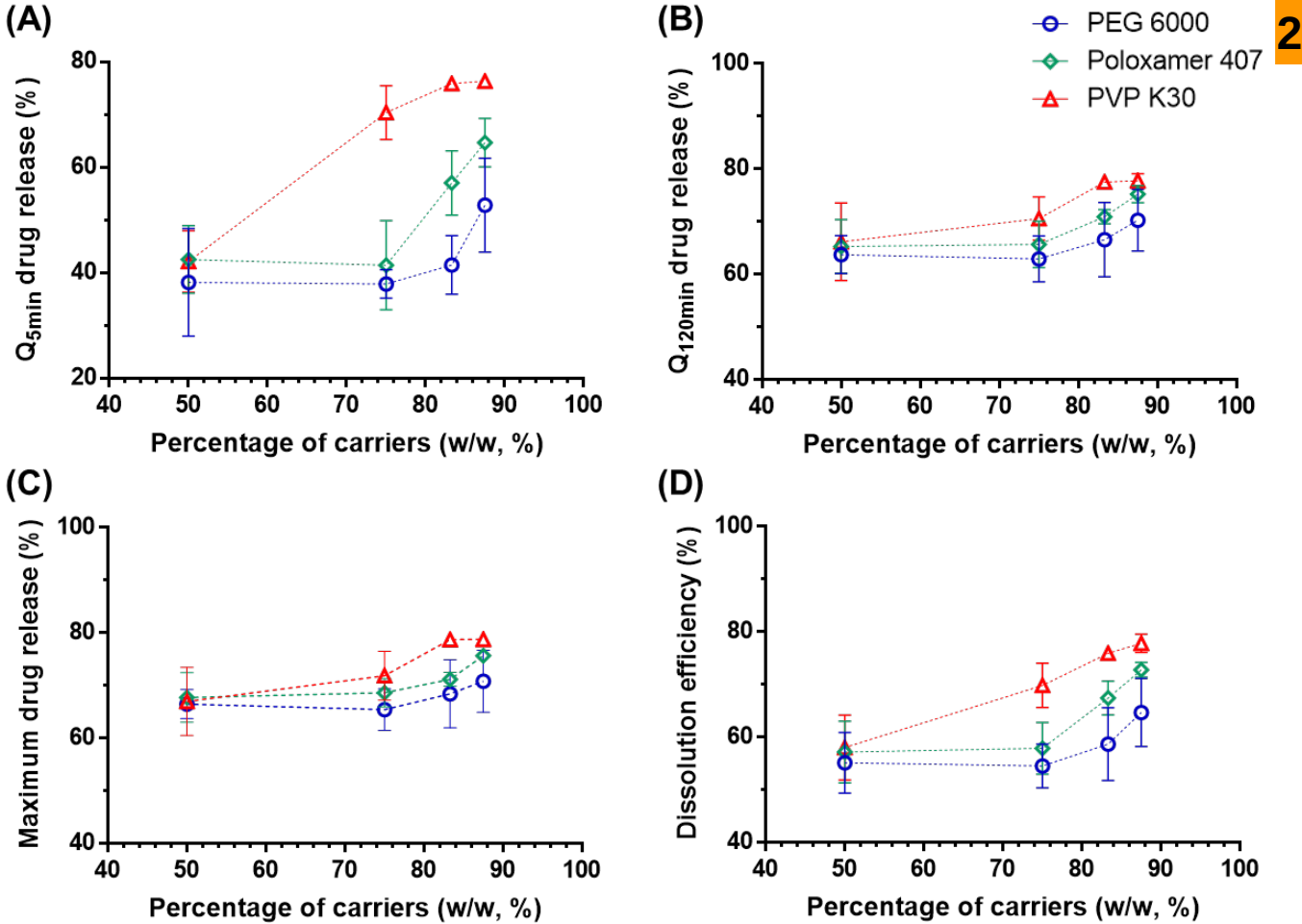


Fig. 5. *In vitro* drug release-percentage of carriers curve of PEG 6000, poloxamer 407 and PVP K30 with (A) Q_{5min}, (B) Q_{120min}, (C) maximum drug release and (D) dissolution efficiency. Data are expressed as the mean ± standard deviation for n=3 per group.

Dissolution test – Different molecular weight

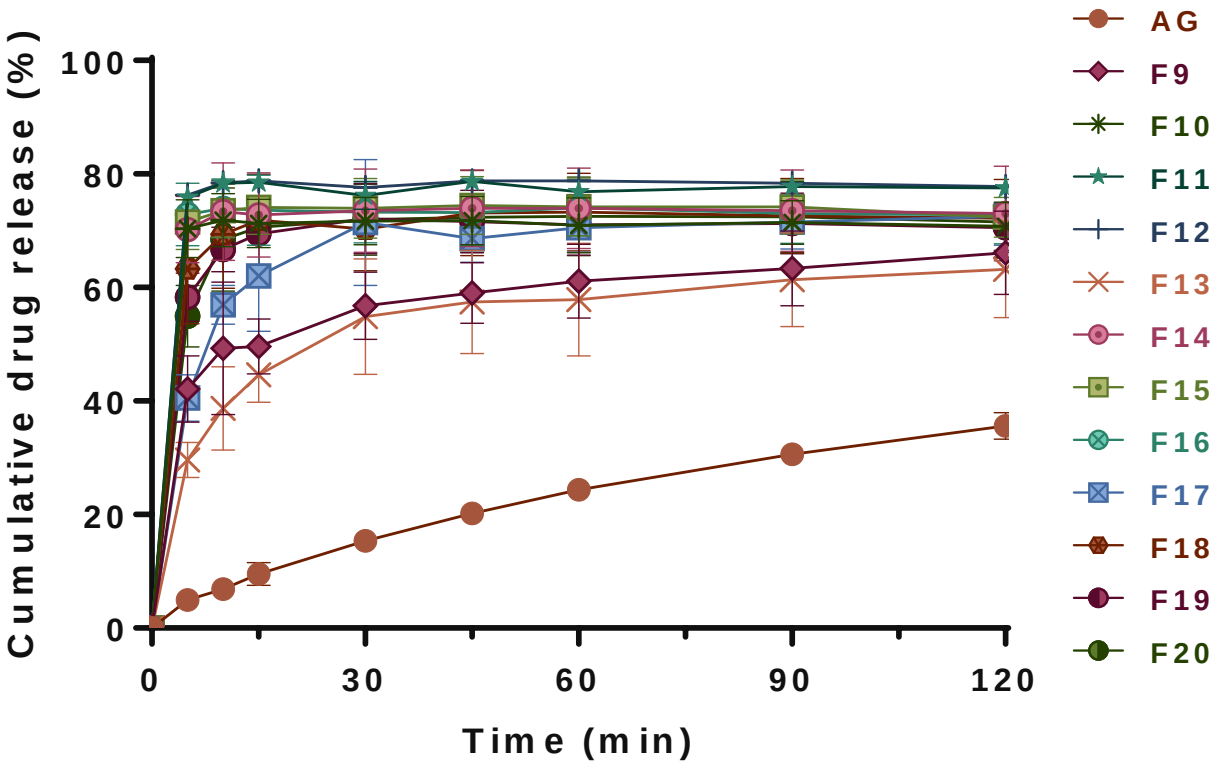


Fig. 6. *In vitro* dissolution profile of andrographolide and F9 to F20 in 0.1 N HCl medium. Data are expressed as the mean ± standard deviation for n=3 per group.

Dissolution test – Different molecular weight

Table 4. *In vitro* releasing values of andrographolide and F9 to F20.

Excipient	-	PVP K30				PVP K15				PVP K90			
Ratio (w/w)	-	1:1	1:3	1:5	1:7	1:1	1:3	1:5	1:7	1:1	1:3	1:5	1:7
Formulation	AG	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20
Q_{5min}	4.9 ± 1.8	42.1 ± 5.8*	70.4 ± 5.1*	75.9 ± 0.2*	76.3 ± 0.9*	29.6 ± 3.1*	70.2 ± 5.9*	71.6 ± 4.9*	72.9 ± 5.5*	40.5 ± 4.1*	63.3 ± 9.7*	58.3 ± 4.3*	55.0 ± 5.4*
Q_{120min}	35.6 ± 2.3	66.2 ± 7.3*	70.8 ± 4.3*	77.5 ± 0.1*	77.8 ± 1.4*	63.2 ± 8.6*	73.0 ± 8.3*	72.4 ± 3.6*	72.8 ± 5.1*	72.5 ± 5.1*	72.2 ± 6.9*	70.6 ± 4.4*	71.5 ± 6.3*
D.E.	22.4 ± 1.7	58.0 ± 6.2*	69.8 ± 4.2*	75.9 ± 0.7*	77.8 ± 1.7*	54.7 ± 7.8*	71.9 ± 8.2*	72.3 ± 4.6*	71.8 ± 5.2*	66.9 ± 5.3*	70.3 ± 7.0*	68.9 ± 5.1*	69.7 ± 6.4*
$t_{70\%}$(min)	-	-	5	5	5	-	5	5	5	30	15	30	15

Note: Q_{5min}, cumulative percentage release in 5 min; Q_{120min}, cumulative percentage release in 120 min; D.E., dissolution efficiency; $t_{70\%}$, time required for 70%release,
Data are expressed as the mean ± standard deviation for n=3 per group, * $p < 0.05$, compared with AG group.

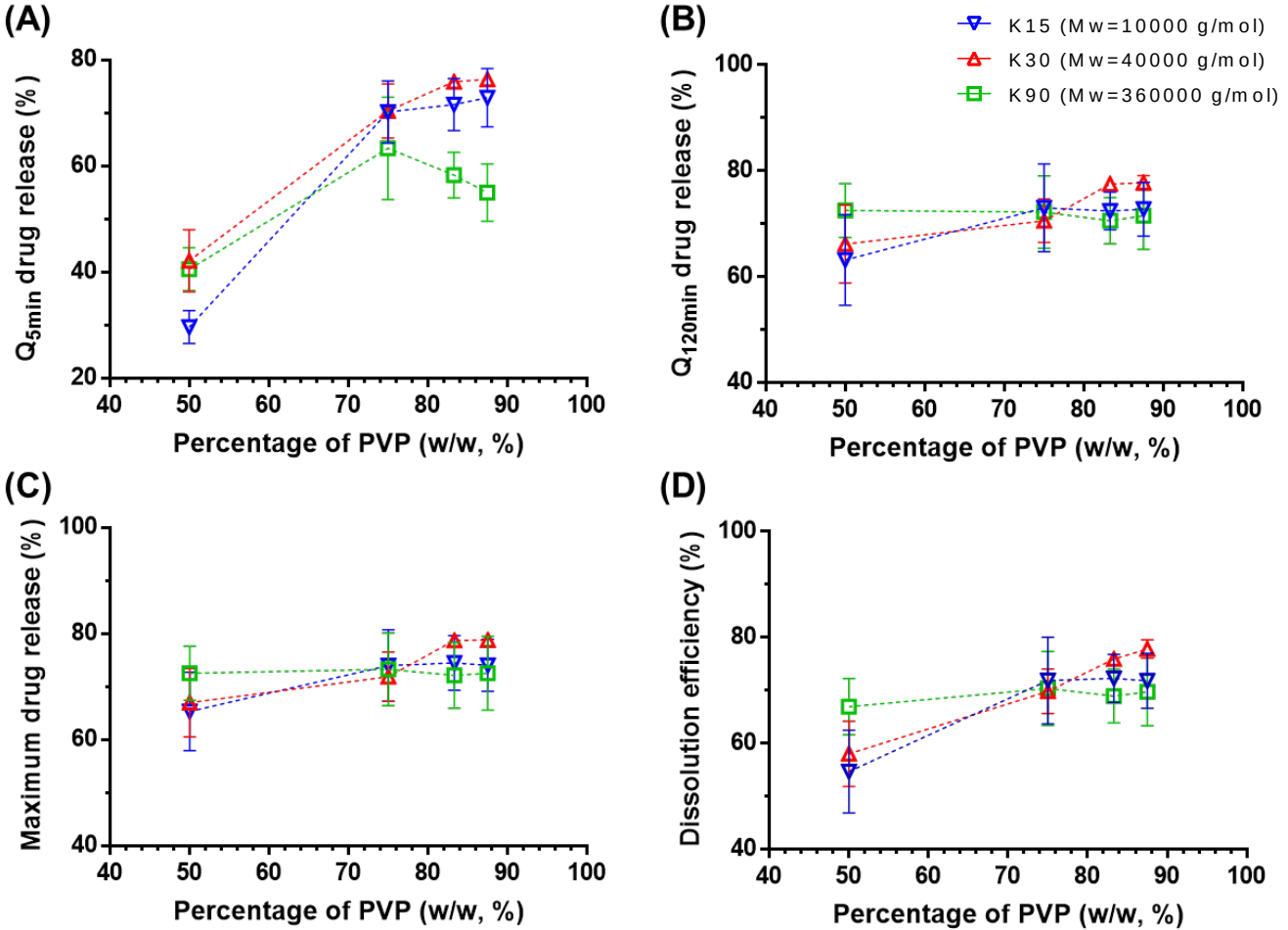


Fig. 7. *In vitro* drug release-percentage of PVP curve of K15, K30 and K90 with (A) Q_{5min}, (B) Q_{120min}, (C) maximum drug release and (D) dissolution efficiency. Data are expressed as the mean ± standard deviation for n=3 per group.

Dissolution test – Optimized carrier

- The F12 (AG:PVP K30=1:7 w/w) demonstrated the highest drug release
- Achieve 70% release in 5 min
- $Q_{5min} \rightarrow 76.3\%$

– Increasing about 15.6-fold comparing with AG

- $Q_{120min} \rightarrow 77.8\%$

– Increasing about 2.2-fold comparing with AG

- Dissolution efficiency $\rightarrow 77.8\%$

– Increasing about 3.5-fold comparing with AG

Dissolution test – Add surfactants

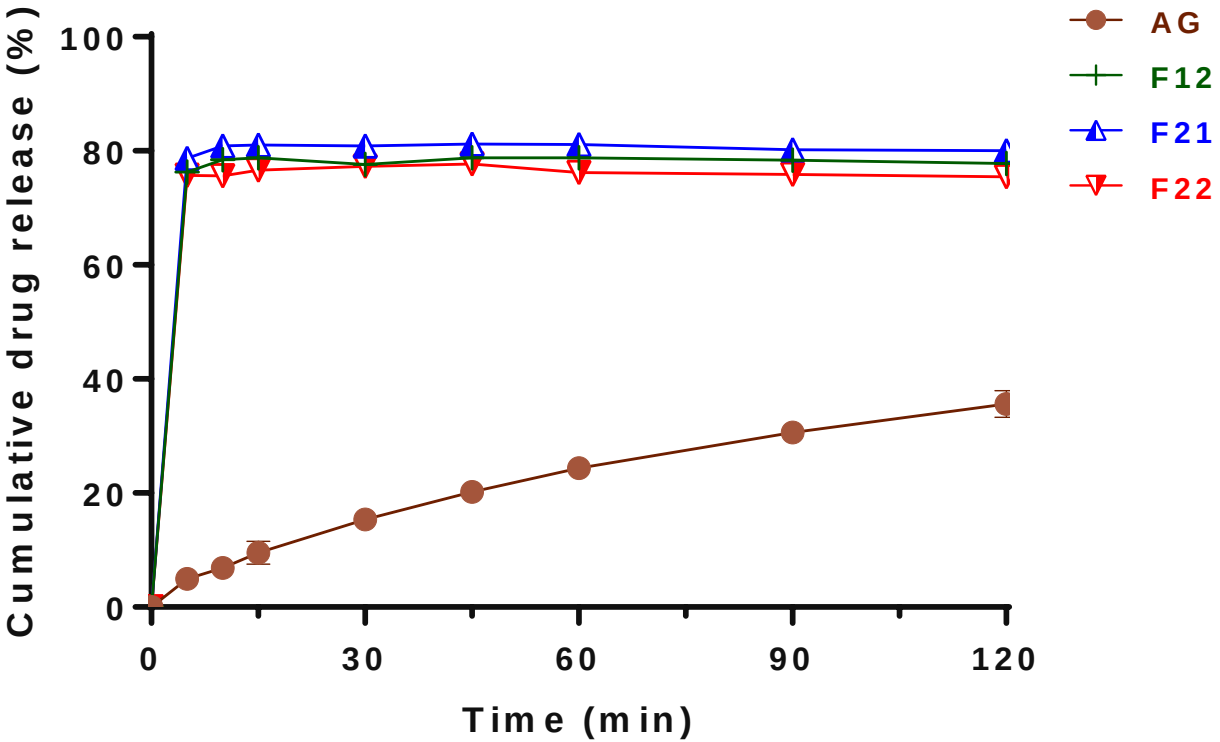


Fig. 8. *In vitro* dissolution profile of andrographolide, F12, F21 and F22 in 0.1 N HCl medium. Data are expressed as the mean ± standard deviation for n=3 per group.

Dissolution test – Add surfactants

Table 5. *In vitro* releasing values of andrographolide, F12, F21 and F22.

Excipient	-	PVP K30	PVP K30/ Cremophor EL	PVP K30/ Tween 80
Ratio (w/w/w)	-	1:7	1:7:1	1:7:1
Formulation	AG	F12	F21	F22
Q_{5min}	4.9 ± 1.8	$76.3 \pm 0.9^{*}$	$78.7 \pm 0.1^{*}$	$75.7 \pm 1.2^{*}$
Q_{120min}	35.6 ± 2.3	$77.8 \pm 1.4^{*}$	$80.0 \pm 0.7^{*}$	$75.4 \pm 0.6^{*}$
D.E.	22.4 ± 1.7	$77.8 \pm 1.7^{*}$	$78.9 \pm 0.5^{*}$	$74.8 \pm 0.1^{*}$
$t_{70\%}$(min)	-	5	5	5

Note: Q_{5min}, cumulative percentage release in 5 min; Q_{120min}, cumulative percentage release in 120 min; D.E., dissolution efficiency; $t_{70\%}$, time required for 70%release,
Data are expressed as the mean ± standard deviation for n=3 per group, * $p < 0.05$, compared with AG group.

Dissolution test – Optimized formulation

- The F21 (AG: PVP K30: Cremophor EL=1:7:1 w/w/w) demonstrated the highest drug release
- Achieve 80% release in 10 min
- $Q_{5min} \rightarrow 78.7\%$

– Increasing about 16.1-fold comparing with AG

- $Q_{120min} \rightarrow 80.0\%$

– Increasing about 2.2-fold comparing with AG

- Dissolution efficiency $\rightarrow$ 78.9%

– Increasing about 3.5-fold comparing with AG

DSC analysis

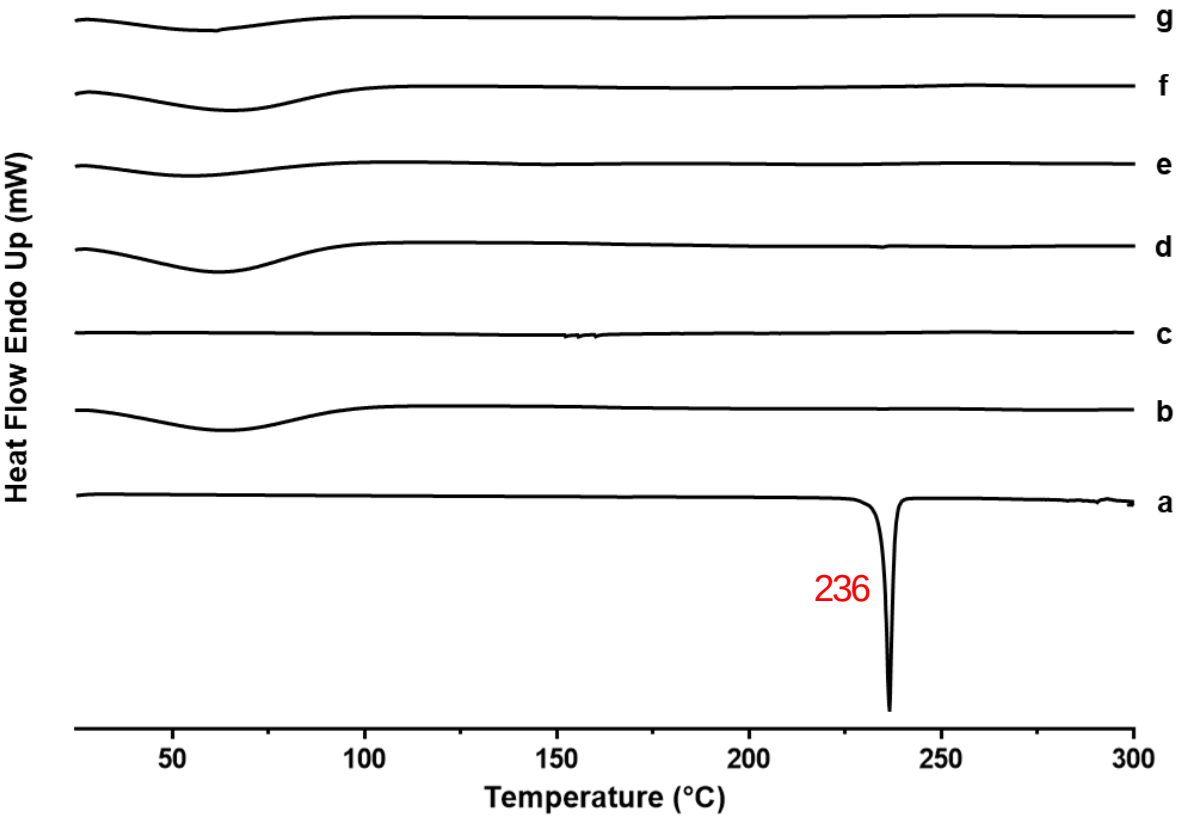


Fig. 9. DSC thermograms of (a) AG, (b) PVP K30, (c) Cremophor EL, (d) PM-AG/PVP (1:7), (e) SD-AG/PVP (1:7), (f) PM-AG/PVP/Cremophor EL (1:7:1), (g) SD-AG/PVP/Cremophor EL (1:7:1).

DSC analysis

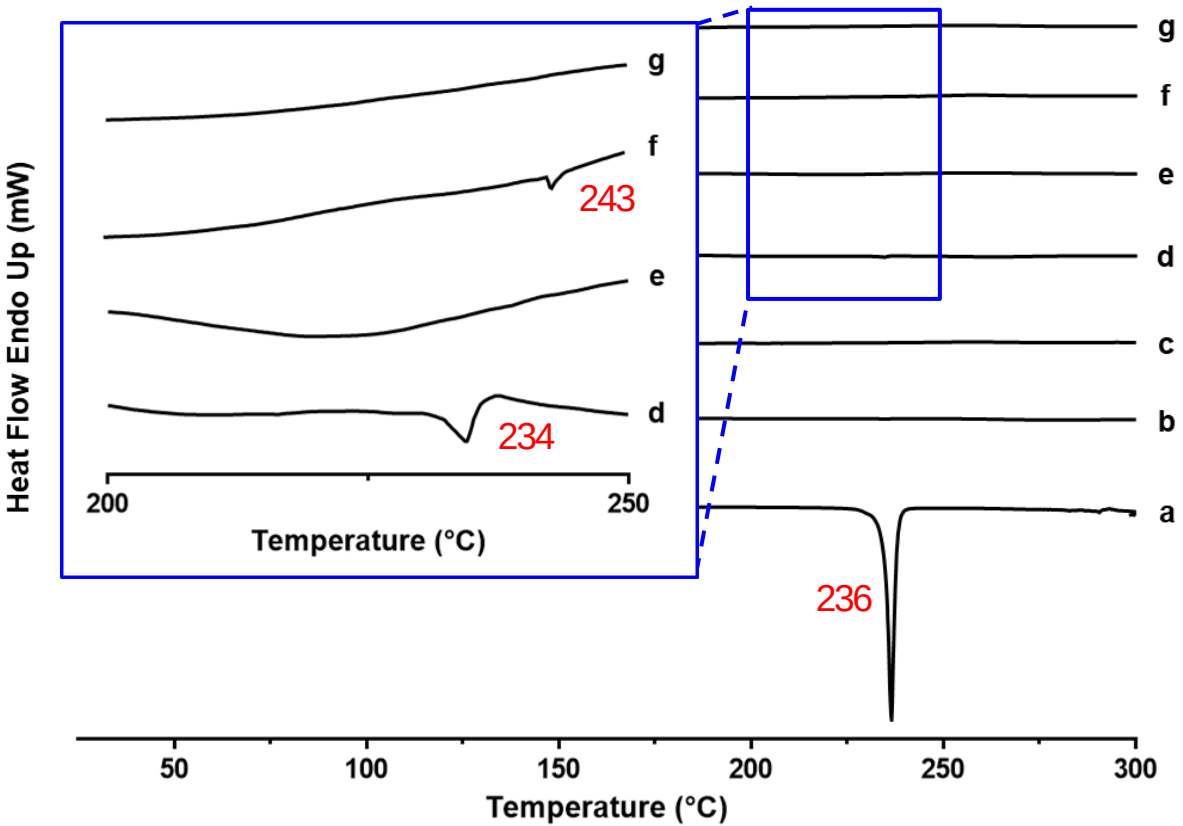


Fig. 9. DSC thermograms of (a) AG, (b) PVP K30, (c) Cremophor EL, (d) PM-AG/PVP (1:7), (e) SD-AG/PVP (1:7), (f) PM-AG/PVP/Cremophor EL (1:7:1), (g) SD-AG/PVP/Cremophor EL (1:7:1).

FTIR analysis

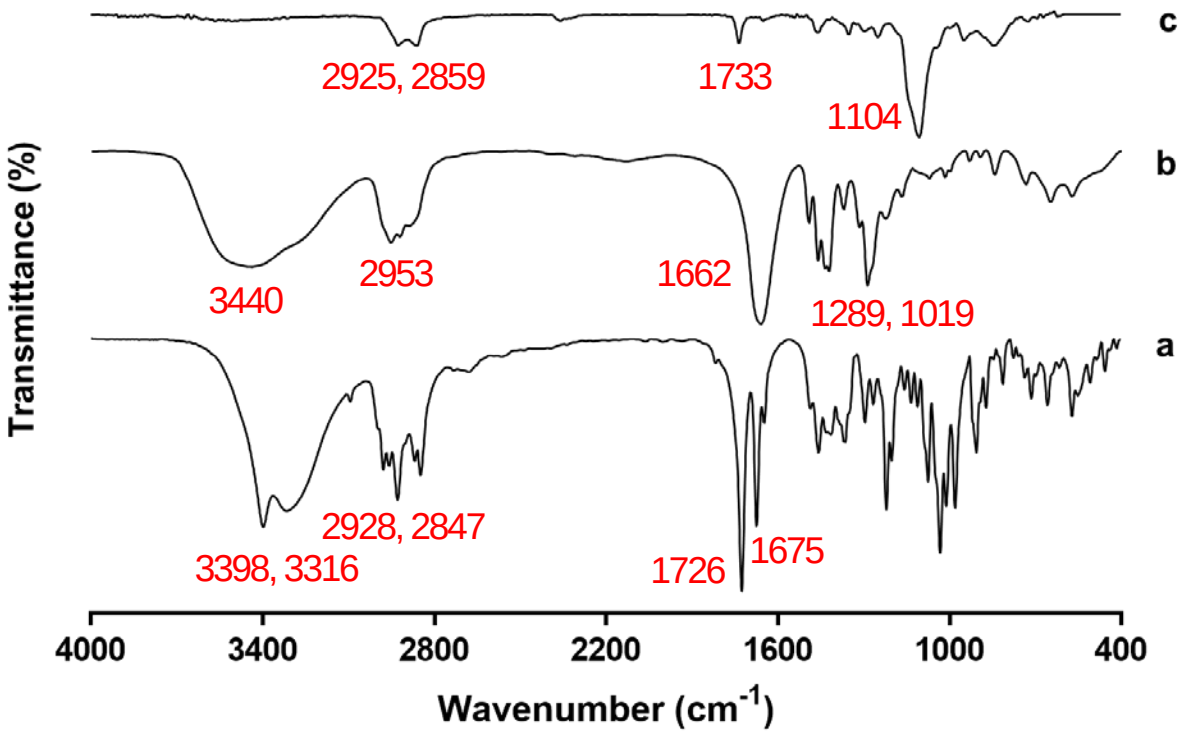


Fig. 10-1. FTIR spectrums of (a) AG, (b) PVP K30 and (c) Cremophor EL.

FTIR analysis

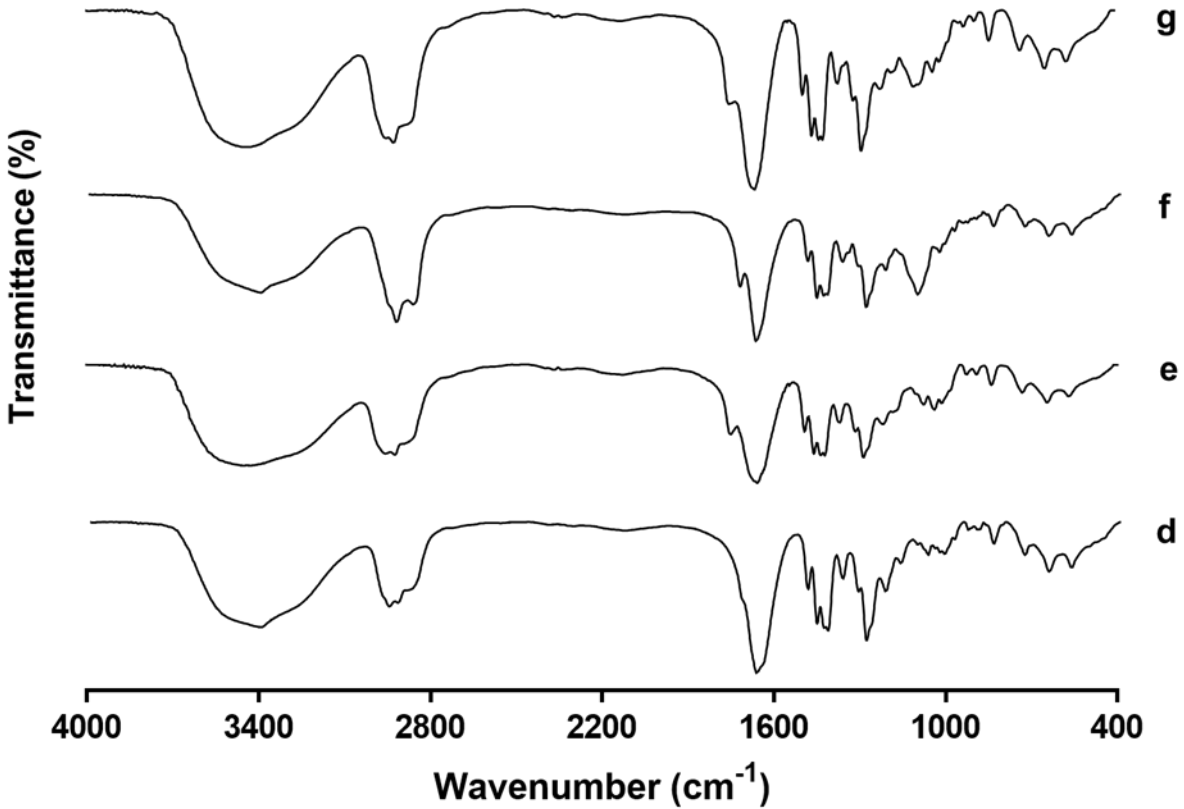


Fig. 10-2. FTIR spectrums of (d) PM-AG/PVP (1:7), (e) SD-AG/PVP (1:7), (f) PM-AG/PVP/Cremophor EL (1:7:1) and (g) SD-AG/PVP/Cremophor EL (1:7:1).

Pharmacokinetic study

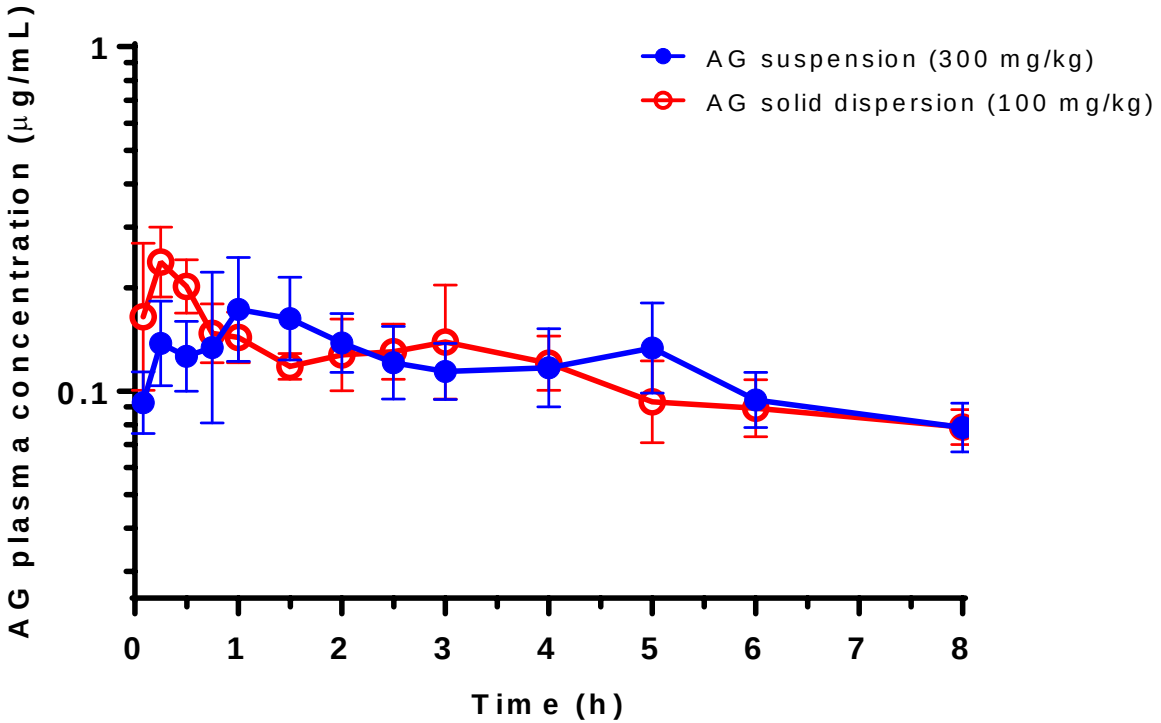


Fig. 11. Plasma concentration-time curve of oral AG suspension (300 mg/kg) and F21 solid dispersion (100 mg/kg). Data are expressed as the mean ± standard deviation for n=5 rats per group.

Pharmacokinetic study

Table 6. Pharmacokinetic parameters of oral F21 solid dispersion and AG suspension.

Parameter	Unit	AG solid dispersion	AG suspension
Dose	mg/kg	100	300
T_{max}	min	15	60
$t_{1/2}$	min	422.1 ± 183.2	428.7 ± 224.0
C_{max}	μg/mL	0.3 ± 0.1	0.2 ± 0.1
AUC_{0-t}	μg·min/mL	55.7 ± 10.9	56.1 ± 7.8
Relative bioavailability	%	298.9	

Note: Data are expressed as the mean ± standard deviation for n=5 rats per group.

Pharmacokinetic study

Table 7. The different formulations of andrographolide of previous studies with pharmacokinetic studies.

Formulations	Excipients	Animals	Relative bioavailability	Reference
Microcrystals	HPMC-E5 and microcrystalline cellulose	Beagle dogs	1.57-fold	*International Journal of Pharmaceutics 493 (2015) 214-223.*
Nanoparticles	Eudragit EPO and Pluronic F-68	Wistar rats	2.22-fold	*European Journal of Drug Metabolism and Pharmacokinetics 35 (2011) 123-129.*
Solid lipid nanoparticles	Compritol 888 ATO, GMS, lecithin and Tween 80	Wistar rats	2.42-fold	*Journal of Pharmaceutical Sciences 102 (2013) 4414-4425.*
Solid dispersion	PVP K30 and Cremophor EL	Sprague-Dawley rats	2.99-fold	*This study*

Conclusion

Conclusion

- We have developed solid dispersion of AG
  - The optimized formulation

    AG: PVP K30: Cremophor EL=1:7:1 w/w/w
  - Present amorphous state
  - Not generate chemical bonding between AG and carriers
  - Release about 79% AG in 5 min in dissolution test
  - Improve the oral bioavailability about 2.99-fold comparing with AG

Thanks for your attention

Biopharmaceutical classification

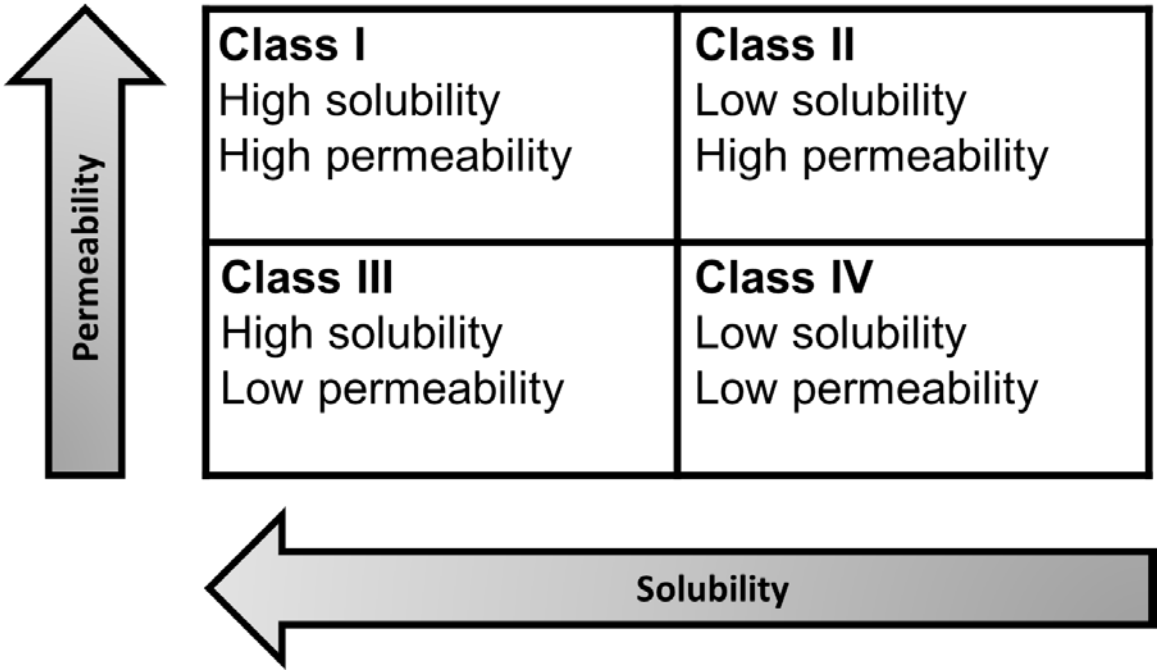


Fig. The diagram of biopharmaceutical classification (BCS).

International Journal of Pharmaceutics 420 (2011) 1-10.

P-glycoprotein

- A member of the ATP binding cassette (ABC) transporter superfamily
- Located within both the intestinal epithelium and the blood brain barrier (BBB)

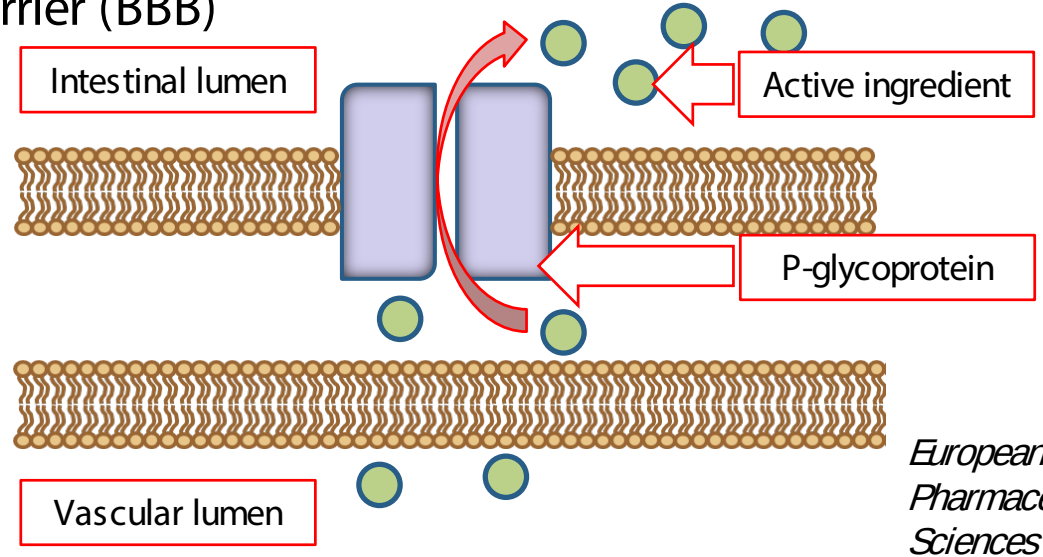


Fig. The schematic diagram of p-glycoprotein efflux.

European Journal of Pharmaceutical Sciences 227 (2006) 392–400.

Saturation solubility test

- Equivalent to content 5 mg andrographolide
- Dissolved in 5 mL water
- Shaking bath for 72 hr → Centrifuged 12000 rpm, 10 min → filtered through 0.45 μm → HPLC-UV analysis

Saturation solubility

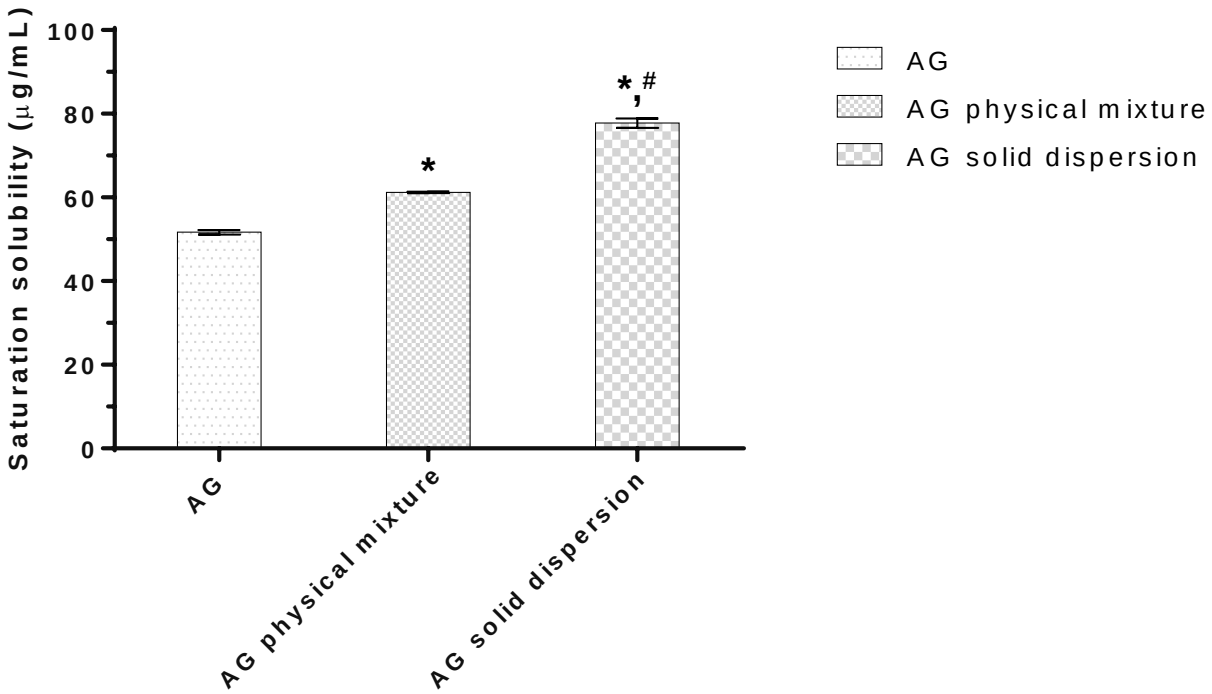
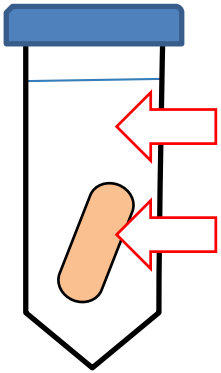


Fig. Saturation solubility of AG, physical mixture and F21 solid dispersion. Data are expressed as the mean ± standard deviation for n=3 per group. * indicates significant difference compared to the AG group ($p < 0.05$) and # indicates significant difference compared to the AG physical mixture group ($p < 0.05$).

Everted gut sac study



Each of the gut sac incubated in gentle shaking water bath at 37°C, 50 rpm for 1 h

Homogenized and centrifuged at 10000 rpm for 10 min

HPLC-UV

Everted gut sac study

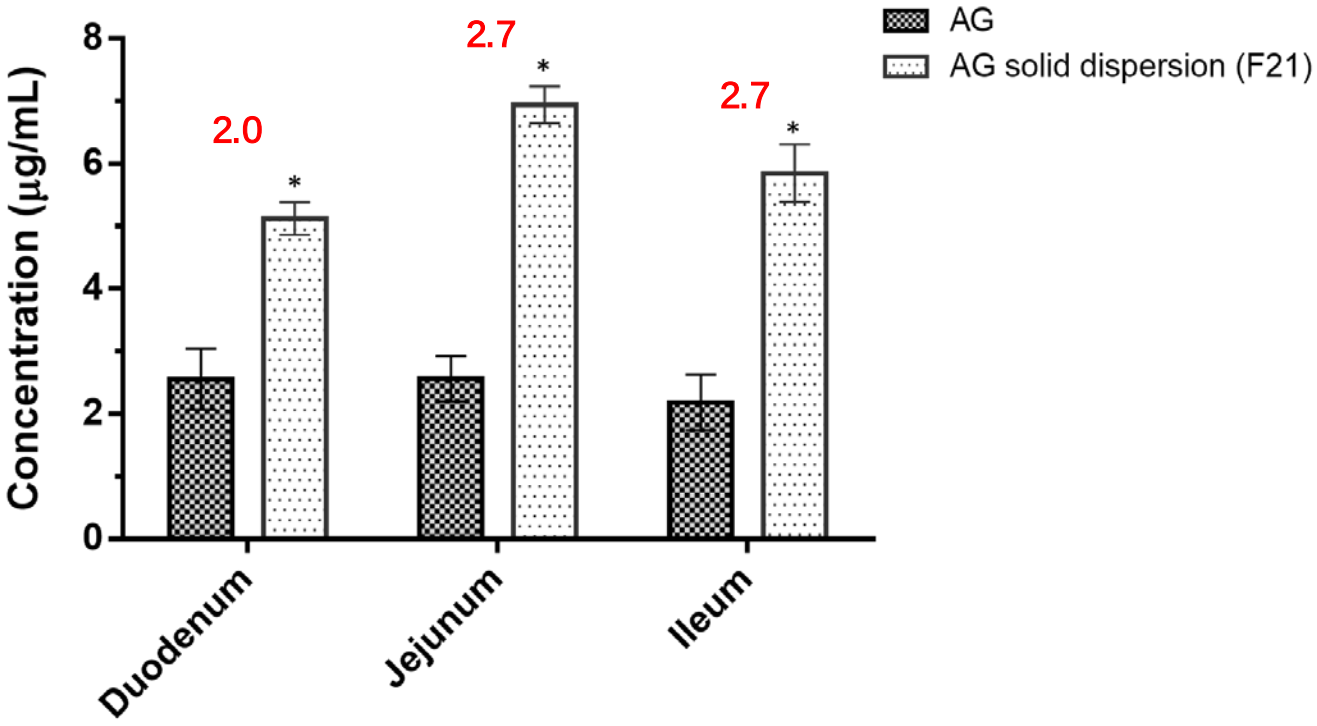


Fig. Everted gut sac study of AG and F21 solid dispersion in duodenum, jejunum and ileum. Data are expressed as the mean ± standard deviation for n=4 per group. * indicates significant difference compared to the AG group ($p < 0.05$).

Dissolution test – Comparison

Table. The trend of maximum dissolution of different molecular weights PVP in previous studies.

Model drug	Weight ratio (w/w)	Trend of maximum dissolution	Reference
Celecoxib	1:3	K30 > K60 > K25 > K17 > K12	*European Journal of Pharmaceutics and Biopharmaceutics 101 (2016) 145-151.*
Probucol	1:9	K30 > K25 > K90	*Chemical and Pharmaceutical Bulletin 44 (1996) 241-244.*
Andrographolide	1:1	K90 > K30 > K15	*This study*
	1:3	K15 > K90 > K30	
	1:5	K30 > K15 > K90	
	1:7	K30 > K15 > K90	

Dissolution test – Drug releasing mechanism

- Mechanism of drug release from solid dispersions
  - **Drug-controlled release** → 1:3, 1:5 and 1:7 of PVP K15 and K30
  - Polymorphic state, particle size, drug solubility
  - **Carrier-controlled release** → PEG 6000, Poloxamer 407, PVP K90 and 1:1 of K15 and K30
  - Gel or concentrated carrier layer
  - Noyes–Whitney equation
  - $\frac{dM}{dt} = \frac{D \cdot A \cdot C_S}{h}$

dM/dt → the rate of dissolution
D → the diffusion coefficient
A → the total surface area
C_s → the aqueous saturated solubility of the drug
h → the height of the boundary (diffusion) layer

European Journal of Pharmaceutics and Biopharmaceutics 85 (2013) 799-813.

Andrographolide

- **Anti-inflammatory**

Andrographolide has the potent anti-inflammatory effect. The study shows that andrographolide could inhibit the activation of NF-κB and STAT3 and interfere with the expression of SOCS1 and SOCS3 signaling to downregulate inflammatory iNOS and COX-2 gene expression.

Journal of Ethnopharmacology 135 (2011) 678–684.

Andrographolide

- **Anti-platelet**

Andrographolide may involve an increase in cyclic GMP/PKG, followed by inhibition of the p38 MAPK/•HO-NF-κB-ERK2 cascade in activated platelets.

Biochemical Pharmacology 84 (2012) 914–924.

Polyvinylpyrrolidone (PVP)

- Polyvinylpyrrolidone is the most common used polymer carriers for preparing solid dispersion
- It could dissolve in both hydrophobic and hydrophilic solvents
- Solid dispersion with PVP generally increased solubility and dissolution rate
- PVP has also been shown to have significant inhibitory effects on the crystallization of drugs

Journal of Pharmaceutical Sciences 104 (2015) 2905–2912.