

## IMPROVING SOLUBILITY AND DISSOLUTION OF A NATURAL PRODUCT APIGENIN VIA PREPARATION OF SOLID DISPERSION BY HOT MELT EXTRUSION

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Received: 24 Sep 2020, Revised and Accepted: 15 Oct 2020

### ABSTRACT

**Objective:** Hot Melt Extrusion (HME) is one of the techniques for preparing a solid dispersion hydrophilic excipient known as a no solvents practical method to increase the solubility of drugs. Apigenin (APG) has properties that thermal stable with melting point 345-350 °C but very low solubility in the water around 1,35 µg/ml. The polymer is stable in the HME method are Soluplus and Kollidon VA 64. The study aims to optimize the kind of polymer in HME formulae to improve the solubility and dissolution rate of apigenin by solid dispersion using hot-melt extrusion.

**Methods:** Apigenin 10–50% w/w and Kollidon®VA 64 or Soluplus® and combination of Kollidon®VA 64 and Soluplus® were mixed, and the resulting blends extruded using a twin-screw extruder (Teach-Line ZK25T). Characterization of apigenin extrudates conducted using scanning electron microscopy, thermogravimetric analysis, differential scanning calorimetry, Fourier transform infrared spectroscopy, powder X-ray diffractometry, and dissolution.

**Results:** Solubility studies presented enhancement in apigenin of 10%/Soluplus®90%; 10% w/w apigenin/Kollidon®VA 64 (90%); and 33,3% w/w apigenin/Kollidon®VA 64 33,3% mix Soluplus® 33,3% increased more than 18,25; 16,18-and 8,52-fold in water, respectively. Furthermore dissolution studies showed enhancement in apigenin percent release of 10%/Soluplus®90%; 10% w/w apigenin/Kollidon®VA 64 90%; and 33,3% w/w apigenin/Kollidon®VA 64 33,3% mix Soluplus® 33,3% tablet apigenin HME up to 34,29%; 69,75% and 30,69%, respectively.

**Conclusion:** The formulation of 10% w/w Apigenin and 90% Soluplus® using hot-melt extrusion able to increase water solubility approximately 18,25-fold than raw material apigenin.

**Keywords:** Apigenin, Solid dispersion, Solubility, Hot melt extrusion

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### INTRODUCTION

Apigenin is a phytochemical compound that contained in some fruits, herbs, and vegetables. The main source of Apigenin is celery (*Apium graveolens* L), parsley, and chamomile [1]. Apigenin (5,7,4-trihydroxy flavone) corresponds to the subclass of flavones, and it is present in minimal amounts in food [2]. The previous research showed that APG with dose 4,8 mg/kg BW has potential as anticalculi [3]. Although APG has much practical use, it faces an obstacle in the formulation because of limited solubility, especially when it is used for oral administration. APG solubility in water is insoluble 0.00135 mg/ml [4]. APG categorized as BSC (Biopharmaceutical Classification system) class II, which has high permeability but low solubility and weak acid with the solubility of 1.43, 1.56, and 2.16 µg/ml in pH 1.0, 6.8, and 7.5, respectively [5]. Apigenin solubility is influenced by pH, particle size, selected solvent, and temperature; hence it needs to overcome different barriers for successful apigenin delivery [6]. Many techniques used for improving chemical and physical properties including particle size reduction, micronization, physical modifications, nanosuspension, modification of crystal habit such as, pseudo polymorphs, polymorphs, salt formation, complexation, solubilization, and use of cyclodextrins which can enhance the solubility and dissolution rate of insoluble drug and solid dispersion [7]. However, limited studies concerning the application of these techniques apigenin for examples apigenin nanocrystals using supercritical antisolvent process [8], inclusion complex of apigenin-hydroxypropyl-β-cyclodextrin by using supercritical antisolvent process [9], cocrystal of apigenin [10], apigenin in binary {Transcutol® + water} mixtures [11], Formulation and characterization of an apigenin-phospholipid phytosomes (APLC) [12], design and evaluation of a self-micro emulsifying drug delivery system for apigenin [13], improved solubility and oral bioavailability of apigenin via soluplus/pluronic f127 binary mixed micelles system [14], preparation and *in vitro* evaluation of apigenin loaded lipid nanocapsules [15]. Lack of several methods than HME are difficult to scale up the process, and requires a lot of organic solvents [16].

The solid dispersion has become an established solubilization technology for poorly water-soluble drugs, and it is the most promising successful in enhancing solubility. Since a solid dispersion is a modifier, a drug-polymer two-component system, the drug-polymer interaction is the determining factor in its design and performance [17]. Some of the practical aspects to consider for the preparation of solid dispersions, such as methods of physicochemical characterization, selection of carrier, along with an insight into the molecular arrangement of drugs in solid dispersions [18]. Dispersion techniques in carriers can conduct by hot spin mixing, co-evaporation, co-precipitation, freeze-drying, spray drying, and hot-melt extrusion (HME) [19]. The technique still rare in Indonesia is HME. Recently, many pharmacies material had prepared their solid dispersion using HME. However, in that consideration, no HME technique applies an APG. HME is an efficient technology for producing solid molecular distributions with considerable advantages over solvent-based processes such as spray drying and co-precipitation [20]. Spray drying has a report to enhance the solubility and dissolution of Ibuprofen microparticle [21]. Advantage of HME is superior to other techniques because the process is easy, no solvent is required, enhance dissolution rate for solid dispersion, mask the taste, and controlling and modifying drug release [22]. APG suitable for HME method using high energy because it has a high melting point 345-350 °C [23].

This study aimed to improve the solubility and dissolution of Apigenin. Two polymeric carriers Soluplus®, polyvinylpyrrolidone-co-vinyl acetate 64 (Kollidon® VA 64), were selected via the hot-melt extrusion process to investigate the solubility and dissolution of apigenin. The matrix use soluplus® and Kollidon® VA 64 and is mixed due to show the best and most suitable polymer for HME and has thermoplastic behavior, proper transition glass (T<sub>g</sub>), high degradation temperature, and low toxicity [24].

### MATERIALS AND METHODS

#### Materials

Apigenin for the standard purchase from Sigma-Aldrich, apigenin row material (Hefei Dielegance Biotechnology Co., Ltd), Soluplus®,

and Kollidon® VA 64 (BASF-Megasetia), reagents used in this study was of analytical grade, twin-screw extruder (Teach-Line ZK25T) in Polytechnique STMI Jakarta.

## Methods

### HME preparation

Hot Melt Extrusion Apigenin 10–50% w/w and Soluplus®, Kollidon® VA 64 (table 1) mix using a mixer (IKA EUROSTAR) for 10 min. The resulting concrete mixture blends extruded using a twin-screw extruder (Teach-Line ZK25T) at the screw speed of 100 rpm, at a

temperature 140 °C and 180 °C. All extrudates were milled and sieved through an ASTM 35 mesh to obtain a uniform particle size [25].

### Thermogravimetric analysis

Thermogravimetric analysis (TGA) perform for apigenin, Soluplus®, Kollidon® VA 64, and mixed to evaluate their stability at the extrusion temperatures. Approximately 10–15 mg of apigenin, polymers, as well as physical mixtures, was heated from 30 °C to 300 °C at a heating rate of 20 °C/min (HITACHI, STA7300) [25].

**Table 1: Apigenin composition for HME**

Formulation	Apigenin (%)	Soluplus®	Kollidon® VA 64
F1	10	90	-
F2	20	80	-
F3	40	60	-
F4	10	-	90
F5	20	-	80
F6	40	-	60
F7	50	25	25
F8	33,3	33,3	33,3
F9	20	40	40
F10	14,2	42,9	42,9

### Differential scanning calorimetry

Differential scanning calorimetry (DSC) studies obtained using Perkin Elmer Pyris 1 DSC equipped with Pyris manager software (PerkinElmer Life and Analytical Sciences). Approximately 2–4 mg of apigenin, extrudates were heated from 30 °C to 400 °C at a heating rate of 10 °C/min [4].

### X-ray diffraction (XRD)

The samples were analyzed by XRD pattern using an X-ray powder diffractometer with a rotating anode (Philips, PW 2213/30) and Cu Ka1 radiation generated at 30 mA and 50 kV. The scanning rate was 5°/min from 5 to 60° [26].

### Scanning electron microscopy

The morphology and physical state of the extrudates evaluated using Scanning electron microscopy (SEM) analysis. Samples were mounted on adhesive carbon pads placed on aluminum and were sputter-coated with gold using ion sputter (HITACHI MC1000) in a high vacuum evaporator. HITACHI SU3500 scanning electron microscope operating (SEM) at an accelerating voltage of 10 kV used for imaging [25].

### Fourier transforms infrared spectroscopy

Fourier transforms infrared spectroscopy (FTIR) spectra of apigenin, polymeric carriers, physical mixtures, as well as extrudates were performed using IR prestige-21 SHIMAZU FTIR. Apigenin 5 mg of each sample was mixed with 200 mg KBR and compressed into pellet and the scanning range was 400–4000 cm<sup>-1</sup>, and the resolution was 1 cm<sup>-1</sup> [25].

### Solubility test

The solubility was determined at saturated water for formulations F1-F10 and optimum formula as well as pure apigenin. Samples equivalent to approximately 100 mg of apigenin each put into a flask containing 100 ml of aquadest shaken in 24 h. Then filtered and diluted using aquadest until 10 ppm by adding ethanol 96%. A sample volume of 1 ml collected and detected with spectrophotometer (SPECORD 200) wavelength for apigenin detection set at  $\lambda_{max}$  336 nm [10, 25].

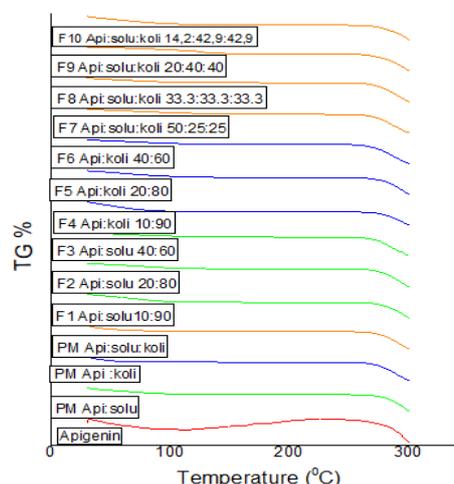
### In vitro drug release

A tablet with 8% primogel equivalent to 58,08 mg apigenin of each extrudate as well as pure apigenin and *in vitro* drug release profiles run using a USP type II dissolution apparatus. The used dissolution

medium was 900 ml of 0.4% SDS maintained at 37 °C. A sample volume of 5 ml taken at 5, 10, 15, 30, 45, 60, 120 min filtered, and analyzed using a spectrophotometer; then, 5 ml of fresh dissolution medium was added back to the dissolution vessel at each time point to maintain the volume [26].

## RESULTS AND DISCUSSION

All formulation conducted processing temperatures, TGA data showed no decrease in sample weight, decomposition held up 250 °C, which indicated that all formulations in the study were stable under all applied extrusion temperatures is 140 °C can be seen on fig. 1.



**Fig. 1: TGA all formulation**

XRD data showed pure apigenin, the physical mixture is crystalline, but with HME change crystallin form to amorf so that solubility can increase, all the extrudates showed in fig. 2.

The solubility of apigenin enhance after prepared by HME, formula 1, formula 4, and formula 8 is the highest solubility (table 2). In this research, we optimize temperature and screw with hot melt extrusion for three formulations are F1 apigenin 10% and Soluplus® 90%; F2 apigenin 10% and Kollidon® VA 64 90%; and F3 apigenin 33,3%, Soluplus® 33,3%, mixed Kollidon® VA 64 33,3%.

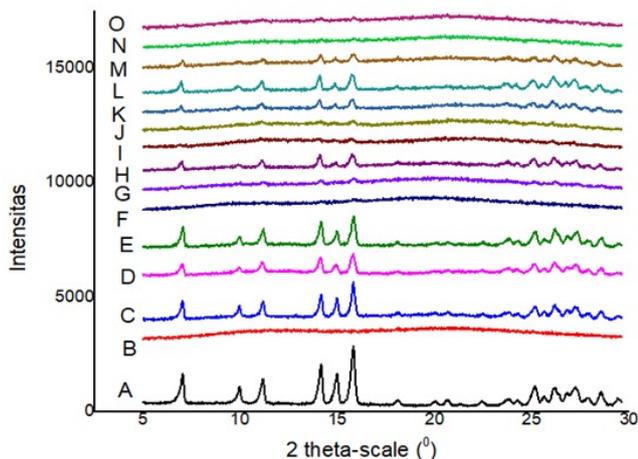


Fig. 2: PXR (a) pure apigenin, (b) Soluplus® and Kollidon® VA64, (c) PM APG-solu, (d) PM APG-koli, (e) PM APG-solu-koli, (f) 10% w/w APG/Soluplus® 90%, (g) 20% w/w APG/Soluplus® 80%, (h) 40% w/w APG/Soluplus® 60%, (i) 10% w/w APG/Kollidon® VA64 (90%), (j) 20% w/w APG/Kollidon® VA64 (80%), (k) 40% w/w APG/Kollidon® VA64 (60%), (l) 50%w/w APG-solu 25%-koli 25%, (m) 33,3%w/w APG-solu 33,3%-koli 33,3%, (n) 20%w/w APG-solu 40%-koli 40%, (o) 14%w/w APG-solu 43%-koli 43%

Table 2: Separated solubility apigenin in water\*

Sample	Dissolve concentration±SD (mg/l)
Apigenin	1,0109±0,0015
Formula 1	17,716±0,0011
Formula 2	4,7031±0,001
Formula 3	2,4682±0,001
Formula 4	16,084±0,0061
Formula 5	7,7754±0,0015
Formula 6	8,9772±0,003
Formula 7	3,8494±0,0030
Formula 8	9,4122±0,0058
Formula 9	6,5846±0,0032
Formula 10	8,5639±0,0055

\*Avg±% in mg/l deviation, n=3

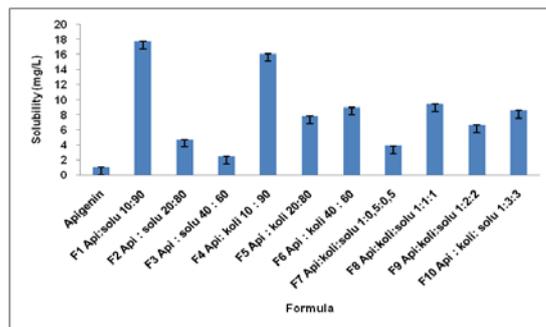


Fig. 3: Solubility of apigenin

Table 3: Processing parameters for HME

Formulation	Temperature (°C)	Screw speed (rpm)	Extrudate image
Apigenin: Soluplus 10: 90	110	5-100	no extrudate
	120	5-100	no extrudate
	130	5-100	no extrudate
	140	5-90	Little bit extrudate
	140	100	
Apigenin: Kollidon VA 64 10: 90	110	5-100	no extrudate
	120	5-100	no extrudate
	130	5-100	no extrudate
	140	5-90	no extrudate
	140	100	
Apigenin: Soluplus: Kollidon VA 64 33,3%:33,3%: 33,3%	110	5-100	no extrudate
	120-170	5-100	no extrudate
	180	100	
	190	100	
	210	100	

The hot-melt extrusion process carried out using a twin-screw extruder (Teach-Line ZK25T) standard screw configuration used in

this study, which consists of five conveying zones and two mixing zones (fig. 4).



Fig. 4: Twin screw extruder

The HME processing conditions for screw speed and extrusion temperatures are stable results good extrudate was greenish-yellow, transparent, and brittle in 140 °C and 100 rpm for polymer Soluplus® and Kollidon® separately although mixed Soluplus® and Kollidon® give the resulting extrudate was brown, brittle, not transparent, opaque in 180 °C and 100 rpm. All extrudates were milled using a coffee grinder and sieved to obtain a uniform particle size (table 3).

Formula 1 has solubility 18,455 µg/ml, apigenin 1,011 µg/ml. Formula 1 has a 18,25-fold solubility increase compare to apigenin without HME, and it can see in fig. 5.

Table 4: Separated solubility apigenin in water for optimum formula

Sample	Dissolve concentration (mg/l)±SD
Apigenin	1,011±0,0249
F1	18,455±0,0652
F2	16,362±0,0188
F3	8,618±0,0282
PM F1	6,100±0,0524
PM F2	0,212±0,0094
PM F3	0,456±0,0188

\*Avg±% in mg/l deviation, n=3

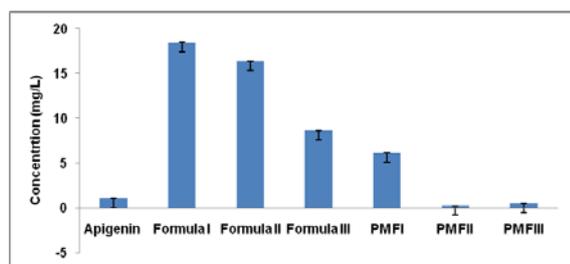


Fig. 5: The solubility of apigenin in the optimum formula

#### Fourier transforms infrared spectroscopy (FTIR)

There is a shift in the spectrum but does not form new bonds, indicating there any chemical change in solid dispersion. The loss of some peak in apigenin-like polymers means that solid dispersion has formed (fig. 6).

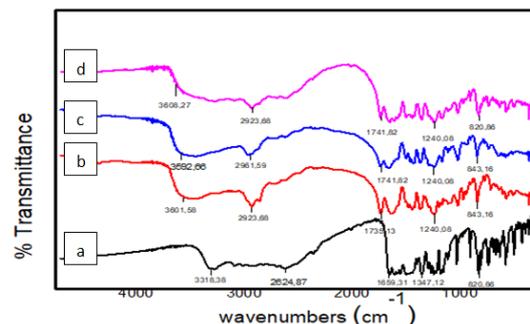


Fig. 6: (a) Fourier transforms infrared spectroscopy (FTIR) spectra of apigenin, (b) 10% w/w APG with Soluplus 90%, (c) 10% w/w APG with Kollidon 90%, (d) 33,3% w/w APG mixed Soluplus 33,3% and Kollidon 33,3%

DSC result results show that Apigenin has an endothermic peak up 347,76 °C means a crystalline structure. In contrast, others do not have sharp peaks that apigenin wit HME is molecularly dispersed into the pores of polymer and possibility amorphous state due to solubility increase. Alternatively, all the extrudates showed the absence of crystalline melting peaks in DSC data (fig. 7).

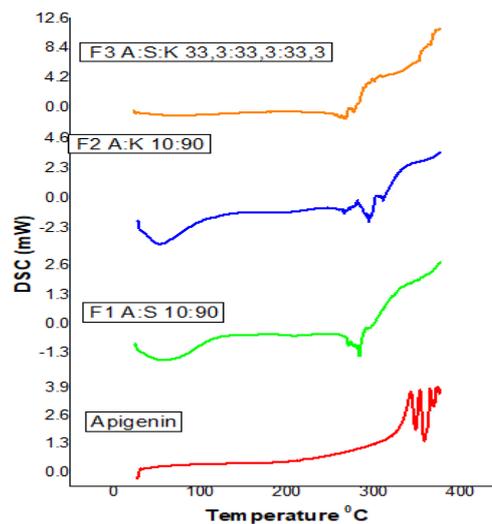


Fig. 7: Differential scanning calorimetry of extrudates

Scanning electron microscopy images showed the absence of crystals in 10% w/w apigenin/Soluplus and kollidon indicating that apigenin was dispersed in the Soluplus and in the kollidon polymer carrier as an amorphous, but when apigenin, soluplus mixed by kollidon with the same comparison still have image crystallin. However, crystals were evident in all other formulations with different ratios. Below are pictures of apigenin before and after HME (fig. 8).

The formula I have particle size smallest than the others is 685,1 nm; the smaller particle size can increase the solubility of the drug.

Table 5: Drug loading and particle size of apigenin

Formula	Drug loading (%)	(Particle size) (nm)
Apigenin	-	1743.33±15.63
F1 (Apigenin: Soluplus 10: 90)	90%	685.1±8.77
F2 (Apigenin: Kollidon VA 64 10: 90)	96%	700.33±13.09
F3 (Apigenin: Soluplus: Kollidon VA 64: 33.3:33.3:33.3)	78.31%	6222.67±337.7

DrugLoading %, particle size in nm; Avg±deviation, n=3

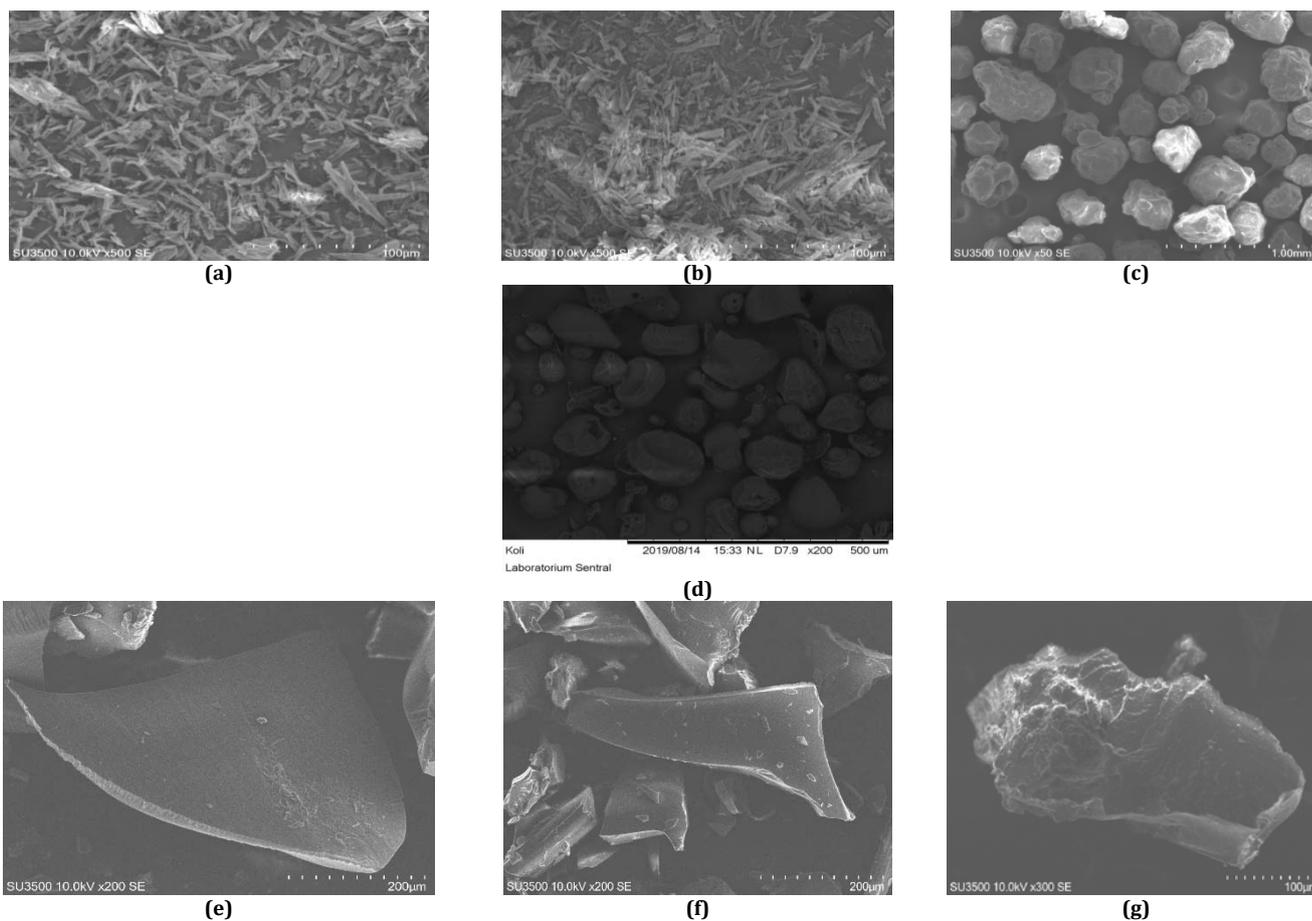


Fig. 8: SEM image (a) pure apigenin, (b) Physical mixture, (c) Soluplus®, (d) Kollidon® VA64, (e) 10% w/w apigenin/Soluplus® 90%, (f) 10% w/w apigenin/Kollidon® VA64, (g) 33,3% w/w apigenin/Soluplus® 33,3% mixed by 33,3% Kollidon® VA64

Dissolution studies demonstrated enhancement in apigenin percent release of 10%/Soluplus®90%; 10% w/w apigenin/Kollidon®VA 64 90%; and 33,3% w/w

apigenin/Kollidon®VA 64 33,3% mix Soluplus® 33,3% tablet apigenin HME up to 34,29%; 69,75% and 30,69%, respectively (fig. 9).

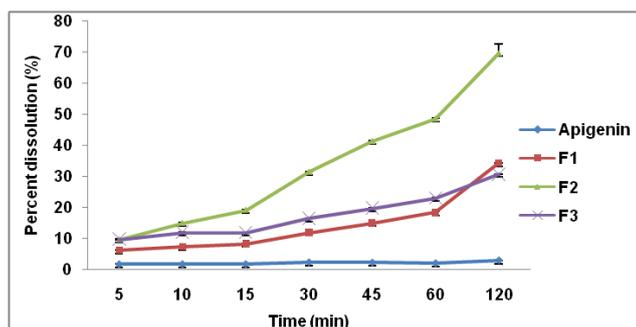


Fig. 9: *In vitro* drug release HME tablet of Apigenin, ◆ pure Apigenin; ■ Formulæ 1; ▲Formulæ 2;><Formulæ 3

**CONCLUSION**

Solid dispersion technique by Hot Melt Extrusion (HME) can improve the solubility and dissolution of Apigenin. Formula 1 with 10% w/w Apigenin/90% soluplus® is the best formula in this research can increase the 18, 25-fold solubility of Apigenin in water.

**ACKNOWLEDGMENT**

We would like to thank the minister of the ministry of research, technology, and higher education for supporting the funding of this research through the doctoral grant 2018 program; we would like to

thank Politeknik STMI Jakarta for supporting equipment twin screw extruder.

**FUNDING**

This research is supported by Universitas Padjadjaran, Bandung Sumedang Indonesia, and the ministry of research, technology, and higher education Indonesia.

**AUTHORS CONTRIBUTIONS**

All the authors contributed equally.

**CONFLICTS OF INTERESTS**

There are no conflicts of interest.

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