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 anticalcull [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) [5]. 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 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 modifying drug release [22]. APG suitable for HME method using high thermoplastic behavior, proper transition glass (Tg), high modified due to show the best and most suitable polymer for HME and its design and performance [17]. Some of the dispersion techniques in carriers can conduct by 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 a has report to enhance the solubility and dissolution of suspension 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 (Tg), 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\textsuperscript{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\textsuperscript{®}, Kollidon\textsuperscript{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\textsuperscript{®}, Kollidon\textsuperscript{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\textsuperscript{VA} 64 |
|-------------|-------------|-----------|-------------------------------|
| F1          | 10          | 90        | -                             |
| F2          | 20          | 80        | -                             |
| F3          | 40          | 60        | -                             |
| F4          | 10          | -         | 90                            |
| F5          | 20          | -         | 80                            |
| F6          | 40          | -         | 60                            |
| F7          | 50          | -         | 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 a pellet and the scanning range was 400–4000 cm\textsuperscript{-1}, and the resolution was 1 cm\textsuperscript{-1} [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 \(\lambda_{\text{max}}\) 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, and 300 °C at a heating rate of 20 °C/min (HITACHI, STA7300) [25].

**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](image)

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\textsuperscript{VA} 64; and F3 apigenin 33.3%, Soluplus® 33.3%, mixed Kollidon\textsuperscript{VA} 64 33.3%.
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).
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           | 16,36±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

**Table 5: Drug loading and particle size of apigenin**

| Formula | Drug loading (%) | (Particle size) (nm) |
|---------|------------------|----------------------|
| Apigenin| -                | 174,3.3±15.63        |
| F1      | 90%              | 685,1±8.77           |
| F2      | 96%              | 700,3±13.09          |
| F3      | 78.31%           | 6222,67±337.7        |

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

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.

**Fig. 4: Twin screw extruder**

**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 with 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. 5: The solubility of apigenin in the optimum formula**

**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%**

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**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.
Dissolution studies demonstrated enhancement in apigenin percent release of 10%/Soluplus®90%; 10% w/w apigenin/Kollidon® VA64 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; ■ Formulae 1; ▲ Formula 2; >Formulae 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.

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AUTHORS CONTRIBUTIONS
All the authors contributed equally.
CONFLICTS OF INTERESTS

There are no conflicts of interest.

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