

Madecassoside and Asiaticoside-Loaded Film-Forming Polymeric Solutions based on Hypromellose E5 and Eudragit® NE 30D

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Abstract

The aim of this work was to prepare and evaluate the physicochemical properties of film-forming polymeric solutions containing madecassoside and asiaticoside. The stability and *in vitro* release were also investigated. Hypromellose E5 and Eudragit® NE 30D were used as film-forming agents. Glycerin was used as a plasticizer. Ethanol is used as a solvent and for shortening film drying. The best formulations composed of 5 % hypromellose E5, glycerin, ethanol, and *Centella asiatica* Cosmélène® in the ratio of 60:20:20:10 (Formulation 11) or 60:20:10:10 (Formulation 12) w/w. It could form a complete film after being applied to the skin within 10 - 15 min. The pH, viscosity, and spreadability values were 7.9, 23.7 cP, 25.1 g·cm²/s, for Formulation 11, and 7.9, 27.1 cP, and 23.4 g·cm²/s, for Formulation 12, respectively. They were stable for at least 6 months. Both formulations released only 13 % of madecassoside and 14 -15 % of asiaticoside to the phosphate buffer pH 7.4. In conclusion, the developed FFPS could be used as the delivery system for madecassoside and asiaticoside for skin application.

Keywords: Madecassoside, Asiaticoside, Film-forming polymeric solutions, Hypromellose, Eudragit® NE 30D

Introduction

Centella asiatica (L.) Urb. (Apiaceae) is a well-known plant used as an herbal drug, food supplement, or cosmetic additive. *C. asiatica* shows a good *in vitro* and *in vivo* wound healing activity similar to the clinical trials [1]. The active compounds mostly reported in *C. asiatica*, which relate to wound healing, are pentacyclic triterpenoids, i.e., asiaticoside, madecassoside, and their aglycones; asiatic acid and madecassic acid [2,3]. Apart from wound healing, it is recommended for the treatment of various skin conditions such as leprosy, varicose ulcer, eczema, psoriasis, etc. Besides, it is relieving anxiety and improves cognitive function [4]. Some *C. asiatica* extract-contained formulations were developed in conventional dosage forms such as ointments [5], creams [5], emulsion [6], gels [5-7], and capsules [8], and modern dosage forms such as transdermal patches [9], transfersome-loaded gel [10], and sprays [11]. Conventional topical formulations have some limitations, such as poor adherence to the skin, poor permeability, and compromised patient compliance. So topical film-forming system is developed [12].

The film-forming polymeric solutions (FFPS) are novel drug delivery systems that can deliver the drug via the skin. The formulation is composed of the active ingredient, film-forming agent, plasticizer, and other excipients such as penetration enhancers or solvents [13,14]. When the formulation in the liquid form is applied to the skin, the solvent is evaporated, the film is formed. Thus, the obtained film can control drug release to the skin [12]. The transparency of the obtained film is an important feature that considerably affects patient compliance [12]. Some drugs can be delivered by this system, i.e., steroid-based drugs such as betamethasone-17-valerate [15] and ethinylestradiol [16], a non-steroidal anti-inflammatory drug such as ketorolac [17], an antifungal agent such as naftifine hydrochloride [18], and drug for smoking cessation such as nicotine [13,19]. Besides, plant extract, the α -mangostin is incorporated into FFPS for antibacterial application [20]. Several polymers are introduced to use as a film-forming agent such as Eudragit®

[14-16,21-23], hypromellose [19,20], deproteinized natural rubber latex [13,19], polyvinyl alcohol [13,24], etc. Hypromellose is widely used in oral, ophthalmic, nasal, and topical formulations [25]. The concentration of 2 - 20 % is used for the film-forming solution to a film-coated tablet. The lower viscosity grades are used in aqueous film-coating solutions, while the higher viscosity grades are used with organic solvents [25]. Eudragit® NE 30D is an aqueous dispersion of neutral copolymer consisting of polymethacrylic acid esters. It swells in aqueous media without dissolving. The film produced is insoluble in water and gives pH-independent drug release [25].

The authors experienced in preparing FFPS of the two Eudragit® grades; Eudragit® RS 30D and Eudragit® RL30D as film-forming agents in film-forming polymeric dispersion formulation of *C. asiatica* extract [26]. However, the formulation was stable for less than 3 months. So we try to modify FFPS properties by mixing the hypromellose with Eudragit® NE 30D and improve the formulation stability. The aim of this work was to prepare and evaluate the physicochemical properties of FFPS containing madecassoside and asiaticoside based on hypromellose E5 and Eudragit® NE 30D. The stability and *in vitro* release were also investigated.

Materials and methods

Materials

Asiaticoside (purity 99.81 %) was purchased from Chengdu Biopurify Phytochemicals Ltd., Sichuan, China. Madecassoside from *Centella asiatica* (purity 95.0 %) was purchased from Sigma, Missouri, USA. *Centella asiatica* Cosmélène® (1.0 % of a mixture of asiaticoside and madecassoside in butylene glycol) (Greentech Biotechnologies, Saint-Beauzire, France) and glycerin were purchased from Namsiang Co., Ltd., Bangkok, Thailand. Polyacrylate dispersion 30 % (Eudragit® NE 30D, poly(ethyl acrylate, methyl methacrylate) 2:1) (Evonik Nutrition & Care GmbH, Essen, Germany) were gifted from Jebsen & Jessen Ingredients, Bangkok, Thailand. Absolute ethanol was purchased from QR&C, Auckland, New Zealand. The other solvents and chemicals were HPLC grade or analytical grade.

Preparation of madecassoside and asiaticoside-loaded FFPS

The 12 formulations of FFPS were prepared: 7 blank FFPS and 5 madecassoside and asiaticoside-loaded FFPS. Hypromellose E5, Eudragit® NE 30D, and their mixtures were used as film-forming agents. Glycerin was used as a plasticizer. Ethanol was used as a penetration enhancer and for the shortening of film drying time. Three formulation factors: Glycerin content (F1 to F3), ethanol content (F3 to F5), and the mass ratio of hypromellose E5 and Eudragit® NE 30D (F5 to F7) were varied to adjust the physicochemical properties of the blank FFPS. The F8 to F12 were madecassoside and asiaticoside-loaded FFPS. In case of these formulations, the effect of the mass ratio of hypromellose E5 and Eudragit® NE 30D (F8 to F10) and ethanol content (F8, F11, and F12) was investigated. The active and inactive ingredients and their contents in FFPS are shown in **Table 1**. Initially, 5 % hypromellose E5 was prepared by dispersed hypromellose E5 in water until a clear solution was obtained, then it was kept in the refrigerator overnight to allow the polymer to swell completely. FFPS was prepared by mixing 5 % hypromellose E5 with Eudragit® NE 30D (if any). Glycerin and ethanol were then added and mixed, respectively. Lastly, *Centella asiatica* Cosmélène® was added and mixed (only for F8-F12).

Table 1 The active and inactive ingredients and their contents in FFPS.

Formula	5 % Hypromellose E5 (g)	Eudragit® NE 30D (g)	Glycerin (g)	Ethanol (g)	<i>Centella asiatica</i> Cosmélène® (g)
F1	30	30	5	5	-
F2	30	30	10	5	-
F3	30	30	20	5	-
F4	30	30	20	20	-
F5	30	30	20	40	-
F6	60	-	20	40	-
F7	-	60	20	40	-
F8	60	-	20	40	10
F9	30	30	20	40	10
F10	-	60	20	40	10
F11	60	-	20	20	10
F12	60	-	20	10	10

Evaluations of madecassoside and asiaticoside-loaded FFPS

Drying time and integrity of film after applied on the skin

The FFPS (0.1 mL) was applied to the forearm skin with an area of 3×3 cm² (n = 3). Drying time was recorded after applying the formulation to the forearm skin with the specific area until it dried. Drying time was categorized into 3 levels, including less than 10 min, 10 - 15 min, and more than 15 min, which was adapted based on the results of the previous work [13]. The obtained film on the skin has categorized the integrity of dried film on the skin; A (a complete film with no cracking and no flaking), B (complete film with cracking or sporadic flaking), and C (incomplete or partially lost film) [13]. Each formulation was performed in triplicate.

pH

The pH of the formulations was measured 3 times using a pH meter (SevenCompact S220, Mettler Toledo, Greifensee, Switzerland). The average value and standard deviation (SD) were reported.

Viscosity and spreadability

The viscosity of the formulations was evaluated using a viscometer (Brookfield DV-II+, Ametek, Inc., Wisconsin, USA). The spindle no. S00 was used. The viscosity was recorded every 10 s for 1 min by the Wingather V2.0 software. Each formulation was measured 3 times. The average value and SD were reported. According to the spreadability value, it was evaluated according to the method reported by Pichayakorn *et al.* [13]. The 1 g of the formulation (W) was dropped onto the glass plate. Then, a second glass plate covered the formulation. Radius (cm) of the formulation that spread by the applied pressure and time (s) used to spread (T) were recorded. Each formulation was evaluated 3 times. Then, the area (A) that the formulation covered was calculated using a radius value. Finally, spreadability was calculated as Eq. (1) The average value and SD were reported:

$$\text{Spreadability} = \frac{W \times A}{T} \quad (1)$$

Analysis of drug content

The 100 mg/mL of FFPS was prepared by dissolving 1 g of FFPS in the 10-mL volumetric flask using methanol as solvent (n = 3). It was extracted by sonication technique, filtered, and analyzed by high-performance liquid chromatography (HPLC). The HPLC method used for the analysis of madecassoside and asiaticoside was already validated in our previous work [27]. Briefly, the Agilent 1260 Infinity (Agilent Technologies, California, USA) was used for the analysis. The ACE 5 C18-PFP (250×4.6 mm², i.d., 5 μm) was used and controlled at 25 °C. The gradient system of acetonitrile and 0.01 % orthophosphoric acid was used at the flow rate of 1 mL/min. The injection volume was 10 μL. The signal was detected at 210 nm. The content of madecassoside and asiaticoside was calculated based on the calibration curves of each standard compound. The theoretical contents of madecassoside and asiaticoside added to FFPS were calculated from the madecassoside and asiaticoside containing in *Centella asiatica* Cosmélène® which were analyzed by HPLC. The loading capacity and loading efficiency were then calculated and reported as Eqs. (2) and (3).

$$\text{Loading capacity (\%)} = \frac{\text{Weight of madecassoside (or asiaticoside)}}{\text{Weight of the FFPS}} \times 100 \quad (2)$$

$$\text{Loading efficiency (\%)} = \frac{\text{Weight of madecassoside (or asiaticoside)}}{\text{Weight of madecassoside (or asiaticoside) added}} \times 100 \quad (3)$$

Stability test

The selected formulations were kept in the refrigerator (4°C) and climate chamber (Mettmert GmbH + Co. KG, Schwabach, Germany) at 25 °C/75 %RH and 40 °C/75 %RH. The formulations were sampled at 1 month, 2 months, 3 months, and 6 months to analyze the remaining content of madecassoside and asiaticoside compared with an initial time point.

Drug release

The release study condition was performed, following our previous work [26]. A modified Franz-type diffusion cell (Teledyne Hanson Research, Inc., California, USA) was used to evaluate the drug release. The effective diffusion area was 1.77 cm². A receptor medium was 12 mL of phosphate buffer solution (pH 7.4) and controlled at 37±0.5°C. The release medium was constant stirring at 600 rpm. The receptor compartment and donor compartment were separated by dialysis membrane (Spectra/Por® Dialysis

Membrane, MWCO 3500, Spectrum Laboratories Inc., California, USA). The 0.5 mL of the FFPS was filled to the donor compartment ($n = 3$). The donor compartment did not occlude during the release study. Drug release was evaluated for 8 h. The 1 mL of release medium was sampled at 1, 2, 3, 4, 5, 6, and 8 h, and the fresh phosphate buffer solution pH 7.4 (37 °C) was replenished. The sampled release medium was filtered and analyzed by HPLC. The release profiles were constructed.

Statistical analysis

The difference between the 2 groups was analyzed using the student's t-test. The difference of more than 2 groups was analyzed using One-way analysis of variance (One-way ANOVA) followed by Tukey HSD post hoc analysis. Data was significantly different when the P-value was less than 0.05 at a 95 % confidence interval.

Results and discussion

Physicochemical properties of madecassoside and asiaticoside-loaded FFPS

The first factor investigated for the blank FFPS formulation was the content of glycerin. Glycerin is a plasticizer that promotes film flexibility. Increasing glycerin content (F1 - F3), could increase the drying time from 10 - 15 min (F1 and F2) to more than 15 min (F3). This occurrence could be described by decreasing ethanol content; Increasing glycerin content decreased ethanol content in the formulation from 7.14 to 5.88 %. Hence, the formulation F3 exhibited a longer drying time compared with F2 and F1. A small amount of glycerin in F1 gave the incomplete film after the film was dried on the skin. While a higher amount of glycerin in F2 and F3 gave the complete film. The pH of the formulation was slightly basic. The viscosity was decreased, while spreadability was increased when glycerin content increased. Due to the long drying time, ethanol content was the second factor investigated to shorten the drying time (F3 - F5). When ethanol content increased, the drying time was decreased from more than 15 min to 10 - 15 min. The drying time results of the developed FFPS were similar to the previous work. Deproteinized natural rubber FFPS containing nicotine as an active pharmaceutical ingredient could be dried within 10 - 15 min to more than 15 min [13]. However, the developed FFPS had a long drying time when compared with the previous publication, that fast-drying time was categorized as less than 5 min [14]. The formulations gave the complete film after being applied to the skin. The pH remained slightly basic. The viscosity and spreadability were decreased. However, too much ethanol promoted the coagulation of the formulation. The film-forming agent's ratio was varied in F5 to F7. In case of F7, the Eudragit® NE 30D was still coagulated due to a high amount of ethanol. The recommended solvent for Eudragit® NE 30D is water [25], so the use of too much ethanol in the formulation might dissolve the Eudragit® particles that make the coagulation of them. Thus, viscosity and spreadability of F5 and F7, as well as the madecassoside and asiaticoside-contained FFPS, F9 and F10, were not evaluated. Comparing between F6 and F8; without vs. with *Centella asiatica* Cosmélène®, drying time, the integrity of film on the skin, pH, and viscosity was the same, while the spreadability was slightly decreased. Therefore, the addition of *Centella asiatica* Cosmélène® did not alter the physicochemical properties of the FFPS.

Ethanol is a well-known penetration enhancer due to it can increase drug solubility, alteration of permeation property of stratum corneum, produce the thermodynamic driving force for drug permeation by evaporation and saturation of the drug solubility in the formulation, and extraction of lipid to improve drug permeation, etc. [28]. The authors are concerned that too much ethanol might alter the defense mechanism of the skin by the extraction of skin lipids. Thus, F11 and F12 were decreased in ethanol content compared with F8. The results showed that the drying time and integrity of film on the skin did not alter. The pH value was decreased from 8.14 to 7.89 due to the alkaline solution of ethanol decreasing. The viscosity was increased, while the spreadability was comparable. The physicochemical properties of blank FFPS and madecassoside and asiaticoside-loaded FFPS are shown in **Table 2**. The physical appearance of formulations F8, F11, and F12 are shown in **Figure 1**. The sample of the transparent film obtained from formulation F12 after being applied on the skin and dried is shown in **Figure 2**.

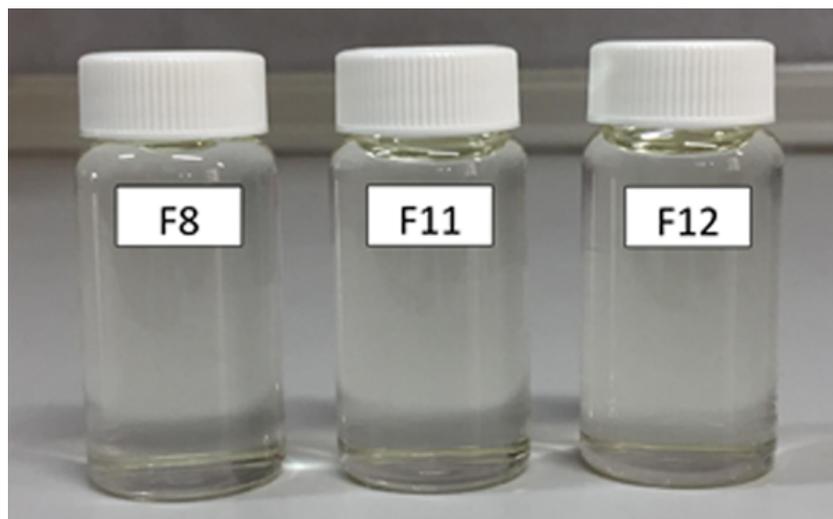


Figure 1 The physical appearance of formulations F8, F11, and F12.



Figure 2 The film obtained from formulation F12 after being applied on the skin and dried.

Table 2 Physicochemical properties of blank FFPS and madecassoside and asiaticoside-loaded FFPS.

Formula	Drying time (min) (n = 3)	Integrity of film* (n = 3)	pH (n = 3)	Viscosity (cP) (n = 3)	Spreadability (g·cm ² /s) (n = 3)
F1	10 - 15	C	8.28 ± 0.02	34.39 ± 0.90	28.47 ± 6.12
F2	10 - 15	A	8.24 ± 0.02	35.56 ± 2.27	31.15 ± 6.56
F3	> 15	A	8.34 ± 0.01	17.77 ± 1.33	37.01 ± 2.48
F4	> 15	A	8.42 ± 0.04	15.73 ± 0.08	30.81 ± 3.01
F5	10 - 15	A	-	-	-
F6	10 - 15	A	8.14 ± 0.01	17.13 ± 0.60	27.09 ± 2.86
F7	10 - 15	A	-	-	-

Formula	Drying time (min) (n = 3)	Integrity of film* (n = 3)	pH (n = 3)	Viscosity (cP) (n = 3)	Spreadability (g·cm ² /s) (n = 3)
F8	10 - 15	A	8.14 ± 0.05	17.11 ± 2.63	24.99 ± 1.19
F9	10 - 15	A	-	-	-
F10	> 15	A	-	-	-
F11	10 - 15	A	7.91 ± 0.00	23.68 ± 1.15	25.12 ± 3.76
F12	10 - 15	A	7.89 ± 0.01	27.07 ± 0.30	23.36 ± 3.45

*A = complete film, B = complete film with crack or sporadic film, C = incomplete film

The loading capacity and loading efficiency were also reported in **Table 3**. The loading efficiency was close to 100 % indicating that there were no madecassoside nor asiaticoside lost during the preparation process. The HPLC chromatogram of formulation F12 is shown in **Figure 3**. The HPLC method used for the analysis of madecassoside and asiaticoside was already validated in our previous work to warrant the reliability of the analysis [27].

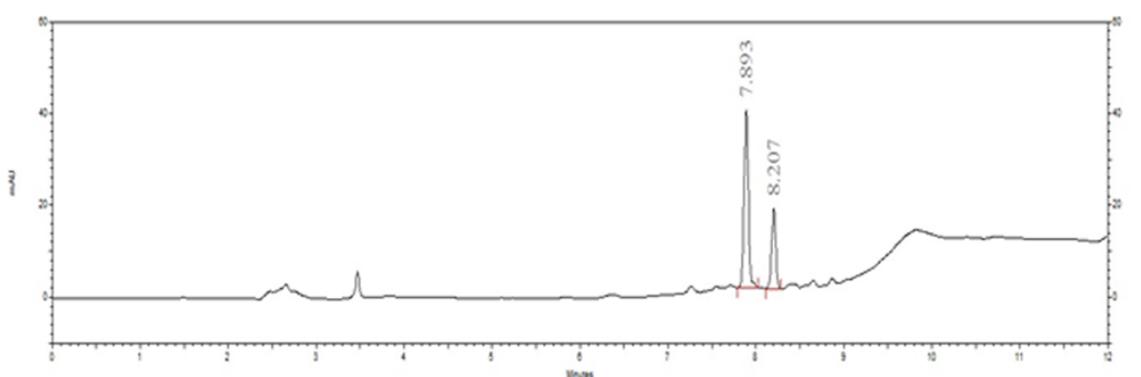


Figure 3 HPLC chromatogram of F12 (100 mg/mL), which identified peaks at the retention time of 7.893 and 8.207 min, were madecassoside and asiaticoside, respectively.

Table 3 Loading capacity and loading efficiency of some madecassoside and asiaticoside-loaded FFPS formulations.

Formula	Loading capacity (%w/w) (n = 3)		Loading efficiency (%w/w) (n = 3)	
	Madecassoside	Asiaticoside	Madecassoside	Asiaticoside
F8	0.0670 ± 0.0009	0.0229 ± 0.0002	104.12 ± 1.40	101.82 ± 0.87
F11	0.0807 ± 0.0001	0.0272 ± 0.0000	106.09 ± 0.14	102.53 ± 0.04
F12	0.0883 ± 0.0001	0.0298 ± 0.0001	105.50 ± 0.12	102.04 ± 0.39

The pH value played an important role in the stability of the formulation and reflected the compatibility with the skin. The pH of the topical formulation was an important factor that should be considered. The pH of the formulation might alter the natural property of the skin or cause irritation. Braun-Falco and Korting [29] reported the normal pH of human skin of 5.4 - 5.9. Lambers *et al.* [30] reported the average pH value of human skin was 4.7. Moreover, the variation of skin pH between 4.0 - 5.96 was reported [31]. The FFPS with a slightly basic pH (approximately 7.6 - 9.0) could be found in the other works [13,19]. However, the skin irritation test should be performed to ensure the safety of the FFPS formulation. The author expected that the basic pH of the formulation perhaps prevents the acid hydrolysis of madecassoside and asiaticoside, hence, it could improve the stability of the FFPS formulation compared with our previous work [26].

Stability of madecassoside and asiaticoside-loaded FFPS

The F8, F11, and F12 were selected to evaluate their stability at different temperatures; 4, 25 and 40 °C, for 6 months. They were sampled to analyze the remaining madecassoside and asiaticoside at 1 month, 2, 3 and 6 months. The stability data are shown in **Figure 4**. The shelf-life of the pharmaceutical products was the time that the active ingredient remained for 90 % or degraded for 10 % of the label claim [32]. In this case, all formulations, F8, F11, and F12 were stable for at least 6 months. The asiaticoside seems to be more stable than madecassoside. Increasing madecassoside and asiaticoside remained in the formulation might relate to the evaporation of ethanol. The high increment of remaining madecassoside and asiaticoside was observed in the formulation stored at 40 °C more than at 25 and 4°C, respectively. Furthermore, the high increment of remaining madecassoside and asiaticoside was observed in F8, the highest ethanol formulation, more than F11 and F12. Our recent work succeeded in developing a more stable formulation of the FFPS system by expanding the shelf-life of them from 2 months to 6 months compared with our previous work. The film-forming polymeric dispersions based on Eudragit® RS 30D and Eudragit® RL 30D were demonstrated in our previous work. This system also contained madecassoside and asiaticoside as active compounds. Results showed that the formulation was stable for more than 2 months but less than 3 months. The pH of the formulation was 5.7, so it could induce the degradation of madecassoside and asiaticoside by acid hydrolysis [26].

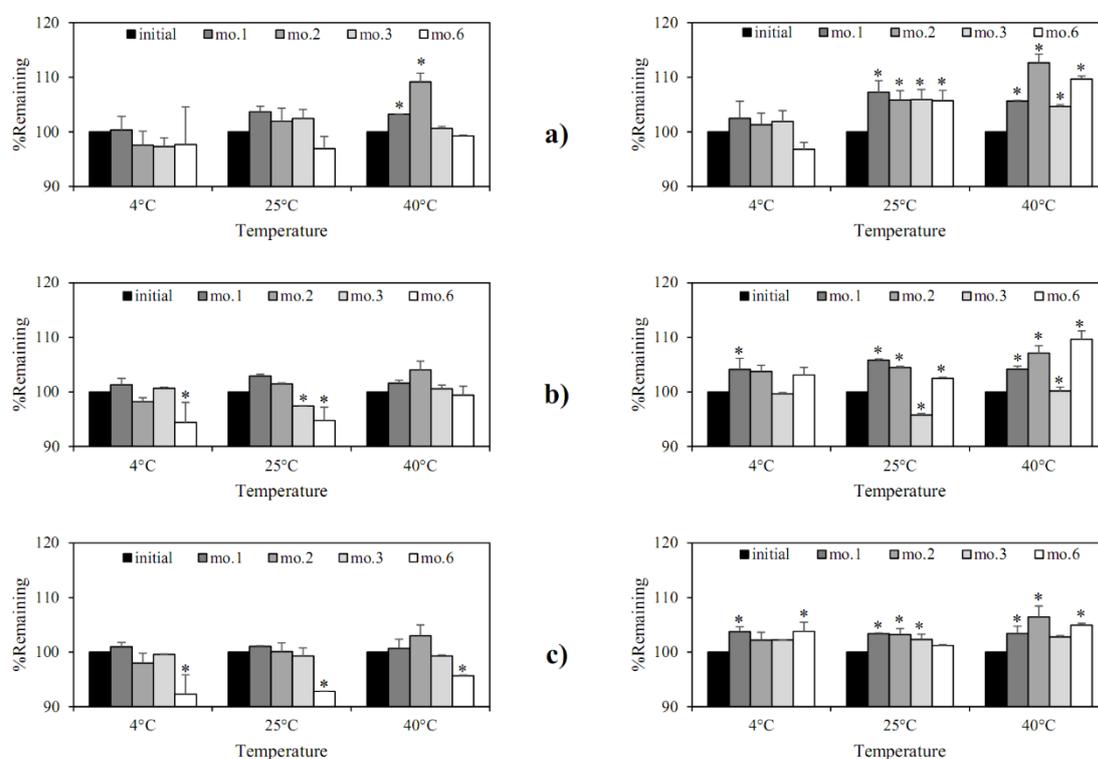


Figure 4 The content of madecassoside (left) and asiaticoside (right) remaining in the formulations (a) F8, (b) F11, and (c) F12 when stored at different temperatures for 6 months. * = significant difference ($p < 0.05$) when compared with at initial time point.

Release of madecassoside and asiaticoside from F11 and F12

Only F11 and F12 were selected to evaluate drug release. **Figure 5** shows the release profile of madecassoside and asiaticoside from F11 and F12. The release of madecassoside and asiaticoside from F11 was comparable to F12. A low percent cumulative release of madecassoside and asiaticoside was observed. The maximum release of madecassoside and asiaticoside from F11 was 12.8 and 13.7 %, respectively. In case of F12, the maximum release of madecassoside and asiaticoside was 13.4 and 14.8 %, respectively. Our study evaluated the release for only 8 h, but the release was higher than the FFPS formulation developed in our previous work that evaluates for 12 h [26]. The retarded drug release was also observed in the

thymoquinone release from FFPS formulation containing 5 % hypromellose E5 blended 0.5 % xanthan gum. It was released only 10 - 15 % for 8 h of drug release study [33].

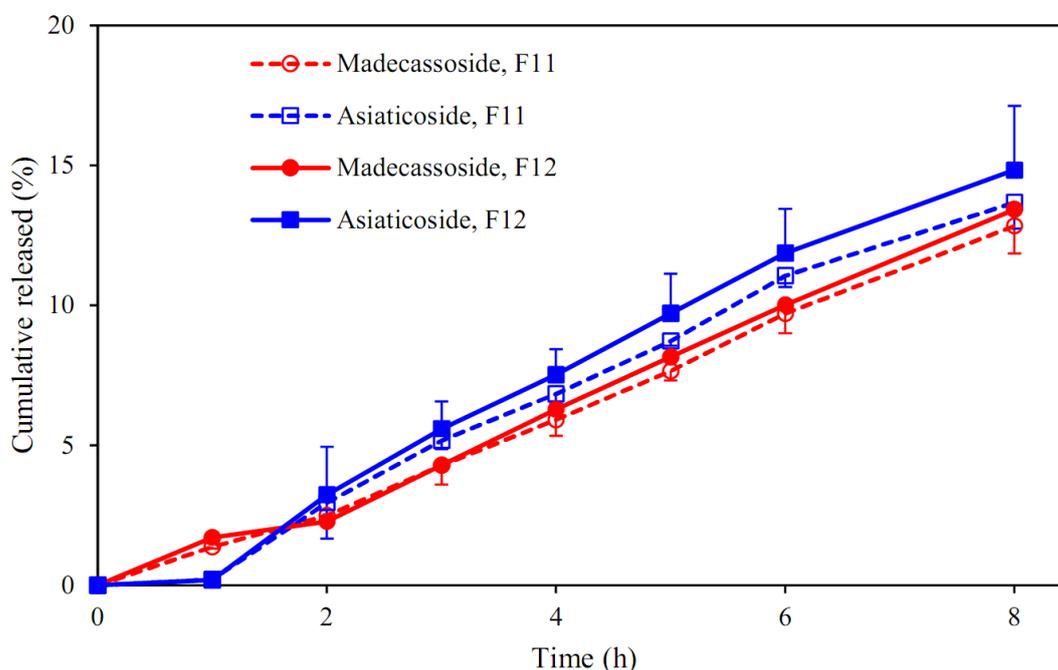


Figure 5 Release profiles of madecassoside (○/●) and asiaticoside (□/■) from F11 (dash line) and F12 (solid line) in phosphate buffer pH 7.4. There was no significant difference of madecassoside as well as asiaticoside released from F11 and F12 ($p > 0.05$).

Conclusions

This work developed the madecassoside and asiaticoside-loaded FFPS. Hypromellose E5 and Eudragit® NE 30D were selected as film-forming agents. The best FFPS developed in this work was composed of 5 % hypromellose E5, glycerin, ethanol, and *Centella asiatica* Cosmélène® in the mass ratio of 60:20:20 (or 10): 10, respectively. The formulations formed a complete film within 10 - 15 min. FFPS was stable for at least 6 months at 4, 25 and 40 °C. The low content of madecassoside and asiaticoside released from FFPS to phosphate buffer pH 7.4 was observed. Therefore, the developed FFPS could be used as the delivery system for madecassoside and asiaticoside for skin application.

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