Indonesian Journal of Pharmacy

VOL 34 (1) 2023: 128–139 | RESEARCH ARTICLE

Clindamycin Peel-Off Mask Film, An Effective Formulation For *C. Acnes* Treatment: Characterization And Microbiological Activity

Boontida Morakul[1]*, Amaraporn Wongrakpanich[1], Veerawat Teeranachaidekul[1], Kritameth Washiradathsathien[2] and Arkaratep Gamolvate[2]

1. Department of Pharmacy, Faculty of Pharmacy, Mahidol University, 447 Sri-Ayuthaya Rajathevi Bangkok 10400, Thailand
2. Faculty of Pharmacy, Mahidol University. 447 Sri-Ayuthaya Rajathevi Bangkok 10400, Thailand

Article Info

Submitted: 22-07-2022
Revised: 07-11-2022
Accepted: 01-02-2023

*Corresponding author
Boontida Morakul

Email:
boontida.mor@mahidol.edu

ABSTRACT

Clindamycin is the antibiotic of choice for treating *Cutibacterium acnes* (*C. acnes*), which is available in various dosage forms such as topical solution, gel, and foam. In this current study, clindamycin was formulated as the peel-off mask gel, intending to form film and providing the drug release in a sustained manner. This would allow a better antimicrobial efficacy as well as peel-off after use. Fifteen formulations of the peel-off mask gel were designed with varying concentrations of film-forming agents. The primary film-forming agent was polyvinyl alcohol (PVA), which was combined with the co-film-forming agents polyvinylpyrrolidone-K30 (PVP-K30) or sodium alginate (SA). The two best formulations were 1 %w/w clindamycin loaded in 10 %w/w PVA with 20 %w/w PVP-K30 (F18) and 10 %w/w PVA with 1.5 %w/w SA (F21). The physical property evaluation of the peel-off mask gel showed that the types and concentrations of film-forming agents influenced the viscosity, spreadability, and film-forming ability. The F18 and F21 formulations showed good to very good spreadability and film-forming ability. The peel-off masks had a pH of 4-5, which were compatible with the skin's pH. The film provided a drying time of approximately 8 minutes, indicating its fast-drying property, and was appropriate for practical use. The determining of the film's tensile strength indicated the suitable preference of use. There was no interaction between the ingredients in the formulation evaluated by FT-IR, XRD, and DSC. Both formulations showed a high antimicrobial susceptibility for *C. acnes* compared to the blank film and the clindamycin solution implying the potential to be used as the new market formulation for acne treatment.

Keywords: Clindamycin, Peel-off mask film, *C. acnes*, PVA, Sodium alginate

INTRODUCTION

The topical antibiotic is a recommended dosage form in the American Academy of Dermatology guidelines of care for managing acne vulgaris 2016, which is suitable for all acne grading scales from mild to severe (Zaenglein *et al.*, 2016). Among antibiotics, clindamycin is the antibiotic of choice endorsed for acne treatment. The market topical dosage form of clindamycin is usually available in gel, topical solution, and foam at a therapeutic concentration of 10 mg clindamycin per gram or millilitre (1 %w/w or %w/v). Currently, the face mask is one of the dosage forms of interest. It would allow concentrated actives to be delivered in a prolonged manner to the concerned area. Thus, a face mask can enhance the treatment. Gloor *et al.* (1979) conducted a study using a 1 %w/w clindamycin face mask on 29 healthy male volunteers. They found that a clindamycin face mask could significantly reduce *C. acnes* and the total bacterial counts compared to the clindamycin solution.

In this present study, 1 %w/w of clindamycin was developed as a peel-off mask film to treat acne vulgaris. The product was designed to be applied to the inflammatory acne lesion, waited for the film to dry, and peeled off after use.

Table I. The compositions and physical characteristics (mean±SD) of peel-off mask gel base (without clindamycin) formulation F1-F15

Formulation		F1	F2	F3	F4	F5	F6	F7
Composition	PVA (g)	7.5	10	12.5	10	10	10	10
	PVP-K30 (g)						8	12
	SA (g)							
	Glycerin (g)	1.1	1.1	1.1	1.1	1.1	1.1	1.1
	Ethanol (g)	20	20	20	20	20	20	20
	DI water (g)	q.s.100	q.s.100	q.s.100	q.s.100	q.s.100	q.s.100	q.s.100
Characteristics	Spreadability	++++	++++	++++	+++	++	++++	++++
	Film-forming ability	++++	++++	++++	++++	++++	++++	++++
	Drying tim (min)	22.01± 0.60	19.03± 0.87	16.57± 0.53	13.05± 0.42	5.64± 0.33	12.19± 0.12	10.57± 0.40
	Viscosity (cps.)	79± 5	532± 129	854± 211	33.568± 1.604	224.467± 22.532	636± 25	371± 39
	pH	6.24± 0.02	5.96± 0.01	6.14± 0.01	6.11± 0.01	5.14± 0.01	4.57± 0.02	4.32± 0.03

Formulation		F8	F9	F10	F11	F12	F13	F14	F15
Composition	PVA (g)	10	10	10	10	10	10	10	10
	PVP-K30 (g)	15	20	25					
	SA (g)				0.38	0.50	0.75	1.00	1.50
	Glycerin (g)	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
	Ethanol (g)	20	20	20	20	20	20	20	20
	DI water (g)	q.s.100	q.s.100	q.s.100	q.s.100	q.s.100	q.s.100	q.s.100	q.s.100
Characteristics	Spreadability	++++	++++	++++	++++	++++	++++	++++	+++
	Film-forming ability	+++	+++	+++	++++	++++	++++	++++	++++
	Drying tim (min)	9.10± 0.47	7.36± 0.12	8.19± 0.14	14.26± 0.27	12.32± 0.10	1.981± 80	3.141± 90	12.09± 0.04
	Viscosity (cps.)	1.000± 281	1.351± 330	3.436± 169	645± 4	3.090± 215	1.981± 80	3.141± 90	9.932± 115
	pH	4.17± 0.03	4.14± 0.05	4.14± 0.05	6.52± 0.06	6.56± 0.35	6.63± 0.15	6.88± 0.08	6.94± 0.03

The different types and concentrations of the film-forming agents of polyvinyl alcohol (PVA) blending with polyvinylpyrrolidone-K30 (PVP-K30) and sodium alginate (SA) were optimized. PVA is a synthetic polymer with excellent film-forming ability and biocompatibility. Therefore, it is chosen as the main film-forming agent. Blending more than one type of polymer is required to achieve desirable properties such as a flexible, thin, transparent, and resistant film. In this study, the synthetic co-film-former (PVP-K30) and the natural co-film-former (SA) were both evaluated. PVP-K30 is commonly used as a film-forming agent for peel-off mask film in various research studies (Rosaini *et al.*, 2021, Hendrawati *et al.*, 2018). It is a water-soluble polymer that provides a glossy, transparent, clear, and hard film with good adhesiveness and high bioavailability. The drawback of PVP-K30 film is its brittleness due to the absence of a plasticizer. This issue can be avoided by blending it with other polymers. However, PVP-K30 is not biodegradable. According to the environmental concerns that have become increasingly relevant in the selection of film-forming materials, the insoluble and non-biodegradable film-forming materials are considered microplastics that can enter wastewater (Sun *et al.*, 2020). Therefore, in this study, SA, a natural polysaccharide film-former obtained from marine algae with a rigid molecular

chain and good film-forming ability, is also in focus. There are fewer studies on applying SA as the film-former in the peel-off mask film formulation. To adjust the mechanical strength and elasticity, blending SA with PVA might be a good candidate. The physicochemical properties of the clindamycin peel-off mask film were evaluated in various aspects, including the spreadability, film-forming ability, drying time, viscosity, pH, tensile strength, the interaction between ingredients in the formulation by fourier-transform infrared spectroscopy (FT-IR), crystallinity using x-ray diffractometry (XRD), and thermal behavior using differential scanning calorimetry (DSC). Additionally, the microbiological activity of the peel-off mask containing clindamycin against *C. acnes* was also evaluated.

MATERIALS AND METHODS

The ingredients used in this study include clindamycin phosphate, which was kindly supported by MacroPhar Co., Ltd., Bangkok, Thailand. PVA was obtained from Sigma-Aldrich, Missouri, United States. PVP-K30 was purchased from Chanjao Longevity, Bangkok, Thailand. SA was achieved from Chemipan, Bangkok, Thailand. D-glucose anhydrous was obtained from Ajax Finechem, Wellington, New Zealand. Ethanol (analytical grade) was obtained from RCI Labscan, Bangkok, Thailand. Glycerin was obtained from Global Green Chemicals, Bangkok, Thailand. Sterile water for irrigation was obtained from the General Hospital Product Public Co., Ltd., Pathum Thani, Thailand.

Preparation of peel-off mask film containing 1 %w/w clindamycin

Fifteen formulations (F1 – F15) of the blank peel-off mask film were prepared (Table I). PVA was used as the main film-forming agent, with the amount varying from 7.5 %w/w (F1) to 20 %w/w (F5). Additionally, the PVP-K30 or SA were combined with PVA to modify the property of peel-off mask film. The PVP-K30 amount was varied from 8 %w/w (F6) to 25 %w/w (F10). The SA amount was varied from 0.38 %w/w (F11) to 1.50 %w/w (F15). The PVA concentration in F6 to F15 was fixed at 10 %w/w due to the appropriate viscosity for application. Films were prepared using the solvent casting technique in the silicone mold. The photographs of a silicone mold and examples of casted films (Formulation F18 and F21) (Figure 1). Briefly, PVA was dissolved in hot water at 70 °C. PVP-K30 or SA was then dissolved in the water and mixed with the dissolved PVA at the predetermined ratio. According to the preliminary study, ethanol and glycerin were fixed at 20 %w/w and 1.1 %w/w as a solvent and plasticizer, respectively. The formulation was gently stirred to obtain a clear and homogeneous mixture.

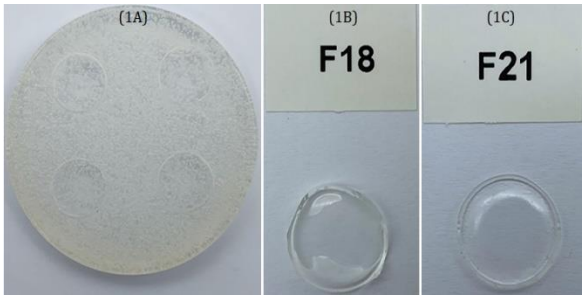

Figure 1. The photographs of a silicone mould (1A) and examples of casted film formulation F18 (1B), and F21 (1C)

The formulations having appropriate properties, including good to very good spreadability and film-forming ability, short drying time (<12.5 min), viscosity in the range of 1,000-10,000 cps, and pH in the range of 4-7, were selected for the 1 %w/w clindamycin incorporation (Table II). The prepared samples were kept in tightly closed glass containers for further evaluation.

Spreadability and film-forming ability

To measure the spreadability and film-forming performance of peel-off mask film, the method was adapted from the study of Beringhs *et al* (2013) and Hariyadi *et al* (2020). A 0.1 g peel-off mask was applied and spread on the glass slide. The spreading area was controlled to obtain the same film thickness. The spreadability and film-forming ability were observed based on a sensorial score. The grading scales were as follows; 0 = poor, + = mild, ++ = moderate, +++ = good, and ++++ = very good.

Drying time

To estimate the time taken for the formulation to dry completely, the *in vitro* drying time evaluation was performed as described by Beringhs *et al.* (2013) with some modifications. A 0.1 g clindamycin peel-off mask was applied and spread on the glass slide. The drying time (in minutes) was quantified and compared.

Table II. The selected formulations incorporated with 1%w/w clindamycin and their physical characteristics (mean±SD)

Formulation	Compositions	Characteristics					
		Spread ability	Film-forming ability	Drying time (min)	Viscosity (cps.)	pH	Tensile strength (g)
Control	F2						3.119.7±479.6
F16	F8 : clindamycin (99:1 %w/w)	++++	+++	11.06±0.07	547±82	4.26±0.02	2.308.2±108.2
F17	F9 : clindamycin (99:1 %w/w)	++++	+++	10.06±0.06	4.023±969	4.22±0.03	2.112.4±146.8
F18	F10 : clindamycin (99:1 %w/w)	++++	+++	8.09±0.03	4.849±342	4.24±0.03	1.509.1±231.7
F19	F13 : clindamycin (99:1 %w/w)	++++	++++	9.34±0.06	2.043±161	5.38±0.02	5.170.9±837.0
F20	F14 : clindamycin (99:1 %w/w)	++++	++++	8.25±0.08	3.804±97	5.32±0.03	5.513.9±612.9
F21	F15 : clindamycin (99:1 %w/w)	+++	++++	8.09±0.12	5.384±112	5.36±0.01	3.410.9±350.0

Viscosity

The viscosity of the clindamycin peel-off mask was measured using a rheometer (HAAKE Roto Visco1, Thermo Fisher Scientific, Germany) (Vieira *et al.*, 2009). The instrument was equipped with a C35/2 Ti rotor and measured at 30 ± 0.5 °C. Each formulation of mask gel (in a gel formulation, before cast as the film) was evaluated without dilution at a shear rate of 5000 s^{-1} for 300 sec. The viscosity value in the centipoise unit was calculated by HAAKE RheoWin Data Manager version 4.87.001 software (Thermo Fisher Scientific, Germany). All samples were measured in triplicate, and the results were averaged.

pH

The gel formulation of the clindamycin peel-off mask (before cast as the film) was measured for the pH value by immersing the electrode directly into the sample using the calibrated pH meter (FiveEasy Plus FP20, Mettler Toledo, Greifensee, Switzerland) at 30±1°C. The measurement was carried out in triplicate, and the results were averaged (Hariyadi *et al.*, 2020).

Tensile strength

The mechanical property of the peel-off mask film was evaluated by determining the tensile strength. The method was adapted from Pichayakorn *et al.* (2012). The clindamycin peel-off mask was prepared as the film to evaluate its tensile strength by casting the mask film in the 20 mm diameter and 0.5 mm thickness-circular shaped silicone mold (Figure 1). After the film was dried, it was peel-off from the silicone mold. The tensile strength was evaluated using a texture analyzer (TA.XT plus C, Stable Micro System, Surrey, United Kingdom) connected with tensile grips (code A/T) at the test speed of 2.00 mm/sec and a distance of 100 mm. All samples were measured in triplicate, and the results were averaged. The 10 %w/w PVA film (without clindamycin, F2) was used as the control.

FT-IR analysis

FT-IR was employed to evaluate the possible interaction between the compositions in the formulation (Singh *et al.*, 2021). FT-IR spectra were obtained over 4000-400 cm^{-1} using a FT-IR spectrometer (Thermo Scientific Nicolet iS5 FT-IR spectrometer, Wisconsin, USA) equipped with a diamond crystal iD7 ATR (attenuated total reflection) accessory. Each measurement represented an average of 32 scans with a resolution of 4 cm^{-1}. The sample of clindamycin peel-off mask formulation F18 and F21 was prepared as the film by casting the mask film in the 20 mm diameter and 0.5 mm thickness-circular shaped silicone mold (Figure 1).

XRD analysis

To evaluate the crystallinity of the film, XRD was applied (Asthana *et al.*, 2021). The sample was scanned between 2θ of 10 ° and 80 ° at a scanning rate of 0.05 °/min by a x-ray diffractometer (MiniFlex 600 Rigaku, Osaka, Japan). CuKα radiation was the x-ray source at a wavelength of 1.54 A°. The experiment was conducted using a rotating anode with the voltage and current setting at 40 kV and 15 mA, respectively. The sample of clindamycin peel-off mask formulation F18 and F21 was prepared as the film by casting the mask film in the 20 mm diameter and 0.5 mm thickness-circular shaped silicone mold (Figure 1).

DSC measurement

DSC was used to evaluate the thermal behavior of the film (Pichayakorn *et al.*, 2012). DSC study was performed using a Mettler TA DSC (Mettler Toledo, Wales, United Kingdom). The samples (approximately 3 mg) were heated from 25 °C to 300 °C at the heating rate of 10 °C/min. The sample of clindamycin peel-off mask formulation F18 and F21 was prepared as the film by casting the mask film in the 20 mm diameter and 0.5 mm thickness-circular shaped silicone mold (Figure 1).

Microbiological activity against *C. acnes*

Formulations with the best results of physicochemical properties (F18 and F21) were evaluated for their antimicrobial activity. The agar disc diffusion method was used according to the M100 Performance standards for antimicrobial susceptibility test, 30th edition (Clinical and Laboratory Standards Institute, 2020). The sample was prepared as a film by casting the mask film in the 20 mm diameter and 0.5 mm thickness-circular shaped silicone mold (Figure 1A). The film was then cut into a small circle with a diameter of 0.6 cm (equal to an area of 0.28 cm^2). The clindamycin peel-off mask formulation F18 and F21 contained clindamycin of approximately 0.89 mg/cm^2. Clindamycin solution at four different clindamycin concentrations ranging from 0.22 – 1.79 mg/cm^2 was used as a positive control. Since the clindamycin amount in each film was calculated based on its average weight per area (mg/cm^2), the clindamycin solutions were also calculated based on weight per area (Table III). Twenty µL of clindamycin solutions were dropped on test papers with a diameter of 0.6 cm. The blank film was introduced as a negative control (F18-blank and F21-blank).

The agar plates were prepared using 20 mL of the Brain Heart Infusion agar (BHI) (Difco™, Maryland, United States) with 1% glucose (Ajax Finechem, Wellington, New Zealand). The samples were placed on the agar and inoculated with *C. acnes* (ATCC 6919) at a density of approximately 1.5 x 10^8 colony forming units (CFU)/mL (adjusted with 0.5 McFarland turbidity standard). The samples were incubated at 35 ± 2 °C in an anaerobic jar for 16-20 h. At the end of the experiment, the inhibition zone was measured and reported in mm.

RESULTS AND DISCUSSION

Physical characterization of the peel-off mask film

Each formulation of the prepared peel-off mask was in a clear and viscous gel. All 15 formulations could form transparent films. The physical properties, including spreadability, film-forming ability, drying time, viscosity, and pH of the peel-off mask gel bases (without clindamycin) (Table I). Increased concentration of film-forming agents, which are PVA, PVP-K30, and SA, enhanced gel viscosity, especially for PVA. For peel-off mask prepared from sole PVA, increasing the concentration of PVA from 7.5 %w/w (F1) to 20 %w/w (F5) resulted in a drastic increase in viscosity. According to the appropriate viscosity for application, PVA 10 %w/w (F2) was chosen to combine with PVP-K30 or SA. When PVA was mixed with PVP-K30 (8-25 %w/w, F6-F10) or SA (0.38-1.50 %w/w, F11-F15), an increment in the viscosity of the gel base was observed. At a similar concentration of PVA, the concentration of PVP-K30, which was higher than the concentration of SA, showed a lesser or relatively equal viscosity when compared to the SA. This indicates that the potential for increasing the viscosity of SA was higher than PVP-K30, with corresponds to the remark of Teodorescu and Bercea (2015) that the PVP melt has very weak flow properties.

Most formulations showed good to very good spreadability and film-forming ability. It was found that an increase in the amount of film-forming agents might bring about a decrease in spreadability, especially for the PVA. Additionally, the spreadability is inversely proportional to the viscosity, similar to the study conducted by Andini *et al.* (2017). In the meantime, increasing the concentration of film-forming agents usually provides an increased film-forming ability. However, in this study, it was found that PVP-K30 co-film-former formulation showed a very good film-forming ability to a certain point, and then the

film-forming ability would decrease. All formulations had appropriate pH in the range of 4 to 7, which is safe for use on the skin. In general, PVA is a water-soluble polymer which has a pH value of 5-8 (Rahmasari *et al.*, 2019). An increase in the concentration of PVA from 7.5 %w/w to 20.0 %w/w resulted in the lower pH of formulations (6.24 vs 5.14) (Table I). Generally, the pH of PVP is in the range of 3-7, depending on the molecular weight and concentration of PVP. For peel-off mask prepared from PVA and SA (F11-F15), increasing the concentration of SA led to an increase in pH. This could be explained due to the alkalinity of sodium alginate. Concerning a drying time, it was reported that a suitable drying time for peel-off mask products ranged from 10 to 15 mins (Roselyne *et al.*, 2014) (in this study, we set the cut-off at 12.5 mins). The viscosity is the main applicability-related characteristic of the formulation, and the viscosity in the range of approximately 1,000-10,000 cps was considered an easy application onto the skin. As a result, F8, F9, F10, F13, F14, and F15 m*et a*l above requirement and they were chosen to incorporate 1 %w/w clindamycin in the preparation of clindamycin peel-off mask gel

After incorporating 1 %w/w clindamycin, the samples were then characterized for their spreadability, film-forming ability, drying time, viscosity, and pH (Table II). The characteristics of the peel-off mask gel after incorporating 1 %w/w clindamycin were comparable with the initial preparation (without clindamycin). Formulations containing SA (Formulation F16 – F18) gave higher viscosity than those containing PVP-K30 (Formulation F19 – F21). The PVA:SA formulations provided a better film-forming property than the PVA:PVP-K30 formulations. An incorporation of 1% w/w clindamycin led to the lower pH of all formulations due to the acidity of clindamycin phosphate. However, all peel-off mask films had an appropriate pH in the range of 4 to 5. The drying time ranged from 8-11 min, and the viscosity was in the range of 547-5,384 cps. Among all 6 formulations, F21 (PVA:SA 10:1.5 %w/w) and F18 (PVA:PVP-K30 10:25 %w/w) gave the fastest drying time at 8.09 ± 0.12 and 8.09 ± 0.03 min, respectively, and provided the viscosity in the optimum range of 1,000-10,000 cps.

Tensile strength

The tensile strength of the peel-off mask film is a potential property that affects the preference of use. The high tensile strength meant the film was tough and might cause pain to the skin during the peeling-off period. Therefore, the appropriate tensile strength for developing peel-off mask film should be considered. The tensile strength was characterized in six formulations of the peel-off mask film containing 1 %w/w clindamycin (F16 to F21) compared with the control group, 10 %w/w PVA film (without clindamycin, F2) as shown in Table II. The clindamycin peel-off mask film of PVA:PVP-K30 formulations presented a lesser tensile strength than that of PVA:SA formulations. Adding PVP-K30 into the formulation resulted in a decreased tensile strength of the film whereas increased amount of SA in the film increased tensile strength as compared to the control. The result indicates that the strengthened potency of the natural polymer (SA) was higher than the synthetic polymer (PVP-K30). Too high tensile strength might cause pain and skin irritation. However, too low tensile strength might cause the film to break easily. The suitable formulation should have a tensile strength ranging between 1,000-4,000 g/cm^2 (Wetchakun *et al.*, 2016, Nursal *et al.*, 2021). Therefore, the formulations F16, F17, F18, and F21 provided the tensile strength in the acceptable range.

After considering the physicochemical properties of 15 peel-off mask film formulations, the formulations F18 and F21 were chosen as the two most suitable formulations. Both were further evaluated for the interaction between the ingredients in the formulation, crystallinity, thermal behavior, and antimicrobial activity of the formulation against *C. acnes*.

FT-IR analysis

The FT-IR spectra (Figure 2A) showed that PVA and PVP-K30 presented the large band located at 3200-3500 cm^{-1} for the O-H stretch vibration. The fingerprint spectrum of PVA showed asymmetrical stretching vibration and symmetric stretching vibration of CH_2 occurred at 2932 and 2908 cm^{-1}, respectively. The coupling of the secondary O-H in-plane bending and the C-H wagging vibrations were observed at 1414 and 1326 cm^{-1}. The peak at 1085 cm^{-1} indicated C-O stretching vibration (Xiao *et al.,* 2006). The main spectrum of PVP-K30 showed the peak at 1661, 1420, 1371, and 1283 cm^{-1} attributed to the C=O symmetric stretching, CH_2 bending, O-H bending (in-plane), and C-H deformation, respectively (Abou_Taleb, 2009). The FT-IR spectrum of clindamycin showed characteristic peaks at 1695 cm^{-1} (C=O stretching), 1090 and

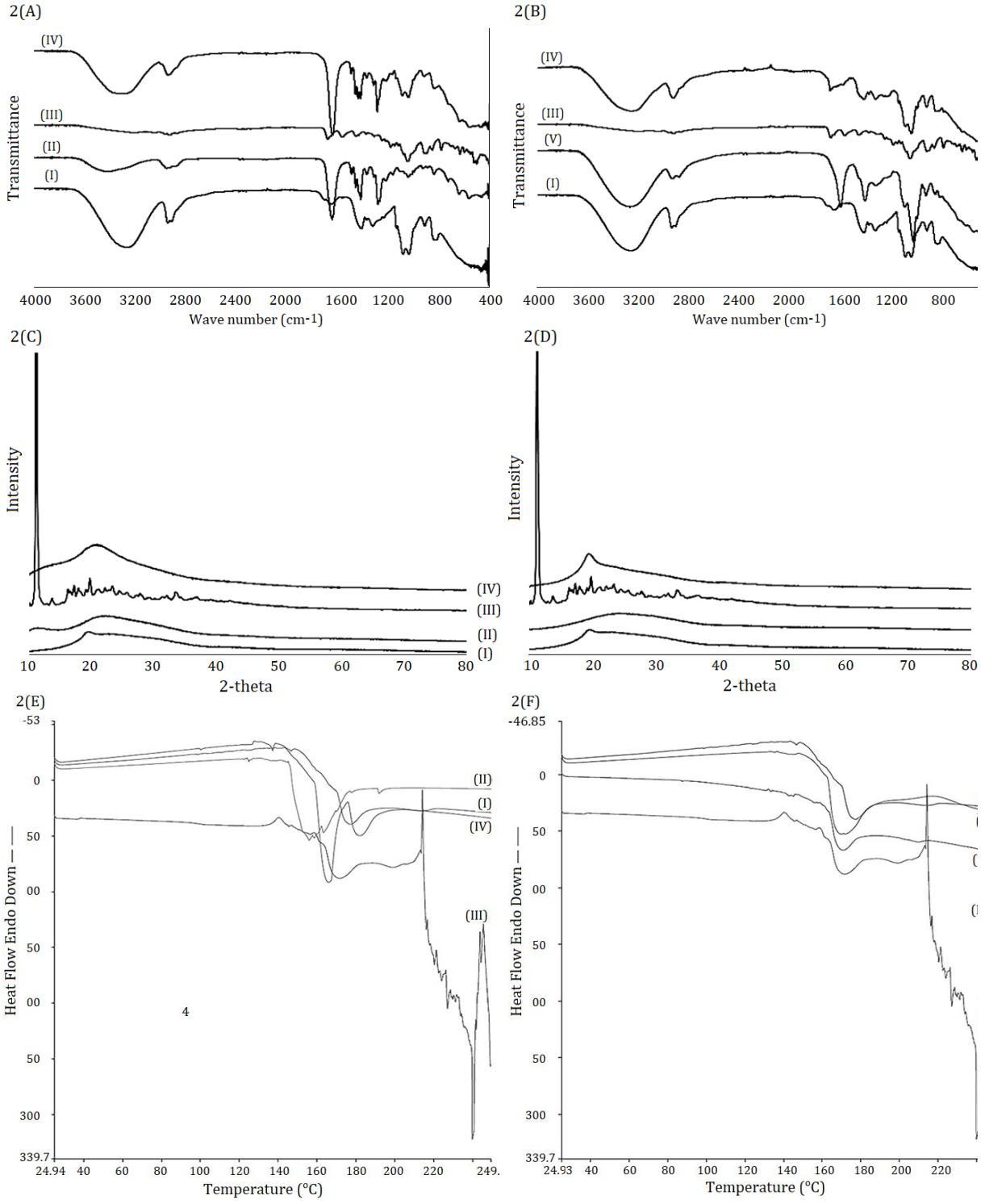

Figure 2. The FT-IR spectra (2A and 2B), XRD diffractograms (2C and 2D), and DSC thermograms (2E and 2F) of PVA (I), PVP-K30 (II), Clindamycin (III), Clindamycin peel-off mask film (PVA:PVP-K30, F18) (IV), Sodium alginate (V), and Clindamycin peel-off mask film (PVA:SA, F21) (VI)

1210 cm^{-1} (C-O stretching), 1561 cm^{-1} (C=C stretching), and 2940 cm^{-1} (C-H stretching) (Patel & Patel, 2015, Mohamed *et al.*, 2017). Peaks were observed at 1661, 1420, 1371, 1283, 1090, and 1085 cm^{-1} in the spectrum of the clindamycin peel-off mask film (PVA:PVP-K30, F18). These peaks were similar to those found in the spectrum of the starting materials, indicating that the active drug was incorporated into the film without changing the chemical structure. There was no interaction between the film composition. Meanwhile, the FT-IR spectra (Figure 2B) showed that sodium alginate gave a wide peak located at 3200-3500 cm^{-1} for the O-H stretch vibration and 2920–2850 cm^{-1} for stretching vibrations of aliphatic C–H. Observed bands in 1649 and 1460 cm^{-1} were attributed to asymmetric and symmetric stretching vibrations of carboxylate salt ions, respectively. The fingerprint band of the alginate structure at 1107 and 935 cm^{-1} was attributed to the C–O stretching vibration of pyranosyl ring and the C–O stretching with contributions from C–C–H and C–O–H deformation (Daemi & Barikani, 2012). The spectrum of the clindamycin peel-off mask film (PVA:SA, F21) showed the peaks at 1695, 1460, 1414, 1326, 1107, 1090, 1085, and 935 cm^{-1} that were similar to the peaks found in the spectrum of the starting materials, indicating no change in the chemical structure of the active drug and no interaction between the compositions in the film.

XRD analysis

XRD measurement is a versatile, non-destructive technique that helps reveal the crystallographic structure of materials and can be used to investigate the complex formation between the polymers. The XRD diffractogram of PVA showed a diffraction peak angle at 2θ = 19.8°C (Figure 2C), which was a strong and broad peak corresponding to the (1 0 1) reflection, a plane that contains the extended planar zig-zag chain direction of the crystallites (Gupta *et al.*, 2009, Sudirman *et al.*, 2020). XRD diffractogram of PVP-K30 exhibited amorphous features characterized by two halos centered at 2θ = 11.7°C and 20.2°C (Jaipakdee *et al.*, 2018). The XRD diffractogram of the clindamycin peel-off mask film (PVA:PVP-K30, F18) implied a decreased degree of crystallinity and showed amorphous patterns. In the meantime, the XRD diffractogram of the clindamycin powder presented the crystalline pattern. After incorporation into PVA:PVP-K30 film, the crystallinity nature of clindamycin In the film matrix was absent. It is possible that the active drug was embedded in the film matrix as a solution. The XRD diffraction pattern of SA showed an amorphous structure with a peak 2θ = 22.9° (Helmiyati & Aprilliza, 2017) (Figure 2D). The clindamycin peel-off mask film (PVA:SA, F21) presented the semi-crystalline peak of PVA. Similar to the F18 film, the crystallinity nature of the drug was absent after incorporating clindamycin.

DSC measurement

The DSC thermograms of PVA, PVP-K30, clindamycin, and clindamycin peel-off mask film (PVA:PVP-K30, F18) (Figure 2E). The DSC curve of clindamycin showed an endothermic melting peak at 170.5 °C. Another sharp endothermic peak at 243 °C and an exothermic peak at 210 °C were also observed (Tamaddon *et al.*, 2015). The DSC plot of PVA exhibited an endothermic melting peak at approximately 180°C and a second small endothermic peak presented at 210 °C, as previously reported by Andrade *et al.* (2020). The thermal behaviour of the PVP-K30 was as expected as hygroscopic substances, with a broad endothermal effect at about 160 °C due to polymer dehydration (Chan *et al.*, 2015). The DSC thermogram of the F18 film exhibited endothermic peaks like the related substances with a little shift to the higher temperatures. The large amounts of polymer in the F18 film covered the characteristic peaks of the clindamycin. Thus, no endothermic peak of clindamycin was detected in the DSC thermogram of the film. In the same way (Figure 2F) the DSC thermograms of PVA, SA, clindamycin, and clindamycin peel-off mask film (PVA:SA, F21). Sodium alginate exhibited one broad endothermic and exothermic peak at 169°C and 220°C, respectively. The first endothermic band corresponded to the evaporation of hydration water molecules, while the exothermic one indicated the oxidative degradation of alginate polymers (Pendekal & Tegginamat, 2013, Alkhatib *et al.*, 2006). The DSC thermogram of F21 film exhibited endothermic peaks similar to the related substances, and the characteristic peaks of the clindamycin were also covered due to the large amounts of polymer. The DSC thermograms showed no interaction between the ingredients in the peel-off mask formulations.

Table III. Antimicrobial susceptibility test for *C. acnes* by disc diffusion method

Formulation	Composition	Formulation type	Calculated clindamycin amount (μg) per sample	Inhibition zone diameter (mm)
F18	F10 : clindamycin (99:1 %w/w)	Film (0.6 cm diameter)	126 μg of clindamycin per 0.6 cm diameter film	56
F18-sol	Clindamycin dissolved in DI water	20 μL of samples dropped in the 0.6 cm test paper	126 μg of clindamycin per 20 μL solution	47.3
F18-blank	F10	Film (0.6 cm diameter)	-	0
F21	F15 : clindamycin (99:1 %w/w)	Film (0.6 cm diameter)	34 μg of clindamycin per 0.6 cm diameter film	53.7
F21-sol	Clindamycin dissolved in DI water	20 μL of samples dropped in the 0.6 cm test paper	34 μg of clindamycin per 20 μL solution	35.7
F21-blank	F15	Film (0.6 cm diameter)	-	0

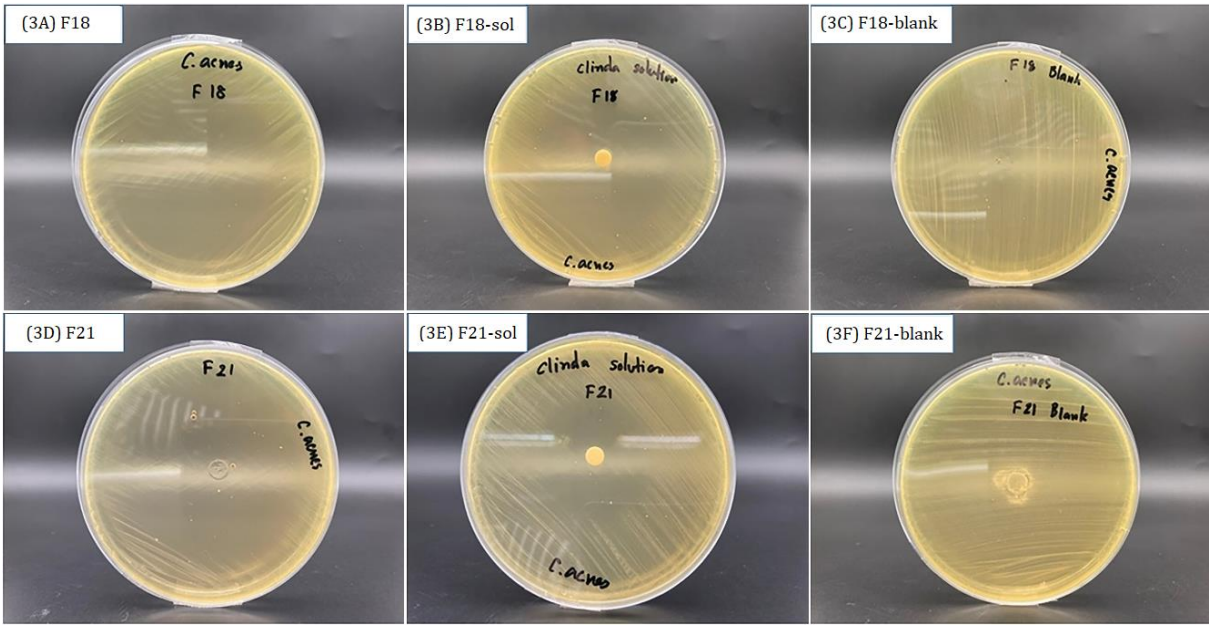

Figure 3. Photos of the agar disc diffusion method represent inhibition zones of C. acnes. F18 (3A) and F21 (3D) are clindamycin peel-off mask film (PVA:PVP-K30, F18) and clindamycin peel-off mask film (PVA:SA, F21), respectively. F18-sol (3B) and F21-sol (3E) are clindamycin solutions at equivalent clindamycin amount to the film. F18-blank (3C) and F21-blank (3F) are blank PVA:PVP-K30 film and PVA:SA film without clindamycin, respectively

Antimicrobial susceptibility test for *C. acnes*

Both clindamycin peel-off mask formulations F18 and F21 were tested for their antimicrobial properties against *C. acnes* using the disc diffusion method (Table III and Figure 3). The film formulations without clindamycin (F18-blank and F21-blank) showed no inhibition zone, indicating no antimicrobial properties. However, films containing 1 %w/w clindamycin having clindamycin amount per area of 0.89 mg/cm^2 showed large inhibition zones with diameters equal to 57.5 mm (for F18) and 59.7 mm (for F21).

Comparing the inhibition zone of films and solution containing the same amount of clindamycin per area (0.89 mg/cm^2), the inhibition zones from films had diameters that were nearly identical to the inhibition zone of the clindamycin solution.

In this experimental set, four different concentrations of clindamycin solutions (0.22, 0.45, 0.89, and 1.79 mg/cm^2) were used as positive control. The clindamycin concentration of 0.89 mg/cm^2 was chosen as an equal concentration of clindamycin in the F18 and F21 films. The rest of the concentrations (folds dilution from 0.89 mg/cm^2) were tested to observe the correlation between the inhibition zone size and the clindamycin concentration.

From the results, it was found that increasing clindamycin concentrations in the solution gave a larger inhibition zone. The inhibition zone obtained from clindamycin solution with a concentration of 0.22 mg/cm^2 was the smallest one (45.5 mm, Figure 3E). The clindamycin solution with a concentration of 1.79 mg/cm^2 had the largest inhibition zone (59.0 mm, see Figure 3H). However, the inhibition zone is not directly proportional to the clindamycin concentration, especially at high concentrations. The clindamycin solution at the concentration of 0.89 mg/cm^2 (56.7 mm, Figure 3G) gave an inhibition zone that was not considerably different from the clindamycin solution at the concentration of 1.79 mg/cm^2 (59.0 mm, Figure 3H).

Since the agar diffusion assay is based on the inhibition zone formation as a result of antimicrobial agent diffusion (Galvao *et al.*, 2016). Various parameters can affect the inhibition zone diameter, such as the diffusion rate, the drug extraction rate from the film (or test paper), the drug solubility in the agar, and the drug's molecular weight (Hudzicki, 2009). It is possible that very high clindamycin concentrations gave the maximum diffusion rate of clindamycin molecules in the agar during a certain incubation period. As a result, there is no difference in the inhibition zone at very high drug concentrations.

From the result above, it can be concluded that both F18 and F21 films could effectively release the clindamycin from the film to inhibit *C. acne* and were good candidates for the treatment of acne.

CONCLUSION

The clindamycin peel-off mask film prepared by blending PVA with PVP-K30 and SA showed a good to very good spreadability and film-forming ability. The increased amount of film-forming agents led to the increased viscosity and reduced spreadability of the gel. When combined with PVA, SA showed a higher viscosity-increasing potential than PVP-K30. The film had a suitable tensile strength that was neither too high to cause pain or skin irritation nor too low to cause ripped film. The data obtained from FT-IR , XRD, and DSC indicates no ingredient interaction and the amorphous pattern of the film matrix. The antimicrobial susceptibility for *C. acnes* demonstrated the superior ability of the mask film against *C. acnes* compared to the blank film and at the same level as the clindamycin solution. The developed clindamycin peel-off mask film offered the advantages of being easy-to-prepare, non-greasy, causing less skin irritation, and having high antimicrobial properties, leading to patient compliance and aesthetic appearance. Hence, it could be further developed as the new market formulation for acne treatment.

ACKNOWLEDGMENTS

This research project is supported by Mahidol University. The authors would like to thank Ms. Nanthawan Jinakul, Department of Microbiology, Faculty of Pharmacy, Mahidol University, for the antimicrobial assay.

REFERENCES

Abou_Taleb, M.H. (2009). Thermal and spectroscopic studies of poly(N-vinyl pyrrolidone)/poly(vinyl alcohol) blend films*. J. Appl. Polym. Sci, 114*(2), 1202-1207. https://doi.org/10.1002/app.30082

Alkhatib, H., Taha, M., Aiedeh, K., Bustanji, Y. & Sweileh, B. (2006). Synthesis and *in vitro* behavior of iron-crosslinked N-methyl and N-benzyl hydroxamated derivative of alginic acid as controlled release carriers. *Eur. Polym. J, 42*, 2464-2474. https://doi.org/10.1016/j.eurpolymj.2006.05.018

Andini, T., Yusriadi, Y. & Yuliet, Y. (2017). Optimization of film forming polyvinyl alcohol and propylene glycol humectant in pumpkin juice (*Cucurbita moschata Duchesne*) peel-off gel mask formula as antioxidants. *J. Farm. Galenika*, *3*(2), 165-173. https://doi.org/10.22487/j24428744.0.v0.i0.8773

Andrade, J., González-Martínez, C. & Chiralt, A. (2020). The incorporation of carvacrol into

poly (vinyl alcohol) films encapsulated in lecithin liposomes. *Polymers, 12*(497), 1-18. https://doi.org/10.3390/polym12020497

Asthana, N., Pal, K., Aljabali, A.A.A., Tambuwala, M.M. Souza, F.G. & Pandey, K. (2021). Polyvinyl alcohol (PVA) mixed green–clay and aloe vera based polymeric membrane optimization: peel-off mask formulation for skin care cosmeceuticals in green nanotechnology. *J. Mol. Struct, 1229*, 129592. https://doi.org/10.1016/j.molstruc.2020.129592

Beringhs, A.O.R., Rosa J.M., Stulzer H.K., Budal R.M. & Sonaglio D. (2013). Green clay and aloe vera peel-off facial masks: response surface methodology applied to the formulation design. *AAPS PharmSciTech, 14*(1), 445-455. http://doi.org/10.1208/s12249-013-9930-8

Chan, S.Y., Chung, Y.Y., Cheah, X.Z., Tan, E.Y.L. & Quah, J. (2015). The characterization and dissolution performances of spray dried solid dispersion of ketoprofen in hydrophilic carriers. *Asian J. Pharm Sci, 10*, 372-385. https://doi.org/10.1016/j.ajps.2015.04.003

Clinical and laboratory standards institute. (2020). *CLSI supplement M100 Performance standards for antimicrobial susceptibility testing* (30th ed.). Wayne, PA: Publisher.

Daemi, H. & Barikani, M. (2012). Synthesis and characterization of calcium alginate nanoparticles, sodium homopoly mannuronate salt and its calcium nanoparticles. *Sci. Iran, 19*, 2023-2028. https://doi.org/10.1016/j.scient.2012.10.005

Galvao, G.V., Saviano, A.M. & Lourenco, F.R. (2016). Reduced incubation time for inhibition zone formation based on diffusion and growth mechanism elucidation. *Anal. Methods, 8*, 3885-3891. https://doi.org/10.1039/C6AY00611F

Gloor, M. & Bauer, W. (1979). The facial mask as a basis of clindamycin therapy for acne vulgaris. *Hautarzt*, *30*(7), 380-383.

Gupta, S., Pramanik, A.K., Kailath, A., Mishra, T., Guha, A., Nayar, S. & Sinha, A. (2009). Composition dependent structural modulations in transparent poly(vinyl alcohol) hydrogels. *Colloids Surf. B, 74*, 186-190. https://doi.org/10.1016/j.colsurfb.2009.07.015

Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. *American Society for Microbiology*. https://asm.org/Protocols/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Pro

Hariyadi, D.M., Isnaeni, I., Sudarma, S., Suciati, S. & Rosita, N. (2020). Peel-off emulgel mask of *Cocos nucifera* L. extract using gelling agent carbomer 940 as antiacne against *Propionibacterium acnes* ATCC 11827. *J. Adv. Pharm. Technol. Res, 11*(4), 220-225. https://doi.org/10.4103/japtr.JAPTR_51_20

Helmiyati. & Aprilliza, M. (2017). Characterization and properties of sodium alginate from brown algae used as an ecofriendly superabsorbent. *IOP Conf. Ser. Mater. Sci. Eng, 188*, 012019. https://doi.org/10.1088/1757-899X/188/1/012019

Hendrawati, T.Y., Nugrahani, R.A., Utomo, S. & Ramadhan, A.I. (2018). Formulation process making of aloe vera mask with variable percentage of aloe vera gel extract. *IOP Conf. Ser. Mater. Sci. Eng, 403,* 012013. http://doi.org/10.1088/1757-899X/403/1/012013

Jaipakdee, N., Pongjanyakul, T. & Limpongsa, E. (2018). Preparation and characterization of poly (vinyl alcohol)-poly (vinyl pyrrolidone) mucoadhesive buccal patches for delivery of lidocaine HCl. *Int. J. Appl. Pharm, 10*(1), 115-123.https://doi.org/10.22159/ijap.2018v10i1.23208

Mohamed, A.I., Abd-Motagaly, A.M., Ahmed, O.A.A., Amin, S. & Mohamed Ali A.I. (2017). Investigation of drug–polymer compatibility using chemometric-assisted UV-spectrophotometry. *Pharmaceutics, 9*(7), 1-13. https://doi.org/10.3390/pharmaceutics9010007

Nursal, F.K., Nining. & Rahmani, A. (2021). Effect of glycerin as plasticizer in formulation of grape seed oil (*Vitis vinifera* L.) emulgel peel-off mask. *IOP Conf. Ser. Earth Environ. Sci, 819*(1),012058. https://doi.org/10.1088/1755-1315/819/1/012058

Patel, P. & Patel, P. (2015). Formulation and evaluation of clindamycin HCl in situgel for vaginal application. *Int. J. Pharm. Investig, 5*(1), 50-56. https://doi.org/10.4103/2230-973X.147233

Pichayakorn, W., Suksaeree, J., Boonme, P., Taweepreda, W. & Ritthidej, G.C. (2012). Preparation of deproteinized natural rubber latex and properties of films formed by itself and several adhesive polymer blends. *Ind. Eng. Chem. Res, 51*, 3393−13404. https://doi.org/10.1021/ie301985y

Pendekal, M.S. & Tegginamat, P.K. (2013). Hybrid drug delivery system for oropharyngeal, cervical and colorectal cancer - *in vitro* and *in vivo* evaluation. *Saudi. Pharm. J, 21*(2), 177-186. https://doi.org/10.1016/j.jsps.2012.07.002

Rahmasari, D., Ermawati, D., Nugraheni, R., Putri, D.R. & Pratiwi, I. (2019). Design and development of peel-off mask gel formulation of citronella oil for acne vulgaris. *Proceedings of the 2nd Health Science International Conference (HSIC 2019),* 157-163. https://doi.org/10.5220/0009126101570163

Rosaini, H., Makmur, I., Lestari, E.A., Sidoretno, W.M. & Yetti, R.D. (2021). Formulation of gel peel fff catechins mask from gambir (*Uncaria gambir* (Hunter) Roxb) with the PVP K-30 concentration variation. *IJRR, 8*(3), 205-211.

Roselyne, M., Marc, P. & Oliver, D. (2014). Film forming complex, cosmetic composition comprising it and their use as peel-off film mask, European Patent 2979685A1.

Singh, S., Chunglok, W., Nwabor, O.F., Ushir, Y.V., Singh, S. & Panpipat, W. (2022). Hydrophilic biopolymer matrix antibacterial peel-off facial mask functionalized with biogenic nanostructured material for cosmeceutical applications. *J. Polym. Environ, 30*, 938–953. https://doi.org/10.1007/s10924-021-02249-5

Sudirman, S., Karo, A.K., Sukaryo, S.G., Adistiana, K.D. & Dahlan, K. (2020). Synthesis of nanofiber from polyvinyl alcohol (PVA)-collagen using electrospinning methods. *J.Kim.Terap. Indones, 21*(2), 55-65. https://doi.org/10.14203/jkti.v21i2.428

Sun, Q., Ren, S.Y. & Ni, G.G. (2020). Incidence ofmicroplastics in personal care products: An appreciable part of plastic pollution. *Sci. Total Environ, 742*, 140218. https://doi.org/10.1016/j.scitotenv.2020.140218

Tamaddon, L., Mostafavi, S.A., Karkhane, R., Esfahani, M.R., Dorkoosh, F.A. & Tehrani, M.R. (2015). Thermoanalytical characterization of clindamycin-loaded intravitreal implants prepared by hot melt extrusion. *Adv. Biomed. Res, 4*(147), 1-9. https://doi.org/10.4103/2277-9175.161563

Teodorescu, M. & Bercea, M. (2015). Poly(vinylpyrrolidone) – a versatile polymer for biomedical and beyond medical applications. *Polym. Plast. Technol. Eng*, *54*(9), 923-943. https://doi.org/10.1080/03602559.2014.979506

Vieira, R.P., Fernandes, A.R., Kaneko, T.M., Consiglieri, V.O., Pinto, C.A.S., Pereira, C.S.C., Bavy, A.R. & Velasco, M.V.R. (2009). Physical and physicochemical stability evaluation of cosmetic formulations containing soybean extract fermented by *Bifidobacterium animalis*. *Braz. J. Pharm. Sci, 45*(3), 515-525.

Wetchakun, C., Puapermpoonsiri, U. & Sila-on, W. (2016). Effect of alcohol and co-film former on the physical and mechanical properties of facial mask formulations. *IJPS*, *11* (Supplement),25-32.

Xiao, S., Huang, R.Y.M. & Feng, X. (2006). Preparation and properties of trimesoyl chloride crosslinked poly(vinyl alcohol) membranes for pervaporation dehydration of isopropanol. *J. Membr. Sci, 286*(1), 245-254. https://doi.org/10.1016/j.memsci.2006.09.042

Zaenglein, A.L., Pathy, A.L., Schlosser, B.J., Stern, M., Boyer, K.M. & Bhushan, R. (2016). Guidelines of care for the management of acne vulgaris. *J. Am. Acad. Dermatol, 74*(5), 945-973. https://doi.org/10.1016/j.jaad.2015.12.037