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FORMULATION OF AEROSOL CONCENTRATES CONTAINING *HARUAN* (*Channa striatus*) FOR WOUND DRESSING

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The objective of this research was to formulate an aerosol concentrate containing haruan (Channa striatus) *water extract that would produce a thin film when sprayed onto a wound and could be used for wound dressing. The aerosol concentrates were formulated with various polymer and plasticiser mixtures and tested in dispersion systems. The polymers evaluated were hydroxypropyl methylcellulose (HPMC), carboxymethylcellulose sodium (CMC Sodium), acacia, tragacanth, chitosan, gelatine and gelatine (bloom 151–160), all at concentrations of 2%. The plasticisers evaluated were polyethylene glycol (PEG) 400 and 4000, glycerine, propylene glycol, and triacetin. Films were prepared from film-forming dispersions by casting techniques. Film-forming dispersions were characterised in terms of pH, density, surface tension, rheological properties, particle size distribution, and tackiness. Based on these evaluations, HPMC was chosen as the best polymer. It produced a film with the expected qualities and was easy to reproduce in the form of dispersions or as thin transparent films. Glycerine was judged as the most appropriate plasticiser because it produced the concentrate having the desired qualities and properties expected from an aerosol concentrate*

Keywords: Haruan, Channa striatus, *Aerosol, Wound dressing*

INTRODUCTION

Traditionally, wound treatment has been carried out by letting the wound dry and create a scab by itself. For the past thirty years or so, wound treatment has undergone changes. New techniques were introduced, one of which includes the use of artificial dressings to cover the wound. The dressing maintains the wound's humidity at a high level, thus stimulating the epidermis to reproduce faster. Even though an ideal wound dressing is the natural skin, artificial wound dressings are being developed from polymers to produce fibres and threads; they contain 85% water and are permeable to air (Turner 1991; Jones and Vaughan 2005; Santos *et al*. 2006). A majority of wound dressings such as plasters and bandages can cause suffering and pain to the patient during the dressing and removal processes. It is possible to minimise these discomforts, especially during

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the dressing process, by spraying a dressing concentrate that would form a film on the wound.

Although a number of spray products or aerosols for wound dressing are currently available in the market, we undertook this project to develop and formulate an aerosol concentrate that contains a mixture of *haruan* extract and a film-forming polymer. The concentrate is expected to form a thin layer of dressing when sprayed onto the wound, and the added *haruan* extract is expected to enhance the healing process (Baie and Sheikh 2000a, 2000b). *Haruan* (*Channa striatus*) is a freshwater, air-breathing and carnivorous fish that is widely consumed in Malaysia and other Southeast Asian countries due to its high source of protein and also for its putative effects on wound healing (Mat Jais, Dambisya and Lee 1997; Dambisya *et al.* 1999; Zakaria *et al.* 2004). The amino acid and fatty acid composition of *haruan*, which can enhance wound healing, has been reported by Mat Jais, McCulloch and Croft (1994). The formulated concentrate that produces a layer of dressing on the wound was expected to have all the advantages of spray dressings, which include causing less pain and discomfort, ability to cover a wound of various sizes and preventing microbial infection.

METHODS

Materials

Haruan extract was obtained from Major Interest (M) Sdn. Bhd. (Malaysia). HPMC, gelatine, triacetin, and α-tocopherol (Vitamin E) were purchased from Sigma Chemical Co. (St. Louis, USA). CMC Sodium was supplied by Metsa-Serla (Sweden). Gelatine bloom 151–160 was obtained from Halagel Sdn. Bhd. Sungai Petani (Kedah, Malaysia). Acacia was acquired from BHD Laboratory Supplies (England). Chitosan low molecular weight was from Fluka Biochemika (Switzerland). PEG 400 and PEG 4000 were obtained from Merck-Schuchardt. Propylene glycol, tragacanth, glycerine, methyl paraben, and propyl paraben were purchased from R&M Chemicals, Essex (U.K.). Ethanol was from Systerm AR. All chemicals were used without further purification.

Polymer Selection

To choose the best polymer (Table 1) to formulate an aerosol concentrate that would produce a film to cover wounds, the films were prepared by a casting technique and evaluated qualitatively based on the ease of preparation and physical appearance, as reported by Pongjanyakul and Puttipipatkhachorn (2008). First, 2% of the dispersed polymer mixtures were prepared by dissolving 2 g of a polymer in distilled water (except for chitosan, which was dissolved in 2% lactic acid solution) to produce 100 g dispersions. The dispersions were continuously mixed for 24 h by using a magnetic stirrer at 25±1˚C (room temperature). Each dispersion (10 g) was poured into a 9.5-cm-diameter petri dish and left to dry at room temperature for 48 h to form a film. The films were peeled off and stored at 25±1 ˚C and 50%—60% humidity until evaluation (Yoo, Dharmala and Lee 2006).

Plasticiser Selection

The plasticisers to be evaluated were PEG 400 and PEG 4000, glycerine, propylene glycol, and triacetin. Other excipients like methyl paraben (0.1%) and propyl paraben (0.02%) were used as preservatives, α-tocopherol (0.01%) as antioxidant, and ethanol and distilled water as solvents. Methyl paraben (100 mg), propyl paraben (20 mg) and α-tocopherol (10 mg) were dissolved in 20 g ethanol. The solutions formed were mixed separately with the selected polymer (2 g), plasticiser (1 g) and distilled water until 100 g of dispersion was obtained. Each dispersion was homogenised by using ultra turrax (IKA T 18 Basic) at 10,000 rpm for 15 min. Each dispersion (10 g) was poured into a petri dish (diameter: 9.5 cm) and dried at room temperature (25±1˚C) for 48 h. The films produced were peeled off and stored at 25±1˚C and 50%—60% relative humidity until evaluation (Yoo, Dharmala and Lee 2006). The films were qualitatively evaluated based on ease of preparation and their physical appearance. Polymer and plasticiser mixtures that formed the films having the expected properties were selected for use in aerosol concentrate formulations and evaluation.

Formulation of Aerosol Concentrate

Specified quantities of methyl paraben (0.1 g), propyl paraben (0.02 g), and α-tocopherol (0.01 g) were dissolved in 20 g ethanol. The polymer

(2 g), plasticiser (1 g) and *haruan* extract (20 g) were added and dispersed into the solution and mixed. The weights of the dispersions were made up to 100 g with the addition of ethanol. The dispersions were homogenised by using ultra turrax (IKA T18 Basic) at 10,000 rpm for 15 min. Density, pH, surface tension, particle size, rheological properties and tack of the dispersions or concentrates were evaluated as described below. Homogenised dispersions or concentrates (10 g) were spread onto 9.5-cm-diameter petri dishes and dried at room temperature for 24 h. The dry films were peeled off and stored at room temperature and 50%–60% relative humidity until evaluation (Yoo, Dharmala and Lee 2006).

Evaluations of Aerosol Concentrate

Measurement of pH and density

The pH of each concentrate or dispersion was measured with a pH meter (Corning Scientific Products, New York). The density of each concentrate or dispersion was measured at room temperature (25±1˚C) by using a pycnometer (British Pharmacopoeia 2007).

Determination of surface tension

The surface tension of each concentrate or dispersion was determined by using a Du Nouy Torsion Balance (Model OS, White Elect. Inst. Co. Ltd, England) at 25±1˚C (Martin, Bustamante and Chun 2001).

Evaluation of rheological properties

The rheological properties of each aerosol concentrate were determined at room temperature 25±1˚C by using a rheometer (Reologica Instrument AB version 5.0.40.38) equipped with a concentric cylinder CC 25 measuring system. About 16 ml of each concentrate sample was carefully poured into the cylinder. The flow profile and viscosity of each aerosol concentrate were determined at the following settings: stress from 1–10 Pa, delay time 5.000 s, and integration time 2.000 s.

Determination of particle size distribution

The particle size distribution of each concentrate or dispersion was determined by using a Mastersizer S V2.19 (Malvern Instruments Ltd.,

UK) fitted with a small sample dispersion unit (MS1) connected to a dispersion unit controller. A beam length of 2.4 mm and 300 RF lens (range 0.05–900 μm) was used. Concentrates were loaded into the small sample dispersion unit and stirred at a speed of 800 rpm until an obscuration value between 12%–17% was obtained. Before each sample was run, the system was aligned, and a background measurement was taken with distilled water, which was used as the dispersing solvent. The determination was carried out in triplicate for each sample.

Tack measurement

The method that we used has been previously reported by others (Wan and Lai 1992a, 1992b; Baie, Al-Fadzol and Khan 2004, and Heng, Wan and Tan 1996), using a texture analyser type TA-XT2 (TA, England). Two millilitres of the sample was placed into a petri dish with an internal diameter of 9 cm and kept in a horizontal position with a metallic clamp. The probe was mounted vertically on the load test shaft of the tensile tester. During the operation of the test, the probe was lowered vertically and brought into contact with the liquid film in the petri dish and allowed to rest against the film for 30 s. The probe was then raised at 5.0 mm/s rate of separation. The maximum force generated during separation was recorded. Six replicate determinations were carried out. The sample was free of air bubbles.

Statistical Analysis

The results were represented as mean ± SD and treated statistically by using SPSS software (version 13, USA). One-way analysis of variance was used to compare the results. When there was a statistically significant difference ($P < 0.05$), the post-hoc Tukey Honestly Significant Difference (Tukey-HSD) test was applied (Khan, Peh and Ch'ng 2000; Petersen 1985).

RESULTS AND DISCUSSION

Polymers Selection

A polymer film for wound dressing should be transparent (Heng, Wan and Tan 1996), so that the wound can be monitored (Balakrishnan *et al.* 2005). Thus, one of the criteria for selecting a suitable polymer was the

transparency of the film produced. Based on the results shown in Table 1, HPMC and CMC Sodium were found to be easily formulated and produced films that were transparent, free from sediments and air bubbles and were thus selected for use in the subsequent studies.

Table 1: Properties of films produced from various polymers.

Polymer used	Physical properties of the film formed	Appearance of the film formed	Physical changes after storage at 25±1^{0}C and 50%–60% relative humidity for one week
HPMC	Could be peeled off easily from the plate	Transparent, soft and tough, flexible and pliable, smooth surface and free from any precipitation or air pockets	No change in colour and texture
CMC Sodium	Could be peeled off easily from the plate	Transparent, soft and tough, flexible and pliable, smooth surface and free from any precipitation or air pockets	No change in colour and texture
Acacia	Difficult to recover from the plate	Transparent, brittle	No change in colour and texture
Tragacanth	Difficult to recover from the plate, sticky	Brown, rough	No change in colour and texture
Chitosan	Sticky, soft, and difficult to recover from the plate	Transparent, light yellow, soft, smooth surface and free from any precipitation or air pockets	Light yellow, no change in colour and texture
Gelatine (bloom 151–160)	Sticky, soft, and difficult to recover from the plate	Transparent, soft, smooth surface and free from any precipitation or air pockets	No change in colour and texture
Gelatine	Difficult to recover from the plate	Transparent, brittle	No change in colour and texture

Plasticiser Selection

Plasticiser is required for producing a good film. It can make the polymer film flawless by reducing the polymer's fragility and improving the polymer's flexibility, toughness, strength, tear resistance, and impact resistance (Wu and McGinity 1999). Formulae B1, B2, B3, B4, and B5 (Table 2) used HPMC (2%) as polymer and PEG 400, PEG 4000, glycerine, propylene glycol, and triacetin, respectively, as plasticiser at 1% concentration. Formulae C1, C2, C3, C4, and C5 used CMC Sodium (2%) as polymer and PEG 400, PEG 4000, glycerine, propylene glycol, and triacetin, respectively, as plasticiser, also at 1% concentration (Table 2). Each of the above formulae also contained methyl paraben (0.1%), propyl paraben (0.02%), α-tocopherol (0.01%) and ethanol (20%). Distilled water was used as a solvent. The properties of the films, which include the strength, transparency, toughness and stability, are presented in Table 2. PEG 4000 produced a non- homogeneous film (B2 and C2) and therefore was not used in subsequent studies. Triacetin produced an opaque but homogeneous film (B5 and C5). Even though the films produced were not transparent, triacetin was used in subsequent studies. Good films were produced when PEG 400, glycerine and propylene glycol were used as plasticiser; thus, these plasticisers were selected for use in subsequent studies.

Formulation of Aerosol Concentrate

The results of visual evaluation of the aerosol concentrates and films prepared with *haruan* extract, the selected polymer (D = HPMC or E = CMC Sodium) and the selected plasticiser (1 = PEG 400; 2 = glycerine; 3 = propylene glycol; or 4 = triacetin) are tabulated and presented in Table 3.

Table 3 shows that all dispersions containing CMC Sodium as polymer were physically unstable; they rapidly separated into two layers and were not used in subsequent evaluations. Films of both polymers that contained triacetin as plasticiser were opaque and not transparent; they contained some sediment and were difficult to peel off from the petri dish. Triacetin was excluded from the subsequent study. The final three formulae that were chosen for final evaluation were D1, D2, and D3.

Table 2: Effects of various plasticisers on the properties of HPMC and CMC Sodium films.

Formula	Physical properties of the film formed	Appearance of the film formed	Physical changes after storage at 25±1^{0}C and 50%–60% relative humidity for one week
B1	Could be peeled off easily from the plate	Transparent, soft and tough, flexible and pliable, smooth surface and free from any precipitation or air pockets	No change in colour and texture
B2	Could be peeled off easily from the plate	There were some sediments, not homogeneous	No change in colour and texture
B3	Could be peeled off easily from the plate	Transparent, soft and tough, flexible and pliable, smooth surface and free from any precipitation or air pockets	No change in colour and texture
B4	Could be peeled off easily from the plate	Transparent, soft and tough, flexible and pliable, smooth surface and free from any precipitation or air pockets	No change in colour and texture
B5	Could be peeled off easily from the plate	Opaque film (not transparent), smooth surface and free from air pockets	No change in colour and texture
C1	Could be peeled off easily from the plate	Light yellow, soft and tough, flexible and pliable, oily surface and free from any precipitation or air pockets	Light yellow, no change in colour and texture
C2	Could be peeled off easily from the plate	There were some sediments, not homogeneous	No change in colour and texture
C3	Could be peeled off easily from the plate	Light yellow, soft and tough, flexible and pliable, rough surface and free from any precipitation or air pockets	Light yellow, no change in colour and texture
C4	Could be peeled off easily from the plate	Transparent, smooth surface and free from any precipitation or air pockets	No change in colour and texture
C5	Could be peeled off easily from the plate	Opaque film (not transparent), smooth surface and free from air pockets	No change in colour and texture

Note: B = HPMC; C = CMC Sodium; 1 = PEG 400; 2 = PEG 4000; 3 = glycerine; 4 = propylene glycol; 5 = triacetin

Table 3: Visual evaluation of the concentrates and films containing *haruan* extract, polymer (D = HPMC; E = CMC Sodium) and plasticiser (1 = PEG 400; 2 = glycerine; 3 = propylene glycol; 4 = triacetin).

Formula	Physical appearance of aerosol concentrate	Physical properties of film formed	Appearance of the film formed	Physical state after storage at 25±1^{0}C and 50%–60% relative humidity for one week
D1	Homogeneous milky white dispersion, not dissevered while storing	Could be peeled off easily from the plate	Transparent, soft and tough, flexible and pliable film with smooth surface and free from any precipitation or air pockets	No change in colour and texture
D2	Homogeneous milky white dispersion, not dissevered while storing	Could be peeled off easily from the plate	Transparent, soft and tough, flexible and pliable film with smooth surface and free from any precipitation or air pockets	No change in colour and texture
D3	Homogeneous milky white dispersion, not dissevered while storing	Could be peeled off easily from the plate	Transparent, soft and tough, flexible and pliable film with smooth surface and free from any precipitation or air pockets	No change in colour and texture
D4	Homogeneous milky white dispersion, not dissevered while storing	Difficult to peel from plate, torn, some film stuck to the plate	Opaque film, there was sedimentation, not homogeneous	No change in colour and texture
E1	Dispersion system separated into two layers, above layer clear, bottom layer milky white	Difficult to peel from plate, torn	Opaque film, not transparent	No change in colour and texture

(continued on next page)

Table 3: *(continued)*

Formula	Physical appearance of aerosol concentrate	Physical properties of film formed	Appearance of the film formed	Physical state after storage at 25±1°C and 50%–60% relative humidity for one week
E2	Dispersion system separated into two layers, top layer clear, bottom layer milky white	Difficult to peel from plate, films were glued to each other	Opaque film, not transparent	No change in colour and texture
E3	Dispersion system separated into two layers, top layer clear and bottom layer milky white	Difficult to peel from plate, torn	Opaque film, not transparent	No change in colour and texture
E4	Dispersion system separated into two layers, top layer clear and bottom layer milky white	Difficult to peel from plate, torn	Opaque film, not transparent	No change in colour and texture

Evaluations of Concentrate for Aerosol

Measurement of pH, density and surface tension

The aerosol concentrates are meant for wound dressing, so their pH value should be as neutral as possible so as to minimise pain and irritation. The pH values of the dispersions of the three evaluated formulae, D1, D2 and D3, were close to neutral pH (Table 4). The pH value of D1 formula was significantly different from those of D2 and D3 formulae ($p < 0.05$), whereas the pH values of D2 and D3 were not significantly different ($p > 0.05$). The density of the D1 dispersion, which contained PEG 400 as the plasticiser, was significantly higher than the densities of the D2 and D3 dispersions (Table 4). The densities of D2 and D3 were not significantly different ($p > 0.05$). The surface tension of the D2 formula was significantly lower compared to the other two formulae ($p < 0.05$). Surface tensions of D1 and D3 were not significantly different ($p > 0.05$). Theoretically, the concentrate with pH close to neutral, having the lowest surface tension and the lowest density should be the most ideal candidate for aerosol delivery. D2 appeared to fulfil these criteria.

Evaluation of rheological properties

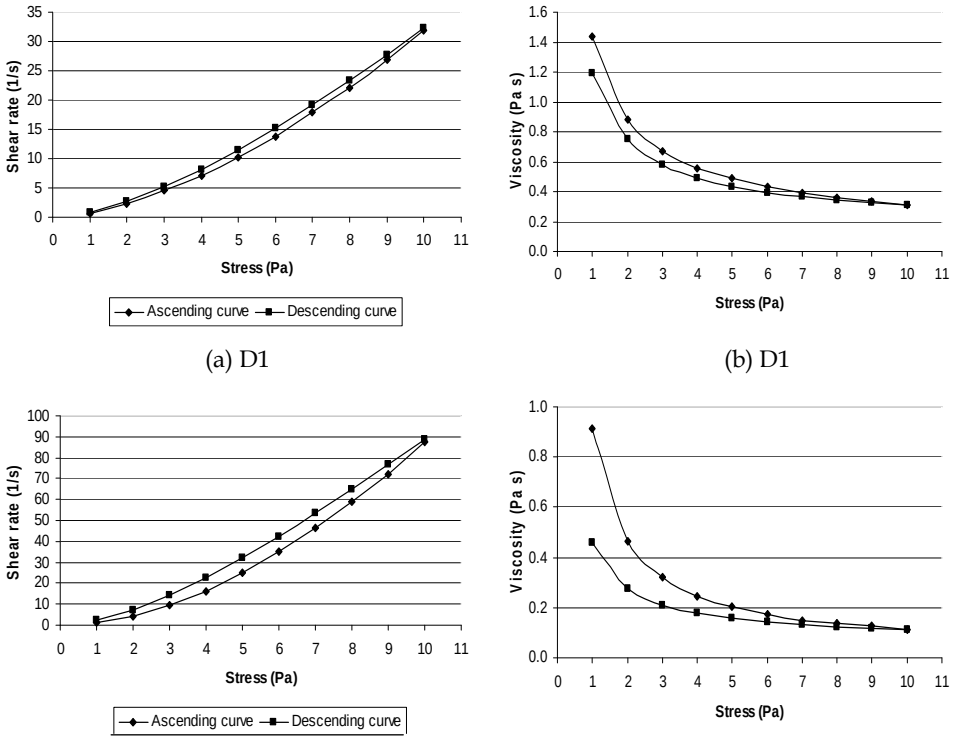
Figure 1 shows rheograms describing the relationship between (a) the shear stress with shear rate and (b) shear stress with viscosity, for the D1, D2, and D3 dispersions. Based on Figures 1(a) D1, 1(a) D2, and 1(a) D3, it can be inferred that D1, D2, and D3 dispersions containing HPMC had a pseudoplastic with tixotropy type of flow (Martin, Bustamante and Chun 2001): the viscosity of the dispersion decreased when a force was applied and did not easily return to the previous state when the external force was

Table 4: Result of pH, density and surface tension measurements of the final three concentrates for aerosol containing *haruan* extract, HPMC and plasticiser. (D1 = PEG 400; D2 = glycerine; D3 = propylene glycol. Mean ± SD, N = 3)

Formula	pH	Density (g/ml)	Surface tension (dynes/cm)
D1	$7.86^{a} \pm 0.07$	$0.86003^{b} \pm 0.00013$	$31.67^{b} \pm 0.29$
D2	$7.99^{b} \pm 0.02$	$0.85594^{a} \pm 0.00005$	$27.83^{a} \pm 0.76$
D3	$7.98^{b} \pm 0.03$	$0.85609^{a} \pm 0.00003$	$31.83^{b} \pm 0.29$

Note: Means within a column with a different letter are significantly different ($P < 0.05$)

reduced. Figures 1(b) D1, 1(b) D2, and 1(b) D3 show that the change in viscosity values along the declining curve was less when compared to the magnitude of the change for the upward curves. Pseudoplastic-tixotropy is the best flow property for a dispersed fluid system (Martin, Bustamante and Chun 2001). Viscosities for D2 and D3 were lower than for D1. Concentrates with lower viscosity are preferred because they are easier to be sprayed than those having a higher viscosity.



(a) D1 (b) D1

(a) D2 (b) D2

Fig. 1: Rheograms showing the relationship between (a) shear stress with shear rate and (b) shear stress with viscosity for dispersions of concentrates D1, D2 and D3.

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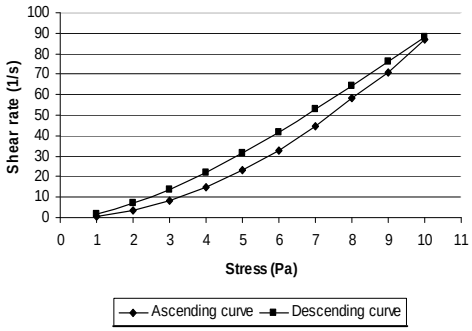
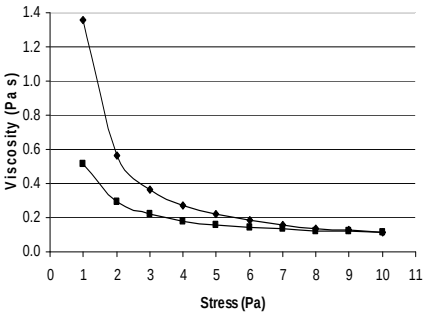


(a) D3



(b) D3

Fig. 1: *(continued)*

Determination of particle size distribution

Table 5 shows the results of particle size analysis for concentrates or dispersions D1, D2 and D3, measured by a Mastersizer S V2.19, Malvern Instruments Ltd., UK.

Topical sprays should have a mean particle size of about 100 μm (Sciarra 1996). The results in Table 5 show that the D2 dispersion had the smallest volume, mean diameter and smallest width of distribution compared to other formulae, indicating that D2 consisted of the smallest and the most uniform particles compared to D1 and D3. A small particle size is an expected property for dispersion of concentrate for aerosols, to enable them to pass through the small orifice of an aerosol valve.

Tack measurement

Tack value is used to determine the force or energy required to separate two surfaces that are initially in contact through a thin liquid film (Baie, Al-Fadzol and Khan 2004). Tackiness is commonly determined by using a probe tack test (Werner, Jones and Paterson 2007), which has been used to measure the tackiness of coating solutions (Wan and Lai 1992b; Baie, Al-Fadzol and Khan 2004). Tackiness of the aerosol concentrates containing different plasticisers was measured because the concentrate is expected to function similarly to a coating solution, i.e., to coat the surface of a wound. The results of tack measurements for D1, D2 and D3 dispersions are shown in Table 6.

The data show that D2 (with glycerine as a plasticiser) had the highest tack value compared to the other two formulae. The tack force value should

Table 5: Particle size distribution for dispersion D1, D2 and D3, Mean ± SD, N = 3.

Parameters	D1	D2	D3
Volume mean diameter (μm)	$45.90^{b} \pm 3.96$	$33.58^{a} \pm 2.21$	$52.85^{b} \pm 3.25$
Surface area mean diameter (μm)	$5.14^{a} \pm 0.60$	$13.54^{c} \pm 0.34$	$6.27^{b} \pm 0.15$
Mass median diameter (μm)	$13.97^{a} \pm 0.35$	$17.45^{b} \pm 0.54$	$22.81^{c} \pm 1.14$
Specific surface area (m^2/g)	$1.18^{c} \pm 0.15$	$0.44^{a} \pm 0.01$	$0.96^{b} \pm 0.02$
Width of the distribution	$9.91^{c} \pm 0.52$	$3.47^{a} \pm 1.91$	$6.10^{b} \pm 0.09$

Note: Means within a row with a different letter are significantly different ($P < 0.05$)

Table 6: Tack force values for concentrates D1, D2, and D3 measured at 25±1˚C, Mean ± SD, N = 6.

Formula	Tack force (mN/mm^2)
D1	$1.9626^{a} \pm 0.4223$
D2	$3.2007^{b} \pm 0.4187$
D3	$2.2764^{a} \pm 0.4032$

Note: Means within a column with a different letter are significantly different ($P < 0.05$)

be quite high, so that the concentrate for aerosol can stick onto the wound surface, dry up and produce a thin film that cannot peel off easily from the skin surface or wound.

CONCLUSION

HPMC was found to be the best polymer to produce the concentrate for aerosol and films with the expected qualities. Glycerine was found to be the most appropriate plasticiser. The chosen polymer and plasticiser were easily formulated as concentrates for aerosol, and they have the required qualities and properties. The films produced from their mixture also possessed the ideal properties of films suitable for wound dressing.

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