Journal of Pharmaceutical Innovation
https://doi.org/10.1007/s12247-021-09593-y

ORIGINAL ARTICLE

Dissolving Microneedle Formulation of Ceftriaxone: Effect of Polymer Concentrations on Characterisation and Ex Vivo Permeation Study

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Accepted: 7 October 2021

Abstract

Purpose This study aimed to develop dissolving microneedle preparations containing ceftriaxone and evaluate the effect of polymer concentrations on characterisation and ex vivo permeation study.

Methods The microneedles were prepared using centrifugation method with different ratios of PVP40% and PVA15%, namely FB1 (50:50), FB2 (60:40), and FB3 (70:30). The microneedles were also prepared with various concentrations of ceftriaxone, namely 10% w/w, 15% w/w, and 20% w/w. The microneedles were evaluated for their mechanical properties and ex vivo permeation profiles.

Results The results showed that the microneedles were in the form of sharp pyramid with size ranging between 712 and 800 μm. The concentration of ceftriaxone was found to affect the mechanical properties of the microneedles where formulations containing 20% w/w of ceftriaxone could not be produced. To achieve high drug loading, 15% w/w of ceftriaxone was selected in this study. Following the microneedle preparations, the formulation containing 15% w/w of ceftriaxone with FB3 polymer mixture exhibited the best mechanical properties with only 12.96 ± 0.56% height reduction after the compression with 30 N pressure. Moreover, this formulation was able to penetrate 4 layers of Parafilm® layers, indicating adequate insertion properties. Importantly, the microneedles were found to dissolve completely after 10 min on the full thickness rat skin. The permeation study showed that the greater the concentration of PVA, the slower the permeation of ceftriaxone from the microneedles. The highest drug permeation was obtained by containing 15% w/w of ceftriaxone with PVP40%:PVA15% with the ratio of 70:30, with the percentage of 99.86 ± 7.6%. The stability studies showed that the microneedles were stable for 1 month in two humidity levels, namely RH 43% and RH 86%.

Conclusion Variation of polymer concentration exhibited a significant effect on the mechanical properties of microneedles. The permeation test showed that the greater the concentration of PVA, the slower the permeation of the active substance from dissolving microneedle. Further studies should be conducted to perform in vivo pharmacokinetic studies.

Keywords Ceftriaxone · Dissolving microneedle · Polymer · Skin permeation

Introduction

Pneumonia is a respiratory infectious disease that is still considered globally as the main cause of death in children under five. The global record showed that around 808,694 children under five died in 2017 due to pneumonia amounting to 15% of total under-five deaths [1]. Meanwhile, in Indonesia, 32 out of 1000 children under five died out of this disease based on data from BPS-Statistics of Indonesia in 2017 [2]. Specifically, in South Sulawesi Province in the same year, there were 31,759 cases of pneumonia with 26 deaths. Pneumonia can be caused by bacteria, viruses, fungi, or other parasites. However, because of its fatality, bacterial pneumonia is believed to be the major cause of morbidity and mortality [3, 4].

Ceftriaxone is a broad-spectrum, third-generation cephalosporin antibiotic used for the treatment of Gram-positive and Gram-negative bacterial infections. Thus, this drug is usually used for severe infections, such as pneumonia at a

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dose of 50 mg–2 g/kgBW/day [5, 6]. Ceftriaxone has good tolerability, but at higher doses or long-term administration, ceftriaxone may cause biliary pseudolithiasis and other quite dangerous side effects [7]. Ceftriaxone is not readily absorbed by mucous membrane, resulting in poor absorption of this drug in the gastrointestinal tract. This drug is categorised as class 3 of Biopharmaceutics Classification System, possessing high aqueous solubility and low permeability. For that reason, ceftriaxone is not available in oral dosage form and only given through intravenous or intramuscular injection [8, 9]. Hence, it is essential to develop a delivery system formulation that has similar effectiveness as parenteral administration, without causing the unnecessary side effects of the injection.

One of the alternative delivery systems that can replace injection delivery is the microneedle delivery system. Microneedle is a novel drug delivery system with a needle-like shape and micron-range size from 100 to 1000 μm. This system can be used as an alternative to the parenteral route because it can penetrate the stratum corneum to blood circulation in the dermis without reaching the nerve endings, so it does not cause pain [10–15]. Among several types of microneedles, the dissolving microneedle is the one that is most often used to deliver active substances [16]. A previous study has shown that gentamicin formulated in the form of dissolving microneedle has a higher AUC than the intramuscular injection, and can deliver 75% gentamicin in 24 h with constant plasma levels, thereby increasing the duration of gentamicin in the body and can reduce the frequency of gentamicin administration [17]. Accordingly, this system could potentially be used as an approach to deliver ceftriaxone transdermally to the systemic circulation.

Dissolving microneedles can be prepared from materials that are readily soluble and biodegradable [18], such as polymers, for example, methyl cellulose, polyvinyl pyrrolidone (PVP), and polyvinyl alcohol (PVA) or sugars such as dextrin, galactose, and many more [19]. Based on a previous study [20], the use of PVP as a microneedle polymer without being combined with other polymers resulted in poor mechanical properties of the microneedle. Meanwhile, the microneedle using a mixture of PVP and PVA as a polymer combination showed adequate mechanical strength. Indeed, in another study reported by Permana et al. [21], it was found that the mechanical properties of microneedles containing a mixture of PVA and PVP were found to be better than microneedles containing only one PVA or PVP polymer.

Based on the aforementioned reasons, this study focused on the formulation of ceftriaxone dissolving microneedles using different concentrations of PVA and PVP. In this study, we also evaluated different drug concentrations to achieve the highest drug loading in the microneedle preparations. Afterwards, the microneedles were characterised for their morphology, mechanical strength, drug loading, and dissolving time. Lastly, the skin permeation study was carried out using a rat skin model to observe the potential of these microneedle formulations to be developed for transdermal delivery.

Materials and Methods

Materials

Ceftriaxone was purchased from Alfa Aesar (Lancashire, UK). Polyvinyl pyrrolidone (PVP), polyvinyl alcohol (PVA), potassium chloride, sodium chloride, potassium dihydrogen phosphate, and disodium phosphate were obtained from Sigma-Aldrich (Singapore). All other reagents used were analytical grade.

Microneedle moulds (Micropoint Technologies, Singapore), centrifuge (LC-04S Centrifuge, Zenith Lab (Jiangsu) Co., LTD.), microscope (Olympus CS33, Olympus Corporation), HPLC (Shimadzu Prominence, Shimadzu, Kyoto, Japan), and column C18 (ODS1) (150 mm × 4.6 mm, with a particle size of 5 μm) (Phenomenex Luna C18 (ODS1)) were used in this study.

Formulation Design and Manufacture of Ceftriaxone Dissolving Microneedle

Initially, blank microneedles were prepared by dispersing 40 g of PVP in 60 g of water at room temperature and 15 g PVA in 85 g of water at 80 ºC to obtain the concentration of 40% w/w and 15% w/w of PVP and PVA, respectively. The polymer solutions were then mixed with the ratio shown in Table 1 to achieve the final weight of 10 g.

The microneedle preparations were made by centrifugation method using a silicone mould. In this study, the specifications of the moulds were needle density of 10 × 10, pyramidal needles: 700-μm height and 200-μm width at the base and 200-μm interspacing (Micropoint Technologies, Singapore). Each mould contained 1 g of formula. The moulds were placed into the centrifuge holder and centrifugation was carried out at 3500 rpm for 30 min (LC-04S Centrifuge, Zenith Lab (Jiangsu) Co., LTD.). Then, the microneedles were dried at room temperature for 1 × 24 h at 37 ºC for another 1 × 24 h without being removed from the mould.

Table 1 Design of dissolving microneedle blank formulas

Material	Ratio		
	FB1	**FB2**	**FB3**
PVP 40% w/w	50	60	70
PVA 15% w/w	50	40	30

Table 2 Design of dissolving microneedle formulas containing ceftriaxone

Formula	Concentration (%w/w)			
	PVP40%:PVA 15% (50:50)	**PVP40%:PVA 15% (60:40)**	**PVP40%:PVA 15% (70:30)**	**Ceftriaxone**
F1	90	-	-	10
F2	-	90	-	10
F3	-	-	90	10
F4	85	-	-	15
F5	-	85	-	15
F6	-	-	85	15
F7	80	-	-	20
F8	-	80	-	20
F9	-	-	80	20

Different formulations were prepared to achieve maximum drug loading of ceftriaxone in the formulations. In this study, the blank formulations were mixed with three various concentrations of ceftriaxone, namely 10% w/w, 15% w/w, and 20% w/w, resulting in nine different formulations, as shown in Table 2. The microneedles containing the active substance ceftriaxone were prepared by mixing ceftriaxone with the polymer mixture. After that, they were sonicated until clear dispersion with no air bubbles was obtained. Then, the polymer-drug mixture was placed on the mould and centrifuged with a similar procedure with the blank microneedles. After the centrifugation was completed, the excess polymer mixture at the top of the mould was removed and replaced with a blank polymer mixture. Lastly, the obtained microneedles were dried at room temperature for 1 × 24 h at 37 ºC for 1 × 24 h without being removed from the mould. Figure 1 describes the preparation of dissolving microneedles and the dimension of the needles.

Morphological Test

This test was performed to determine the shape and size of the needle. The microneedles were observed using a microscope (Olympus CS33, Olympus Corporation) that has been calibrated with an Optilab® camera with a magnification of 4 ×.

Mechanical Strength and Penetration Ability Test

Mechanical strength and penetration ability tests were carried out to ascertain the strength of the microneedle against the applied pressure. This evaluation was carried out by measuring the ability of the microneedle to penetrate 8 layers of Parafilm®, possessing the same thickness as the human skin layer. Microneedle was applied under a pressure equivalent to 30 N for 30 s. To achieve this pressure, a weight of 3.06 kg was applied on the top of the microneedles. It is important to note

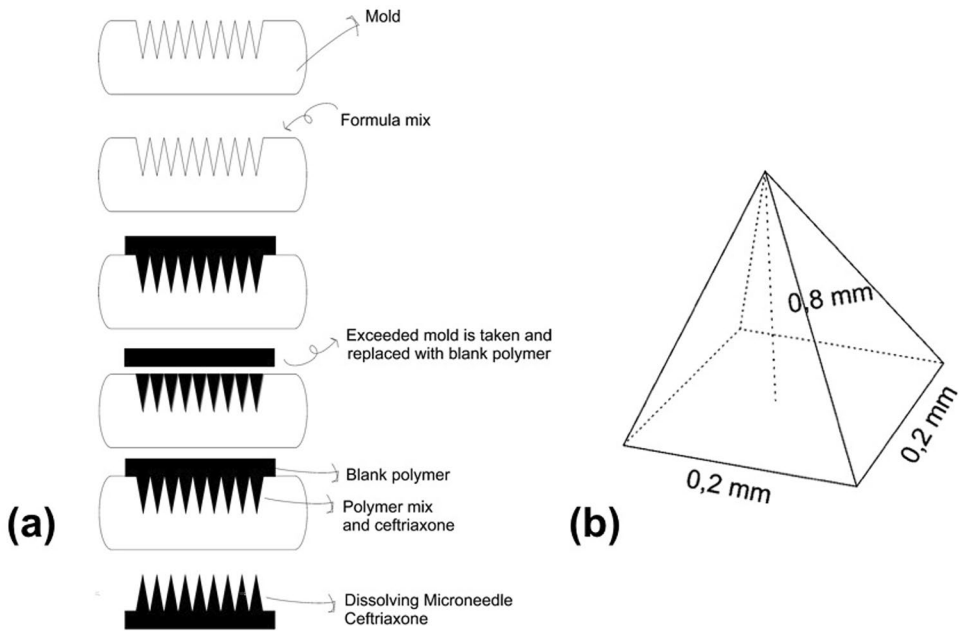

Fig. 1 **a** Dissolving microneedle manufacturing scheme. **b** Microneedle dimensions

that all sides of microneedles received the same pressure during the study. Then, the number of holes formed was observed in each Parafilm® layer and the microneedle shape and size were observed using a microscope according to method 2.3 [22]. The percentage of mechanical strength and microneedle penetration ability is calculated using the following equation [17]:

$$\%compression = \frac{initial\ height - height\ after\ pressure\ applied}{initial\ height} x100\% \quad (1)$$

$$\%penetration\ of\ n\ layer = \frac{number\ of\ holes\ in\ n\ layer}{total\ number\ of\ holes} x100\% \quad (2)$$

Density Determination

First, the polymer mixture was moulded in a mould in the form of a flat block, then weighed and dried. The dry mould was weighed again, and the length, height, and width were measured to determine the volume of the block, then its density (ρ) was calculated using the following equation:

$$\rho = \frac{weight}{volume} \quad (3)$$

Determination of LOD and Percentage of Total Ceftriaxone in Dry Mass

The percentage of total water lost (LOD) after drying was calculated using the following equation:

$$\%loss\ on\ drying\ (LOD) = \frac{weight\ in\ wet\ form - weight\ in\ dry\ form}{weight\ in\ wet\ form} x100\% \quad (4)$$

while the percentage amount of ceftriaxone in dry mass was calculated using the equation:

$$\%amount\ of\ ceftriaxone\ in\ dry\ mass = \frac{ceftriaxone\ weight}{100\% - \%LOD} x100\% \quad (5)$$

Determination of Volume, Needle Weight, and Weight of Ceftriaxone

The determination of the volume of the needle was calculated using the following equation:

$$V = \frac{1}{3} \times \text{lenght} \times \text{width} \times \text{height} \quad (6)$$

$$V_{\text{total}} = \text{V} \times 100 \ \text{needles} \quad (7)$$

Then, the needle weight was calculated using the density equation. Furthermore, the weight of ceftriaxone in dry mass was calculated using the following equation:

$$\text{Total weight of ceftriaxone} = \%\text{ceftriaxone in dry mass} \times \text{weight of 100 needles} \quad (8)$$

Determination of Drug Content in the Microneedle System

Preparation of Phosphate Buffer Saline (PBS) pH 7.4

KCl, NaCl, KH_2PO_4, and Na_2HPO_4 were weighed with the amount of 0.2 g, 8 g, 2.4 g, and 1.44 g, respectively. The salts were then placed in a beaker glass and dissolved with ± 800 mL CO_2-free water. After that, the pH of the solution was adjusted to 7.4. Next, the volume of the solution was made up to 1 l with CO_2-free water [23].

Preparation of Ceftriaxone Stock Solution

Ceftriaxone (10 mg) was placed into a 100-mL fluidized flask, then dissolved and filled with PBS solution pH 7.4 to the limit mark, achieving 100 µg/mL of ceftriaxone stock solution [24].

Preparation of Ceftriaxone Standard Curve

Stock solution of ceftriaxone (100 µg/mL) was pipetted as much as 50, 100, 200, 400, 600, 800, and 1600 µL then put each into a 10-mL flask and the volume was add up to the mark using PBS, so that the solutions were obtained with concentrations of 0.5, 1, 2, 4, 8, 16, and 32 µg/mL. Then, the solutions were filtered using Millipore®. The standard solutions were injected into HPLC (Shimadzu Prominence, Shimadzu, Kyoto, Japan) with a stationary phase of column C18 (ODS1) (150 mm×4.6 mm, with a particle size of 5 µm) (Phenomenex Luna C18 (ODS1)); the mobile phase was a mixture of acetonitrile and trifluoracetic acid in 0.1% v/v water (40:60) with a flow rate of 1.0 mL/min. The detector used was a UV detector with a wavelength of 220 nm. After that, each concentration was taken as much as 20 µL and injected into the injector. Lastly, curve of the relationship between concentration and area was plotted [24].

Determination of Drug Content in the Dissolving Microneedle

The needle was taken out by dredging, then weighed (as theoretical weight) and dissolved in distilled water up to 50 mL.

After that, 1 mL of the solution was taken and made up to 5 mL, then the solution was filtered using Millipore®. Furthermore, 20 µL of this solution was analysed using HPLC as in the standard curve (2.8.3) then determined the amount of ceftriaxone contained in the needle. The percentage of drug content in the needle was calculated using the following equation:

$$\%drug\ content = \frac{analysed\ drug\ concentration}{theoretical\ drug\ concentration} x100\% \quad (9)$$

Dissolving Time Test

This test was carried out using a full-thickness rat skin model with an average thickness of 2.3 cm. Microneedle was inserted into the skin using a pressure equivalent to 30 N for 30 s. Afterwards, a circular weight of 5 g was placed on the top of the microneedles. Afterwards, at 2 min, 4 min, 6 min, 8 min, and 10 min, the microneedle was removed and observed using a microscope [22].

Ex Vivo Permeation Test

This test was conducted to determine the permeation of drug released from dissolving microneedles on the skin. The test was carried out using a full-thickness rat skin model with an average thickness of 2.3 cm. Rat skin was placed in the Franz diffusion cell donor compartment (area of 4.9 cm^2) using cyanoacrylate glue with the stratum corneum facing up in the donor compartment (Fig. 2). Then, the microneedle consisting of 100 needles was applied to the skin with manual pressure for 30 s. A weight of 5 g was placed on top of the microneedle to hold the microneedle in place. The donor compartment was covered using Parafilm® and attached to the receptor compartment of the Franz diffusion cell.

The medium used in the receptor compartment was PBS solution pH 7.4 and the temperature was maintained at 37 ± 0.5ºC under constant stirring at 600 rpm. Samples (1 mL) were taken through Millipore® at 15, 30, and 45 min as well as 1, 2, 3, 4, 6, 8, and 24 h. At each sampling, the medium in the receptor compartment was refilled with the same volume and temperature of PBS. Furthermore, the samples were analysed using HPLC as described in the "Determination of Drug Content in the Dissolving Microneedle" section [17].

Stability Study

The stability studies of microneedles containing ceftriaxone were carried out at controlled room temperature (25 ºC) with two different relative humidity (RH), namely RH 43%, produced using a saturated solution of potassium carbonate,

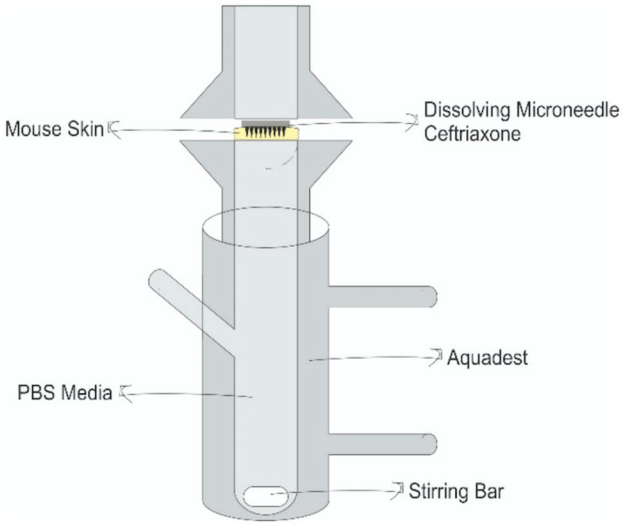

Fig. 2 Ex vivo permeation test scheme

and RH 86%, produced using a saturated solution of potassium chloride [25]. The microneedles were placed into these conditions for a 1-month period in an airtight container. The mechanical properties and drug recovery were assessed and compared to the initial properties.

Data Analysis

The research data were collected, tabulated, and then analysed using a statistical approach. Data were analysed using the One-sample Kolmogorov–Smirnov Test (1 sample K-S) to determine the normality of the data distribution. If the data distribution was normal, then one-way ANOVA was performed for data with more than 2 groups and independent *t*-test or paired *t*-test analysis for data consisting of 2 groups. If the data distribution was not normal, then the Kruskal–Wallis test analysis was performed for data with more than 2 groups and the Mann–Whitney *U* test analysis for data consisting of 2 groups.

Results and Discussion

Morphological and Mechanical Tests

Morphological tests were conducted to determine the shape and size of the blank dissolving microneedle. The morphology of the dissolving microneedle was conical and sharp like a needle with a very small size as shown in Fig. 3a–c which shows a homogeneous mixture of PVA and PVP polymers.

The morphology of the dissolving microneedles following the incorporation of ceftriaxone was also assessed. In our preliminary study, to achieve the highest loading for the

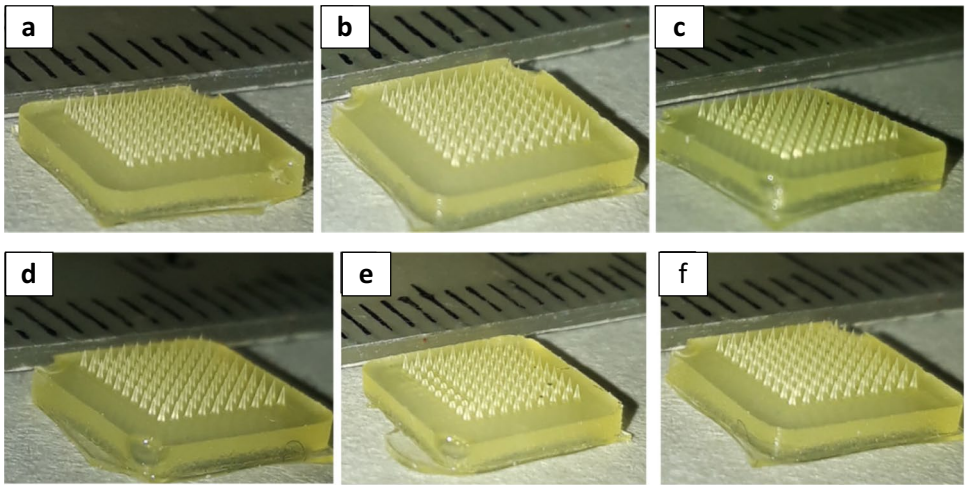

Fig. 3 Morphology of blank dissolving microneedle. **a** FB1, **b** FB2, **c** FB3, **d** F4, **e** F5, and **f** F6

microneedles, three different concentrations (10% w/w, 15% w/w, and 20% w/w) of ceftriaxone were used. The results showed that the use of 20% w/w of ceftriaxone resulted in very viscous solutions, leading to difficulty in the microneedle preparations. No change in the visual of the microneedle was observed following the addition of the active substance. Additionally, the microneedles showed a homogenous mixture of polymer and the drug as shown in Fig. 3d–f.

These results are in accordance with an investigation reported previously by Permana et al. [20], showing that microneedles have sharp edges like needles. With such a shape, it is hoped that the microneedle can penetrate the stratum corneum, obstruct the skin, and form holes up to the epidermis or upper dermis [20, 26]. Figure 5 shows that the addition of ceftriaxone in the formula did not affect the physical appearance of dissolving microneedle when 15% w/w of ceftriaxone was used, seen from the colour of the microneedles that did not change.

Table 3 shows that the height of the microneedles obtained increased with the higher PVP to PVA ratio. FB3, with the highest PVP40% to PVA15% ratio, produced the highest microneedles. This shows that the concentration of the polymer affects the size of the resulting microneedles. The statistical analysis found that the initial heights of the blank microneedles are significantly different ($p < 0.05$). Following the addition of ceftriaxone, there were no significant differences between mechanical properties between microneedles containing 10% w/w and 15% w/w of ceftriaxone. Accordingly, to achieve higher drug loading, formulations containing 10% w/w (F4, F5, and F6) were selected for further studies. After incorporation of the active substance ceftriaxone, the formula F4, F5, and F6 also showed a similar pattern of height increase. However, it was interesting to note that the addition of ceftriaxone also caused a height increase in all three formulas compared to blanks. It is possible that the addition of 15% ceftriaxone to each formulation caused an increase in viscosity, thus affecting the ability of the mixture to enter each mould hole even though it had been centrifuged. Based on the results of ANOVA, it was found that each formula had a significant difference in microneedle height compared to others ($p < 0.05$). The size of the microneedle is in accordance with the length of the needle required to penetrate the skin. The average microneedle is made with a length of 150–1500 μm [27]. This size is adjusted to the thickness of the skin to the dermis without reaching the nerve endings, so it does not

Table 3 Size reduction of dissolving microneedle (blank and with ceftriaxone) (mean ± SD, $n = 3$)

	Formula	Before mechanical strength test (μm)	After mechanical strength test (μm)	Size reduction of *needle* (%)
Blank	FB1	716.66 ± 1.88	258.77 ± 9.59	63.89 ± 1.30
	FB2	725.00 ± 1.09	635.78 ± 2.11	12.31 ± 0.41
	FB3	749.04 ± 1.05	689.45 ± 0.93	7.96 ± 0.22
With ceftriaxone	F1	736.67 ± 9.02	264.32 ± 8.89	64.15 ± 1.64
	F2	762.33 ± 9.29	614.33 ± 9.07	19.41 ± 1.35
	F3	793.33 ± 7.09	694.32 ± 3.62	12.52 ± 10.6
	F4	734.36 ± 4.83	253.12 ± 3.23	65.53 ± 0.66
	F5	755.24 ± 0.65	614.77 ± 8.93	18.60 ± 1.11
	F6	798.07 ± 1.64	694.64 ± 5.34	12.96 ± 0.56

cause pain [28]. Therefore, the possible needle length of microneedles obtained was not considered to result in pain when administered.

The mechanical strength of dissolving microneedles is an initial formulation study conducted to determine the ability of microneedles to effectively penetrate the *stratum corneum* for effective drug delivery [20]. Evaluations of mechanical strength and penetration ability were carried out to ensure the strength of the microneedle against the applied pressure. The condition of the needle of each formula after being given a load equivalent to 30 N is shown in Fig. 4.

Figure 4a–c show all the blank needles of each formula were found to bend, especially FB1 following the application of the pressure. This was probably due to the low mechanical strength of each needle, so it was not able to penetrate the Parafilm® layer and could only penetrate a few layers. The mechanical strength of the dissolving microneedle was measured by the percentage reduction in needle height after the application of the pressure of 30 N.

Table 3 shows that the greatest percentage of the reduction in needle height was found in FB1 with reduction percentage of $69.41 \pm 4.25\%$. On the other hand, the reduction in needle height of FB3 was the lowest, which was $7.95 \pm 0.22\%$, suggesting the greatest mechanical strength of FB3. FB1 was prepared with PVP 40%: PVA 15% with a ratio of 50:50 while FB3 was made of PVP 40%: PVA 15% with a ratio of 70:30. This shows the possibility that the more PVA used, the worse the mechanical strength of the microneedle. The results of this study are in accordance with a study reported by Wang et al. [29], which stated that microneedles containing only PVA as a polymer had a large reduction in mechanical strength when stored in areas with high humidity because PVA is a hygroscopic polymer. Therefore, FB3, which contained only 30% PVA, had the highest mechanical strength among other formulations. In addition, PVA also has a low flexural modulus which causes PVA to be flexible and cannot withstand the external pressure applied. Accordingly, microneedles containing a higher PVA concentration tend to bend more easily when pressure was applied [30]

From Fig. 4d–f, a similar pattern in terms of the correlation between PVP to PVA ratio and the mechanical strength of the resulting microneedles was observed in those containing ceftriaxone when compared to blanks, showing that no significant difference was found between blank microneedles and microneedles containing ceftriaxone ($p > 0.05$).

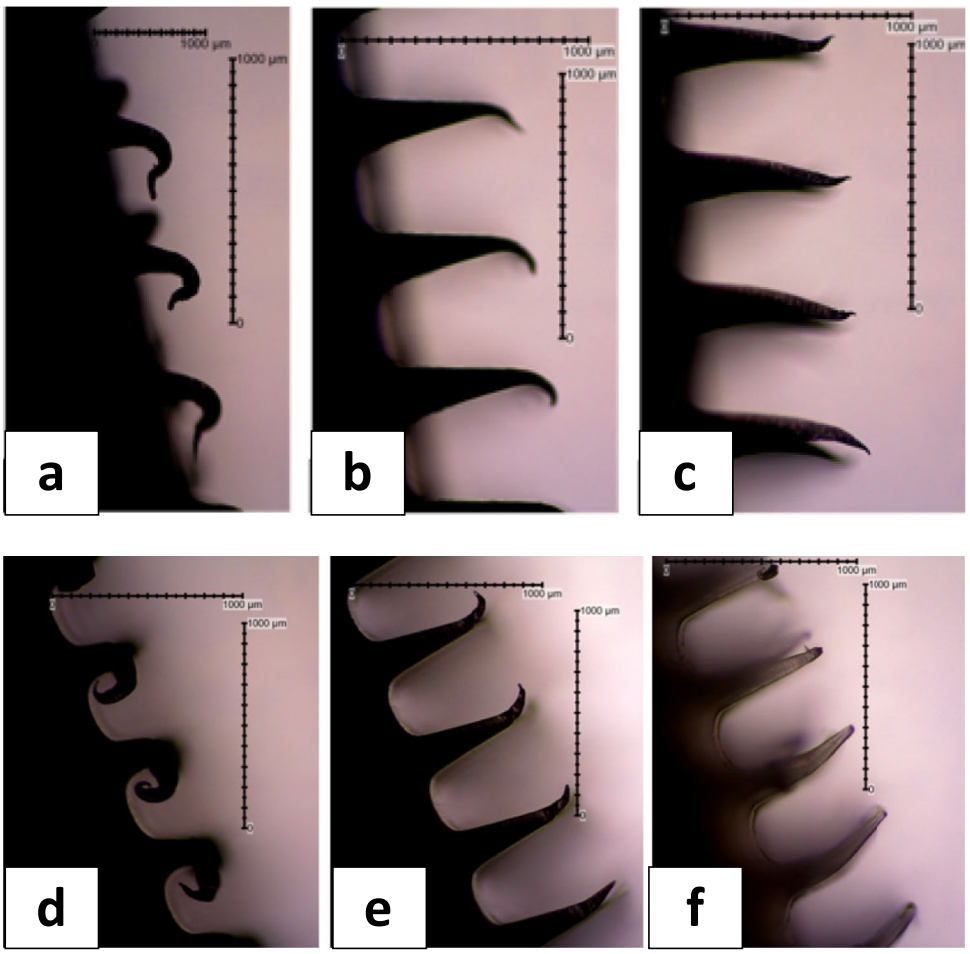

Fig. 4 Morphology of dissolving microneedle formulations. **a** FB1, **b** FB2, **c** FB3, **d** F4, **e** F5, and **f** F6 after mechanical strength test

Table 3 also shows that F4, F5, and F6 had a percentage reduction in needle height of 58.39 ± 0.53%, 18.60 ± 1.11%, and 12.96 ± 0.55%, respectively. It was important to note that the height reduction values of formulations containing 10% w/w of ceftriaxone were not statistically different compared to 15% w/w. Therefore, 10% w/w was chosen for further characterizations. Specifically, the formula with the greatest mechanical strength was F6 with the lowest height reduction. Based on the Kruskal–Wallis test analysis, the height reduction in F6 was significantly different compared to other formulations ($p < 0.05$). Interestingly, comparing the needle height reduction in ceftriaxone dissolving microneedles to that of the blanks, the mechanical strength of all the formulas seemed to decrease following the incorporation of the active ingredient.

The mechanical strength test was also carried out to observe changes in the microneedle base plate after applying pressure. The microneedle base plate did not experience any change after being stressed, which indicated that the microneedle base plate had good mechanical strength. This is in accordance with a research reported by Permana et al. [20] that mechanical tests on several formulas containing PVA and PVP as polymers and did not find any changes in the microneedle base plate. The combination of PVA and PVP in the manufacture of microneedle could potentially improve the mechanical properties of the formulation, due to the interaction of hydrogen bonds between the hydroxyl group (-OH) of PVA and the carbonyl group (C = O) of PVP. This bond creates a strong and robust structure of the microneedle [31].

Penetration Ability Test

Penetration ability test was carried out to confirm the results of the mechanical strength test and determine the depth of the hole produced by the microneedle. This test was based on observing the holes formed in each Parafilm® layer. The results of the percentage of penetration test for dissolving microneedle ceftriaxone are presented in Table 4.

FB1 and FB2 could penetrate up to the third layer by forming 79 holes (79%) and 82 holes (82%). Moreover, FB3 could penetrate up to the fourth layer by forming 69 holes (69%). This result is in accordance with the mechanical strength test which showed that FB3 had the greatest mechanical strength among the blank microneedle formulas. Following the addition of ceftriaxone, it was found that F4 and F5 could penetrate up to the third layer by forming 61 holes (61%) and 73 holes (73%), respectively, while F6 could penetrate up to the fourth layer by forming 62 holes (62%) in that layer. However, F4, F5, and F6 showed a reduction in the number of holes formed in the second layer. This is in accordance with the mechanical strength test of dissolving microneedle ceftriaxone which experienced a reduction in mechanical strength due to the addition of 15% ceftriaxone.

The average thickness of each Parafilm® layer is around 126 µm. Accordingly, 8 Parafilm® layers have a thickness of 1008 µm which is equivalent to the thickness of the skin layer from the *stratum corneum* to the upper dermis [21, 32]. Thus, FB1 and FB2 could penetrate up to 378 µm and FB3 could penetrate up to 504 µm. F4 and F5 can penetrate up to 378 µm and F6 can penetrate up to 504 µm. This means that all formulations could penetrate the skin until the upper dermis. In this part, there are no nerve endings. Therefore, all the microneedle formulations were most likely to be painless but still able to deliver the drug to the blood vessels. Similar results were also shown in studies by Permana et al. [21] and Volpe-Zanutto et al. [26]

Density of Dissolving Microneedle

Before determining ceftriaxone content in the needle, the theoretical amount of ceftriaxone should be determined first. This determination required the density value of each formula. Based on the calculation results, F4, F5, and F6 respectively have a density of 1.14 ± 0.04 mg/mm^3, 1.17 ± 0.06 mg/mm^3, and 1.24 ± 0.05 mg/mm^3. Furthermore, based on the density, the dry weight of the microneedle was calculated. The results of the calculation of the density and dry weight of microneedle can be seen in Table 5.

Table 4 The percentage of penetration test for dissolving microneedle formulas (blank and with ceftriaxone)

Layer	%penetrated					
	FB1	FB2	FB3	F4	F5	F6
1	100	100	100	100	100	100
2	100	100	100	93	98	100
3	79	82	92	61	73	98
4	0	0	69	0	0	62
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0

Loss on Drying and Percentage of Ceftriaxone in Dry Weight

LOD needs to be determined to calculate the percentage of ceftriaxone in dry weight. The LOD values of F4, F5, and F6 were 57.6 ± 1.75%, 57.1 ± 1.50%, and 57.8 ± 1.19%, respectively. From that, the calculated percentage of ceftriaxone in dry weight was found to be 35.44 ± 1.46%, 35.01 ± 1.20%, and 35.57 ± 1.00% for F4, F5, and F6, respectively.

In addition to calculating the percentage of ceftriaxone in dry weight, the LOD value was also related to mechanical strength because during drying, each monomer unit of PVP provides intermolecular stiffness so that dissolving microneedles can harden well during drying and increase the mechanical strength of dissolving microneedles. Therefore, in the mechanical strength test, the dissolving microneedle which had the best mechanical strength was F4 with the highest LOD value.

Volume, Needles Weight, and Ceftriaxone Weight

It was necessary to know the volume and weight of the needle before calculating the theoretical weight of ceftriaxone. The theoretical weight of ceftriaxone in the needle would be used to calculate the percentage of ceftriaxone contents in the dissolving microneedles. Each needle has a volume of 0.00934 mm^3, so that for 100 needles, the volume is 0.934 mm^3. Each F4, F5, and F6 contained 0.39 ± 0.01 mg of ceftriaxone, 0.39 ± 0.02 mg, and 0.39 ± 0.02 mg, respectively. Based on statistical analysis using one-way ANOVA, it was found that the amount of ceftriaxone in F4, F5, and F6 was not significantly different ($p > 0.05$), showing the reproducibility of the preparation method.

Ceftriaxone Content in the Dissolving Microneedle Formulations

Following the theoretical calculations, the ceftriaxone content was determined using HPLC. Initially, the standard curve solution of ceftriaxone was prepared. The standard curve equation obtained was $y = 335.05x - 24.559$ with linearity (R^2) of 0.999 (Fig. 5). Due to the excellent linearity obtained, the resulting standard curve could be used as a reference in determining ceftriaxone content.

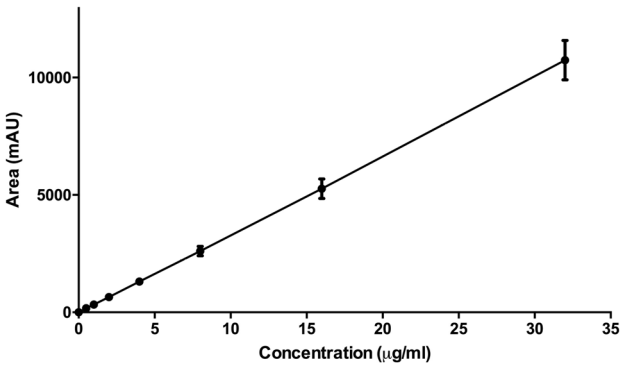

Fig. 5 Standard curve of ceftriaxone (mean ± SD, $n = 3$)

The results of determination of ceftriaxone content in the dissolving microneedle formulations using Eq. 9 showed that F4, F5, and F6 contained 99.79 ± 4.50%, 99.28 ± 4.68%, and 99.72 ± 4.75% ceftriaxone, respectively. Thus, it confirms good homogeneity during the mixing process. This content can be used as a reference in the use of microneedle according to the dose to be used and the development of the size of the base plate and the number of needles for the unit dose of dissolving microneedle containing ceftriaxone.

Dissolving Time Test

Dissolving time test was conducted to determine the time required for the microneedle to completely dissolve in the skin after application. The results of the dissolving time test are depicted in Figs. 6 and Table 6.

After 10 min, F4 has not dissolved completely in rat skin tissue, while F5 and F6 have, evidenced by the absence of needle residue located at the base of the microneedle plate. However, F6 showed a faster dissolving time than F4. Accordingly, it could be concluded that F6 is a formula that may dissolve well when applied to biological tissues.

PVP and PVA are polymers that can dissolve well in body fluids. This is due to the hygroscopicity and absorption of moisture from PVP and PVA. When PVP and PVA are applied to the skin, PVA and PVP would immediately absorb the surrounding interstitial fluid and then dissolve [17]. However, PVA in the semi-crystalline form dissolved more slowly than PVP. Thus,

Table 5 Density, LOD, percentage of ceftriaxone in dry weight, weight of 100 needles, and ceftriaxone weight in 100 needles of dissolving microneedles formulations containing ceftriaxone (mean ± SD, $n = 3$)

Formula	Density (mg/mm^3)	LOD (%)	Percentage of ceftriaxone in dry weight (%)	Weight of 100 needles (mg)	Ceftriaxone in 100 needles (mg)
F4	1.14 ± 0.04	57.60 ± 1.75	35.44 ± 1.46	1.08 ± 0.05	0.39 ± 0.01
F5	1.17 ± 0.06	57.10 ± 1.50	35.01 ± 1.20	1.08 ± 0.05	0.39 ± 0.02
F6	1.24 ± 0.05	57.80 ± 1.19	35.57 ± 1.00	1.15 ± 0.06	0.39 ± 0.02

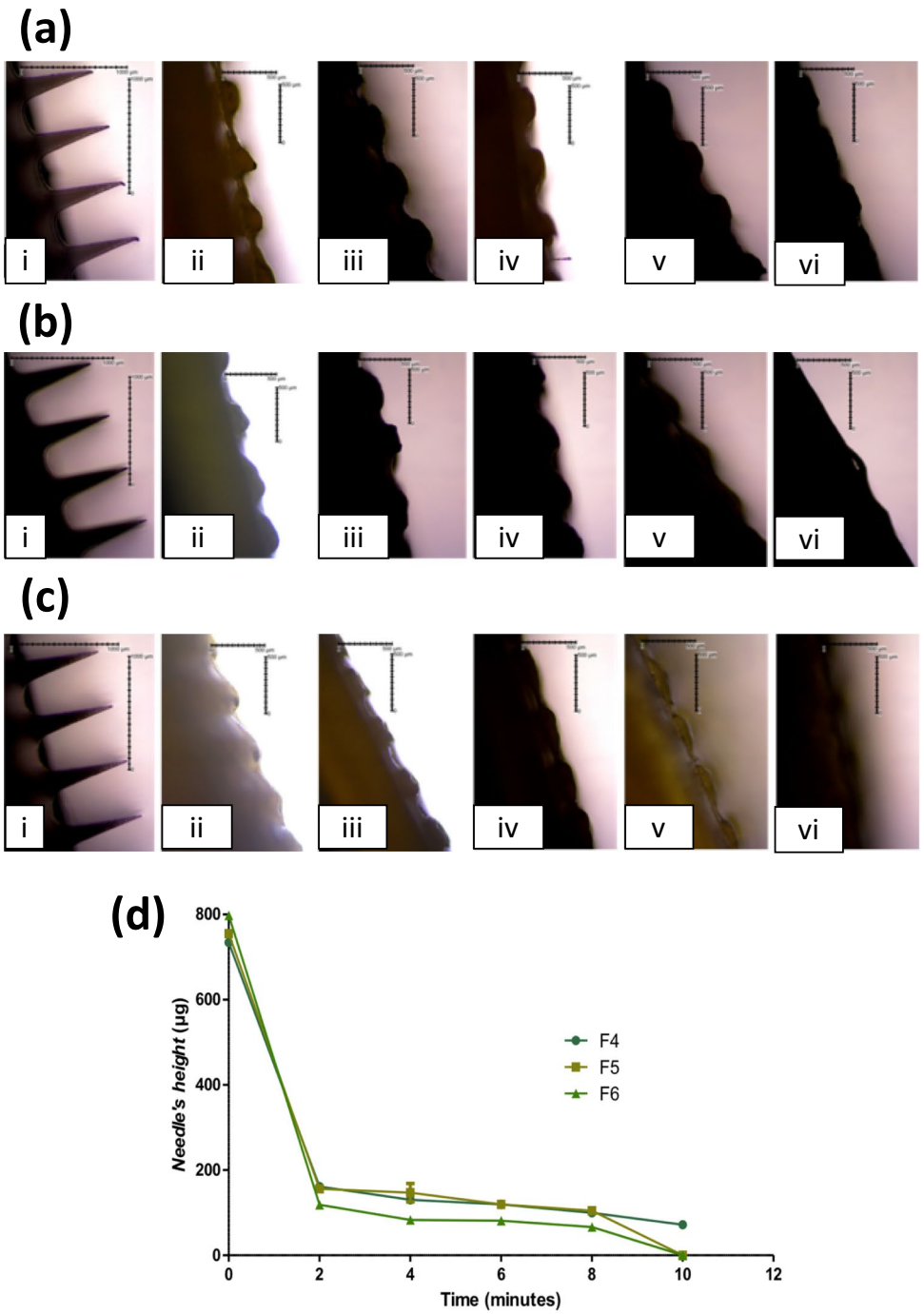

Fig. 6 **a** Morphology of dissolving microneedle containing ceftriaxone F4 at (**i**) 0 min, (**ii**) 2 min, (**iii**) 4 min, (**iv**) 6 min, (**v**) 8 min, and (**vi**) 10 min. **b** Morphology of dissolving microneedle containing ceftriaxone F5 at (**i**) 0 min, (**ii**) 2 min, (**iii**) 4 min, (**iv**) 6 min, (**v**) 8 min, and (**vi**) 10 min. **c** Morphology of dissolving microneedle containing ceftriaxone F6 at (**i**) 0 min, (**ii**) 2 min, (**ii**) 4 min, (**iv**) 6 min, (**v**) 8 min, and (**vi**) 10 min. **d** Dissolving time profile of F4, F5, and F6

F4, containing a higher concentration of PVA, dissolved slower than F5 and F6. The results of this study are in accordance with research conducted by Shim et al. [33] and Shu et al. [34] which showed that a formula with a high concentration of PVP resulted in an increase in the dissolution rate of the microneedle. Based on the results of the Kruskal–Wallis analysis, the three formulas had significantly different dissolving times ($p < 0.05$).

Ex Vivo Permeation Test

Ex vivo permeation test was carried out to determine the drug release profile from the microneedle after application to the skin. This assay describes the release of ceftriaxone from a microneedle applied to rat skin in the donor compartment into the systemic circulation, which in this case the receptor compartment. The experiment used Franz diffusion cells with time intervals of 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, and

Table 6 The height of dissolving microneedle containing ceftriaxone at several time intervals (mean ± SD, $n = 3$)

Formula	Height of dissolving microneedle of ceftriaxone at (μm)					
	0 min	2 min	4 min	6 min	8 min	10 min
F4	34.36 ± 4.83	161.34 ± 3.76	130.24 ± 1.29	126.83 ± 12.56	99.84 ± 6.46	71.94 ± 3.14
F5	755.24 ± 0.65	155.97 ± 8.89	147.03 ± 21.61	119.77 ± 2.44	104.84 ± 5.29	0
F6	798.07 ± 1.64	118.83 ± 7.73	83.27 ± 3.70	81.28 ± 6.05	66.41 ± 1.55	0

24 h. Permeation percentage of ceftriaxone was measured using HPLC with a UV detector at a wavelength of 220 nm. The results of ceftriaxone permeation from the dissolving microneedles are presented in Fig. 7A.

Figure 7 shows the percentage of drug permeated at the sampling time interval. F4 showed a slow and relatively constant permeation rate after 2–4 h but continued to increase up to 24 h, while F5 and F6 showed that the permeation rate continued to increase from the beginning to 2 h. After 24 h, F4, F5, and F6 were permeated by 65.87 ± 5.41%, 78.86 ± 5.7%, and 99.86 ± 7.61%, respectively. Based on the post hoc one-way ANOVA, the permeation percentage value of F6 was significantly different from those of F4 and F5 ($p < 0.05$).

Ceftriaxone permeated from F6 was found to be higher compared to other formulations. This was in accordance with the results of the dissolving time test which exhibited that F6 dissolves faster than other formulas due to the high concentration of PVP. The rate of dissolving of the polymer determines the amount of drug released per unit time. The faster the polymer dissolves, the faster the drug would be released. The formula with high dissolution and permeation speed allows an increase in the amount of drug in the systemic circulation. Drug delivery through dissolving microneedle depends on its ability to penetrate the stratum corneum as demonstrated by mechanical strength tests. Based on mechanical strength test and penetration ability test, F6 can penetrate the fourth Parafilm® layer which causes F6 to reach deeper layers of skin compared to F4 and F5. Accordingly, the release of ceftriaxone from F6 microneedle was faster and more abundant in comparison with other formulations. It is interesting to note that although the microneedles completely dissolved after 10 min, a controlled release manner was observed for 24 h. It could be potentially beneficial to provide a constant plasma level of ceftriaxone. This might be due to the poor permeability of ceftriaxone [8, 9], providing slow permeability in the dermis layer to the receiver compartment although the drug has been delivered to the dermis layer using the microneedles. Additionally, we also compared the permeation profile of ceftriaxone from the microneedles with needle-free polymeric matrix containing polymeric mixture similar to F6. As shown in Fig. 7B, due to the poor permeability of ceftriaxone, only 0.53 ± 0.08% of ceftriaxone permeated after 24 h. A previous study has also reported that the incorporation of the drugs into dissolving microneedles exhibited a greater skin delivery compared to the drugs incorporated into the needle-free patches [20].

Stability Study

In the stability study, F6, containing 15% w/w of ceftriaxone and PVP40%:PVA15% with the ratio of 70:30, was selected. With respect to the mechanical strength, at

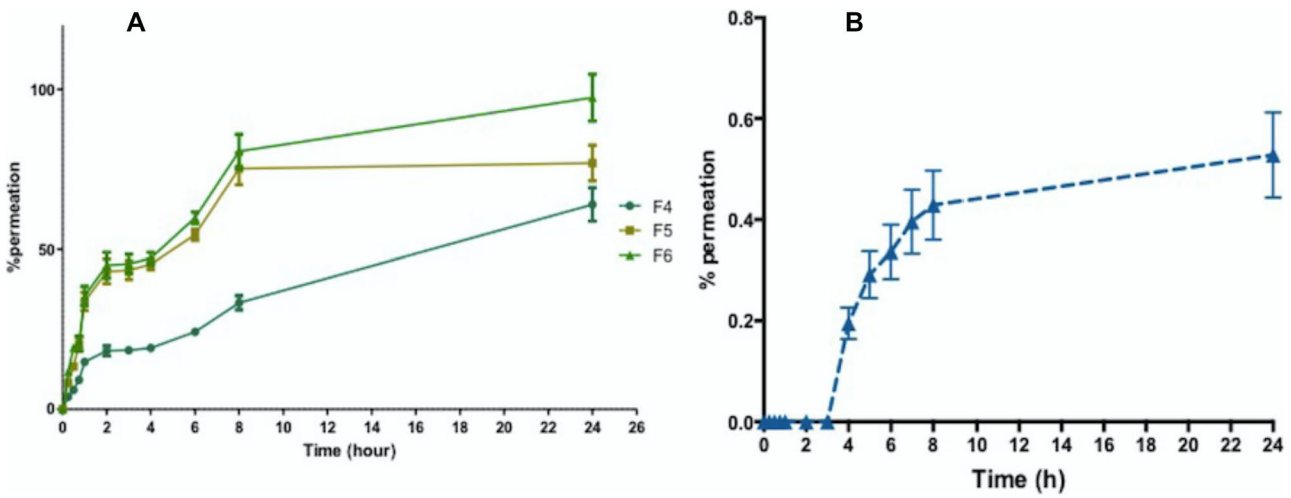

Fig. 7 Ceftriaxone permeation profile from the dissolving microneedle formulations (**A**) and polymeric matrix (**B**) (mean ± SD, $n = 3$)

RH 43%, the percentage of height reduction values of F6 was 13.43 ± 1.31%, 12.87 ± 1.28%, 13.23 ± 1.19%, and 13.76 ± 1.43% after 1 week, 2 weeks, 3 weeks, and 4 weeks, respectively. At RH 86% 12.76 ± 1.22%, 13.09 ± 1.17%, 13.32 ± 1.28%, and 12.93 ± 1.32% of height, reductions were found after 1 week, 2 weeks, 3 weeks, and 4 weeks, respectively. In terms of the insertion profiles, the formulations were able to penetrate 4 layers of Parafilm® after 4-week storage in these conditions, with the percentage penetration values between 61.43 ± 5.47% and 62.98 ± 5.39%. Analysed statically, there was no statistical difference in the mechanical and insertion properties of the microneedles in these two conditions for 4 weeks, showing the excellent stability of the formulations. Importantly, after 4-week storage, the drug recoveries were 98.78 ± 1.23% and 99.82 ± 1.18% at RH 43% and RH 86%, respectively. Accordingly, it can be concluded that ceftriaxone could maintain its stability in the microneedle formulations.

Based on the description above, it is found that the dissolving microneedle ceftriaxone formula using PVA and PVP polymers showed good mechanical strength, penetration ability, dissolving time and amount of permeation, and importantly, stability profiles. Therefore, dissolving microneedle has the potential as a new route of delivery of ceftriaxone to the systemic circulation for the treatment of pneumonia in infants. However, to prove its effectiveness, several studies are required. In vivo pharmacokinetic studies should further be carried out to investigate the plasma concentration of ceftriaxone following the administration of the microneedles. Afterwards, the determination of the size of the microneedle patches can be determined according to the plasma level achieved. Other important studies such as skin irritation test and in vivo pharmacodynamic activity test in appropriate animal models should also be conducted. Importantly, the acceptability and usability investigations should be performed to assure the effect of this approach before reaching the clinical studies.

Conclusion

This study was conducted to determine the effect of variations in PVP and PVA concentrations on morphology, mechanical strength, penetration ability, dissolving time, and ex vivo permeation profile of ceftriaxone from dissolving microneedle formulations. Based on the results of the study, it can be concluded that the microneedle is conical in shape and sharp like a needle and visually homogenous. Variation of polymer concentration showed a significant effect on mechanical strength, penetration ability, and dissolving time. The permeation test showed that the greater the concentration of PVA, the slower the permeation of the active substance from dissolving microneedle. F3 which is a formula containing PVP40%:PVA15% with a ratio of 70:30 is the best formula because it has good penetration ability, dissolving time and skin permeation profile.

Author Contribution The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Declarations

Ethical Approval We have no ethical issue to declare.

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