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DEVELOPMENT OF DISSOLVING POLYMERIC MICRONEEDLES AS AN APPLICATOR FOR OCULAR DRUG DELIVERY SYSTEM

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Abstract: Dissolving polymeric microneedles are micron scale technology and very attractive for minimally invasive intraocular tissue. The application and development of dissolving polymeric microneedles by using biodegradable polymer has been explored as a novel physical method for ocular drug delivery. The purpose of this study was to investigate the appropriate polymer for fabrication of the dissolving polymeric microneedles as an applicator for ocular drug delivery system. The microneedles were fabricated by micro-molding technique using hydroxypropyl methylcellulose (HPMC) mixed with polyvinylpyrrolidone K30 (PVP K30) in various concentrations: C1 (10%HPMC), C2 (10%HPMC:10%PVP K30), C3 (10%HPMC:15%PVP K30) and C4 (10%HPMC:20%PVP K30). The physical appearance under microscope, mechanical strength by texture analyzer, and dissolution study by measuring the microneedle height inserting in the ocular tissues at predetermined time were evaluated. All formulations could produce microneedle arrays (11 rowsx11 columns) with 10 mm^2 of microneedle patch area and conical shape needle. The height and base diameter of microneedle were 530 $\pm$ 25 μm and 300 $\pm$ 3 μm, respectively. The change of percent needle height of C1, C2, C3 and C4 in the mechanical strength (bending force and fracture force; 1.8, 2.8, 3.8, 5.8 and 8.8 N) showed that formulations were added 15% and 20%PVP K30 changing percent needle height significantly less than C1 and C2. However, some needle of C4 could be fractured since 5.8 N. The dissolution result showed that dissolving polymeric microneedles were completely dissolved within 180 seconds. In conclusion, the fabrication of dissolving polymeric microneedles by mixing HPMC and PVP K30 polymer in the appropriate ratio provided the suitable microneedle properties for ocular drug delivery.

Keywords: Dissolving polymeric microneedles, Ocular microneedles, Hydroxypropyl methylcellulose (HPMC), Polyvinylpyrrolidone K30 (PVP K30)

INTRODUCTION

Ocular diseases, one of the most important health problems in the world, cause a visual impairment approximately 285 million people, including 39 million blind and 246 million suffering from low vision (Pascolini and Mariotti, 2012). Ocular diseases have been classified into anterior and posterior segment diseases, depending on eye anatomy. Firstly, anterior segment diseases can cause critically serious vision impairment or morbidity such as corneal neovascularization (CNZ), bacterial/fungal keratitis, glaucoma, uveitis, herpes simplex keratitis, blepharitis and dry eye syndrome. Secondly, posterior segment diseases can lead to permanent loss of vision and blindness such as age-related macular degeneration (AMD), diabetic retinopathy, cytomegalovirus retinitis and other chorioretinal diseases (Yellepeddi *et al.*, 2015). Most of the ocular drug delivery systems such as topical, systemic or local

administration have been used to deliver drugs into the eye. Topical ocular drug delivery is suitable system to cure anterior segment diseases. However, the limitation of topically applied drugs are low ocular bioavailability (less than 5 %) and low patient compliance due to the required frequency of application (Järvinen *et al.*, 1995). The poor ocular bioavailability after topical application to specific intraocular treatment mainly results from a number of inherent anatomical and physiological ocular barriers namely transient residence time in the culde-sac, blinking and nasolacrimal drainage. Moreover, the barrier at the anterior segment of eyes is cornea, and the posterior segment of eyes is sclera, Bruch's-choroid complex as well as the blood-retinal barrier (BRB). Therefore, these tight barriers are main hindrance for efficient drug delivery, limiting the penetration and permeation of ophthalmic agents. Intraocular injections, namely subconjunctival and intravitreal injections, have been largely applied to improve ocular bioavailability in treating posterior segment ocular disorders. However, these methods need to repeat injections by specialist in clinical administration for maintaining effective concentrations of drugs, leading to toxicity from high dose of drug and hemorrhage from invasive ocular injection (Maurice, 2001).

To date overcoming these hindrance and improving ocular drug bioavailability, microneedles have been used as a physical force to create transport pathways and enhance the permeability of ocular drugs. Microneedles are very attractive for minimally invasive ocular tissue and widely used for fifteen years to enhance transdermal drug. The main mechanism of microneedles is the creation of transport pathways in microdimensions, leading to increased drug permeability by bypassing barrier function. Microneedles are typically 25–2000 μm in height and have been fabricated from many materials such as silicon, steel, glass or polymer (Thakur *et al.*, 2016). In general, the microneedles can be divided into five types following to their delivery mechanism including solid, coated, hollow, gel-forming and dissolving polymeric microneedles (Cheung and Das, 2016). Various types of microneedles have been developed to improve the ocular drug delivery. Some drawbacks of using coated, hollow and solid microneedles are complicated and difficult to fabrication. Therefore, dissolving microneedles could avoid the drawbacks of those microneedles. After insertion dissolving microneedles in the ocular tissue, the encapsulated drugs in the microneedle matrix are released. Moreover, these microneedles are fabricated from safe and biodegradable polymers (Thakur *et al.*, 2016).

In this study, the microneedles were fabricated by using hydroxypropyl methylcellulose (HPMC) mixed with polyvinylpyrrolidone K30 (PVP K30). These polymers are FDA accepted biocompatible polymers and have been widely used in pharmaceutical fields (Mansour *et al.*, 2010). The purpose of this study was to investigate the optimal formulation of polymers for fabrication of the dissolving polymeric microneedles as an applicator for ocular drug delivery system. The microneedles were investigated in terms of their physical appearance under microscope, mechanical strength by texture analyzer. The dissolution of microneedles was also studied by measuring the microneedle height inserting in the ocular tissues at predetermined time.

MATERIALS AND METHODS

Materials

Polyvinylpyrrolidone (PVP K30, MW 40,000 Da), hydroxypropyl methylcellulose (HPMC, MW 10 kDa) and polyethylene glycol 400 (PEG400) were purchased from P.C. drug center (Bangkok, Thailand). Fresh porcine eye balls were purchased from slaughterhouse (Nakornpathom, Thailand).

Preparation of dissolving polymeric microneedles

The microneedles were fabricated by micro-molding technique. The silicone micromold contains 121 conical shaped needles (11 rows × 11 columns), with an average height of 600 μm, a base width of 300 μm and an interspacing of 600 μm. HPMC was dissolved in water to obtain 10%(w/w) HPMC solution. Various concentrations of PVP K30 at 0%, 10%, 15% and 20% (w/w) were mixed with HPMC solution. 2%(w/w) of PEG was used as a plasticizer and was added to HPMC hydrogel (Table 1). Approximately, 500 mg of HPMC or HPMC/PVP hydrogel were poured into the micro-molding. Then, the micro-molding was centrifuged (ALC, PK121R, UK) at 4,000 rpm, 25 °C for 30 min to remove bubbles. The mold was placed in a vacuum chamber (TÜV Rheinland, Thailand) for 30 min (60 psi, 25 °C) to fill the sample in the microneedle cavities. The microneedles were dried in the mold at room temperature for 48 h. After that, the microneedle array was carefully removed from the mold and kept in the container to assess physical appearance, mechanical strength and dissolution.

Table 1. Dissolving polymeric microneedle formulations

Formulation code	HPMC (%w/w)	PVP K30 (%w/w)	PEG (%w/w)
C1	10	-	0.2
C2	10	10	0.2
C3	10	15	0.2
C4	10	20	0.2

Mechanical strength in bending force and fracture force

The dissolving polymeric microneedles were tested the mechanical strength at various forces (1.8, 2.8, 3.8, 5.8 and 8.8 N) in compression mode by Texture Analyzer (TA.XT plus, Stable Micro Systems, UK). Microneedle array was carefully attached on the Texture Analyzer probe (P/20P) at downward position. The probe was moved down at a speed of 1 mm/s until a flat stainless steel touch microneedles. Then, the probe was held on microneedles to maintain force for 20 s and moved up at a speed of 1 mm/s. The height of microneedles was measured by digital microscope (Dino-lite digital microscope, Taiwan). The percentage of height change was calculated by following equation.

$$\% \text{ Height change} = \frac{\text{Height before test} - \text{Height after test}}{\text{Height before test}} \times 100$$

Preparation of ocular tissues

Porcine eye balls were kept at -20 °C in phosphate buffer saline (PBS pH 7.4) until used. The adherent muscle tissue was removed from the eye bulb by using surgical scissors. Then, the eye was cut into two halves. The anterior sclera tissues were soaked in PBS and kept in sealed container at -20 °C until further used. The frozen ocular tissues were used within 3 months.

Dissolution of dissolving polymeric microneedles

The dissolution of dissolving polymeric microneedle was investigated in sclera tissue. The sclera tissues were thawed in room temperature for 2 h, cut into square pieces by using a disposable scalpel, soaked in PBS, and then dried by tissue paper and cotton bud before used.

Before microneedles were tested dissolution by Texture Analyzer, sclera tissues were dried by tissue paper and cotton bud and placed on a flat stainless steel baseplate. The same protocol with the mechanical strength test was used at the appropriate force in the eyes (3.8 N) (Nicoli *et al.*, 2009). Microneedles were inserted into the middle of the tissue and held for

predefined time periods (0, 20, 60, 120 and 180 s). After microneedles were withdraw from sclera tissue, height of microneedles was measured. The percentage of decreased height was calculated and reported as microneedle height remaining versus time.

Statistical analysis

Each experiment was repeated three times and the data was analyzed by using the one-way ANOVA with post-hoc comparisons after tested normality by Kolmogorov-Smirnov test. A level of significance was set at *p* value lower than 0.05.

RESULTS AND DISCUSSION

Physical appearance of dissolving polymeric microneedles

As shown in Figure 1, the physical appearance of all formulations exhibited a complete microneedle array with the same size and shape. The microneedle arrays showed 121 conical-shaped needles (11 rows x 11 columns) with 10 mm^2 of microneedle patch area. The height and base diameter of each microneedle was 530 $\pm$ 25 μm and 300 $\pm$ 3 μm, respectively. HPMC has been successfully used to prepare dissolving polymeric microneedles (Kim *et al.*, 2016). At the concentration lower than 5% (w/w) of HPMC, microneedles were soft and bent easily when gently touched with the fingers. At HPMC concentration more than 15% (w/w), microneedles were hard and brittle fracture (data not shown). Therefore, 10% (w/w) of HPMC was suitable to prepare the dissolving polymeric microneedles. However, the texture of 10% HPMC microneedle array was slightly soft. Therefore, PVP K30 was added to HPMC microneedles to increase the mechanical strength.

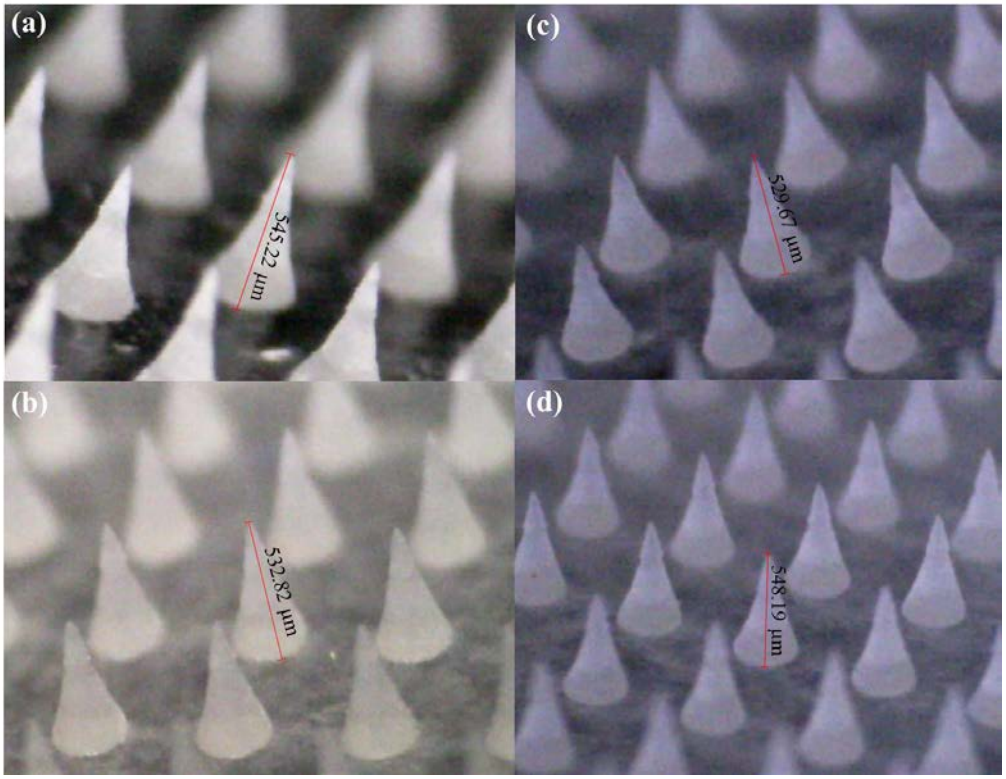

Figure 1. The physical appearance of dissolving polymeric microneedles under digital microscope **(a)** C1 (10%HPMC) **(b)** C2 (10%HPMC:10%PVP K30) **(c)** C3 (10%HPMC:15%PVP K30) and **(d)** C4 (10%HPMC:20%PVP K30).

Mechanical strength in bending force and fracture force

Mechanical strength is one of the microneedle properties to resist the forces for insertion into a biological tissue or any breakable MNs forces. This property is the important factor in the development of microneedle fabrication (Sullivan *et al.*, 2010). In this study, all dissolving polymeric microneedles were evaluated in the mechanical strength (bending force and fracture force). The texture analyzer (in compression mode) was applied the predefined forces for microneedles insertion in sclera (1.87 N), cornea (3.8 N) and other high forces (5.8 and 8.8 N) (Nicoli *et al.*, 2009). The change of percent needle height was calculated and plotted against the applied compression force as shown in Figure 2. 15% and 20% PVP K30 in 10% HPMC formulations (C3 and C4) significantly exhibited the changing percent needle height less than control (C1) and 10% HPMC with 10% PVP (C2). However, no significant difference in the mechanical strength was observed between control (C1) and 10% HPMC with 10% PVP (C2) ($p > 0.05$). The mechanical strength of MNs can be affected by many factors including polymer type or concentration (Lee *et al.*, 2008). In this study, the dissolving polymeric microneedles fabricated from HPMC and PVP K30 showed the most rigid and the least brittle, leading to withstanding of the high forces with minimal height change. Only PVP K30 polymers was sufficiently used to form rigid microneedles, however, brittle microneedles was found (data not know). Mixing PVP K30 with HPMC in the suitable ratio could improve this limitation. The best microneedle formulation was C3 (10%HPMC: 15%PVP K30), which could withstand applying forces from 1.8 to 8.8 N with the slightly height change from 11.23% to 17.71 %. The physical appearance of C3 microneedles after applying predefined forces was used to confirm as presented in Figure 3. However, some needles of C4 fractured or broke when using the applying force since 5.8 N, because the high concentration of PVP K30 caused the brittle and fragile microneedles.

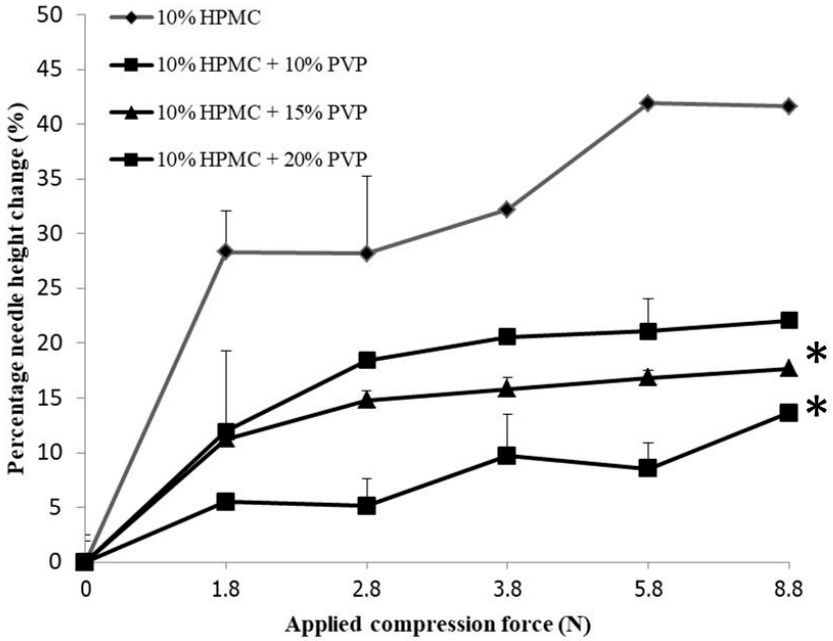

Figure 2. The percent needle height change of dissolving polymeric microneedles versus the applied compression force. Data were reported as mean ± SD (n = 3).

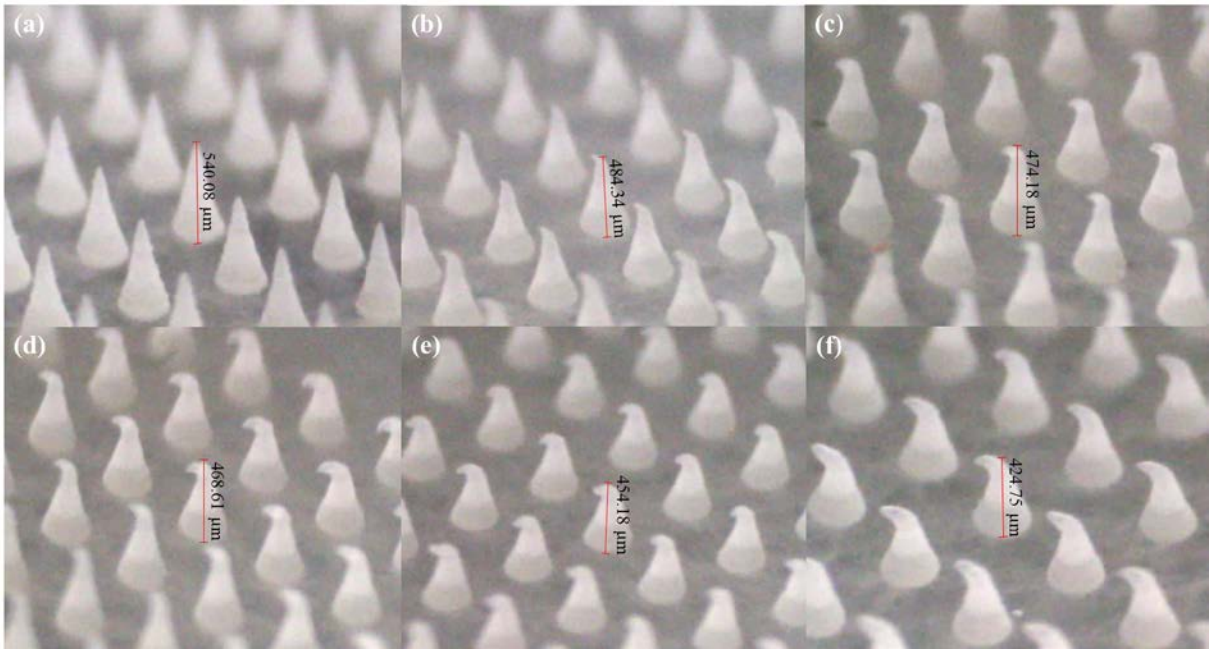

Figure 3. Dissolving polymeric microneedles of C3 (10%HPMC:15%PVP K30) after applying predefined forces; **(a)** 0 N, **(b)** 1.8 N, **(c)** 2.8 N, **(d)** 3.8 N, **(e)** 5.8 N and **(f)** 8.8 N. Data were reported as mean ± SD (n = 3).

Dissolution of dissolving polymeric microneedles

Porcine eye balls have been widely used in many experiment, because it has a similar collagen bundle, histology and water content to the human eye (Nicoli *et al.*, 2009; Sanchez *et al.*, 2011). The dissolution of dissolving polymeric microneedles is important to design and develop the effective microneedle for the eye application. From previous test, the best formulation of dissolving polymeric microneedles, C3 (10%HPMC: 15%PVP K30), was chosen to evaluate the dissolution. The result showed that microneedles perfectly dissolved within 180 s after insertion into the sclera (Figure 4 and 5). The water content of porcine eye tissue is around 75-89 % (Pethig and Kell, 1987). This result indicated the ability of C3 microneedle to insert into the ocular tissue with rapid dissolution.

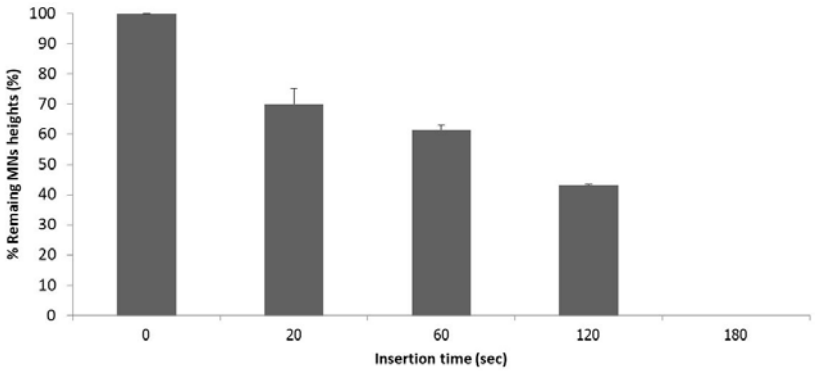

Figure 4. The dissolution of C3 (10%HPMC: 15%PVP K30) microneedles reported as percentage of remaining microneedle heights versus insertion time after applied to sclera tissue. Data were reported as mean ± SD (n = 3).

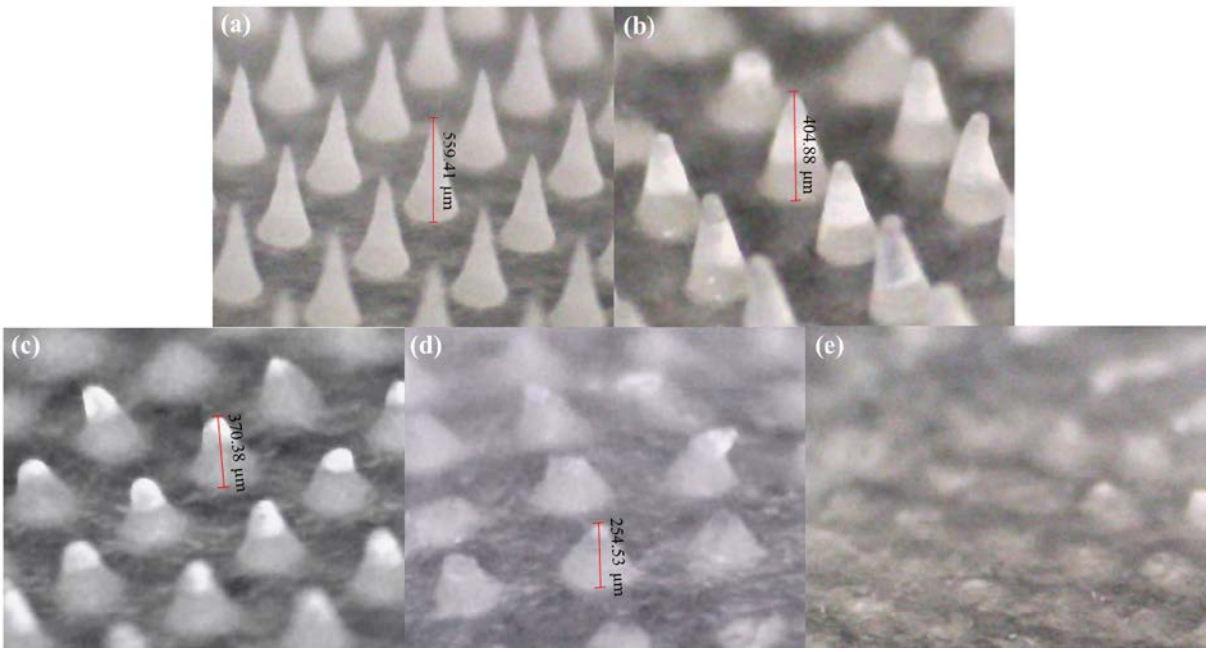

Figure 5. The dissolution of C3 (10%HPMC: 15%PVP K30) microneedles after applied in ocular tissue at predefined times; **(a)** 0 s, **(b)** 20 s, **(c)** 60 s, **(d)** 120 s and **(e)** 180 s. Data were reported as mean ± SD (n = 3).

The appropriate polymer for fabrication of the dissolving polymeric microneedles as an applicator for ocular drug delivery system with is very important**.** Dissolving polymeric microneedles made from biocompatible polymer to ocular tissues are safe and non-irritated to the eyes**.** The minimal reduction of needle height without fracture presented the insertion ability into the eyes**.** In addition, the completely dissolved of microneedles in the ocular tissue might be the suitable dissolving microneedles for ocular drug delivery system. In our study, the drug release from the dissolving polymeric microneedles will be investigated in the further studies**.**

CONCLUSION

In this study, the optimal formulation to fabricate the dissolving polymeric microneedles was 10%HPMC mixed with 15%PVP K30 due to the suitable physical appearance and ability to withstand the higher compression force needed to insert into the eyes. These microneedles were completely dissolved in the ocular tissues within 180 s. Therefore, the fabrication of dissolving polymeric microneedles by mixing HPMC and PVP K30 polymer in the appropriate ratio provided the suitable microneedle properties for ocular drug delivery.

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