공학석사 학위논문

# STUDY ON DRY TABLET FORMULATION CONTAINING POLYMERIC NANOPARTICLES WITH A PREOCULAR APPLICATOR FOR OPHTHALMIC DRUG DELIVERY

전안부 약물 전달을 위한 어플리케이터에
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공과대학 협동과정 바이오엔지니어링 전공

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이 논문을 공학석사 학위논문으로 제출함

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BY

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INTERDICIPLINARY PROGRAM IN BIOENGINEERING

THE GRADUATE SCHOOL

SEOUL NATIONAL UNIVERSITY

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## Abstract

# STUDY ON DRY TABLET FORMULATION CONTAINING POLYMERIC NANOPARTICLES WITH A PREOCULAR APPLICATOR FOR OPHTHALMIC DRUG DELIVERY

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Topical administration in a form of solution and suspension is a common route for ophthalmic drug delivery. However, due to rapid tear clearance, less than 5% of the administrated drug can reach the ocular interior target tissue. In order to deliver an accurate dosage of the drug and enhance ocular drug bioavailability, I propose a combined system of a rapidly-dissolving dry alginate tablet containing drug-loaded poly (lactic-co-glycolic acid) (PLGA) nanoparticles and an ease-of-use preocular applicator. The dry tablet herein was prepared via lyophilization of dexamethasone-loaded PLGA nanoparticle in the alginate medium on

the tip of the PDMS applicator.

The nanoparticles prepared by solid-in-oil-in-water emulsion were loaded with 85.45 μg/mg dexamethasone, and the dry tablet exhibited a controlled drug release for 10 h. To evaluate *in vivo* efficacy, after topical administration of the dry tablet to rabbit eye with the preocular applicator, dexamethasone concentration in the aqueous humor was measured and compared to that of Maxidex®, a commercially-available dexamethasone eye drops. When applied to the rabbit eye, the dry tablet was completely detached from the applicator and dissolved in tear on the eye surface. The nanoparticles remained on the preocular space up to 2 h due to enhancement of tear viscosity from the interaction between alginate in tablet and calcium ion in the tear, resulting in more than 2.5-fold increase in ocular drug bioavailability compared to that of Maxidex®.

Through this study, I envision that the preocular applicator combined with the dry alginate tablet containing drug-loaded PLGA nanoparticles can be a promising system for aseptically delivering an accurate dose of the ophthalmic drug with enhanced bioavailability.

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**Keywords** : PLGA, Nanoparticles, Dexamethasone, Alginate, Ophthalmic drug delivery, Sustained drug release,

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# 1. Introduction

## 1.1 Background knowledge

Topical administration in a form of solution and suspension is a widely preferred and common route for ophthalmic drug delivery for its ease of administration. However, due to rapid tear clearance and blinking, the entry of the drug into the interior of the eye is highly restricted [1, 2]. After topical instillation, a major portion of the drug is rapidly removed from the preocular surfaces due to lacrimation and tear turnover within a few minutes, leading to a short drug residence time [3]. Moreover, to reach the intraocular tissues, the remaining drug on the preocular surface should penetrate the stratified corneal tissue comprising of 5 to 7 layers of epithelial cells [4]. Ultimately, less than 5% of topically administrated is delivered to the intraocular target tissue, resulting in limited ocular drug bioavailability [5, 6].

To enhance ocular drug bioavailability, it is necessary to extend the precorneal drug residence time in the cul-de-sac to enable prolonged the drug adsorption. Ophthalmic drug carriers, such as micro and nanoparticles, have been suggested to prolong drug retention on the preocular space of the eye and release the drug in a sustained manner [3]. However, when delivered in a form of a suspension, the loaded drug could be released from its carrier when dispersed in the aqueous medium [7]. Furthermore, an additional fluid would expedite solution drainage into the nasolacrimal duct, promoting the clearance of drug-loaded particles [2, 8]. Therefore, dry tablet formulation has been suggested as an alternative strategy. The drug carrier would be formed in a dry tablet

formulation, which would be dissolved in tear and release the drug-loaded particles on the eye surfaces [9]. As the tablet medium dissolves the tear viscosity would increase and delay tear clearance, eventually improving the preocular residence time of the drug. Nevertheless, the administration of a dry tablet might irritate the sensitive eye causing inconvenience for patients. Besides, as the tablet would be fetched with the bare fingers for application, the eye would be exposed to a great risk of bacterial and fungal infections which might cause serious diseases such as corneal ulceration [10-12]. These complications demonstrated the need of a better tablet formulation and a proper applicator to deliver the drug hygienically.

## 1.2 Strategy

Herein, I proposed a combined system of a rapidly-dissolving dry tablet containing drug-loaded nanoparticles with an ease-of-use preocular applicator. The applicator was designed to consist of two sections, a handle and a tablet-loading tip. When administrated, the dry tablet on the applicator tip would touch the preocular surfaces to deliver the formulation while the handle is held. This would allow an easy and, more importantly, aseptic topical administration of the dry tablet.

To test the proposed system and strategy, I prepared a preocular applicator using the biocompatible polydimethylsiloxane (PDMS) in this study. The applicator was designed to be similar shape and dimension to that of the commercially-available, single-use applicator of artificial tear fluid for patients' familiarity [13]. The applicator consists of two parts: a tip where a dry tablet formulation is loaded and a handle where patient could hold while applying. For the sustained drug release formulation, I prepared poly(lactic-co-glycolic acid) (PLGA) nanoparticles to be loaded with dexamethasone, a widely used corticosteroid drug for the eye inflammation treatment [14, 15]. To produce the rapidly-dissolving tablet, the nanoparticles were dispersed in a solution containing a mixture of polyvinyl alcohol (PVA), and alginate, which was then freeze-dried on top of the applicator tip. PLGA has been widely used for polymeric drug delivery for its good biocompatibility and biodegradability to produce non-toxic by-products [16, 17]. PVA has been approved for clinical use in various ophthalmic drug products [18-20]. In this study, I also exploited highly-biocompatible alginate in the

tablet medium as a viscosity enhancer of tear fluids. Alginate is a polysaccharide derived polymer [21] that can be crosslinked in the presence of the multivalent cations to form a gel [22, 23]. Since the dry tablet was a physical composite of the alginate polymer chains with drug-loaded nanoparticles held within the structure, when delivered to the preocular surfaces, the tablet dissolved in the $Ca^{2+}$-abundant tear fluid, and the alginate could increase tear viscosity, thereby synergistically extending the preocular residence time of the drug-loaded nanoparticles.

Dexamethasone-loaded PLGA nanoparticles (DX/NP) were prepared by the solid-in-oil-in-water (S/O/W) emulsion method and characterized by scanning electron microscopy (SEM) and dynamic light scattering (DLS), which were employed to assess nanoparticle size and morphology. The DX/NP was examined for its cytotoxicity using human corneal epithelial cells (HCECs). To examine the effect of alginate on tear viscosity enhancement, two distinct tablet formulations containing DX/NP were prepared with two different tablet media containing both PVA and alginate (DX/NP AL_TAB) and only PVA (i.e., without alginate) (DX/NP TAB), respectively. For *in vivo* experiments, these tablet formulations were applied to rabbit eyes, and drug concentration in the aqueous humor was assessed and compared to the commercially-available ophthalmic dexamethasone medication, Maxidex®, (i.e., an aqueous suspension of dexamethasone itself).

## 2. Materials and Methods

### 2.1. Materials

PLGA (lactic acid:glycolic acid = 50:50; i.v. = 48,000) was purchased from Evonik Industry (Germany). Dexamethasone and Nile red were purchased from Tokyo Chemical Industry (Japan). PVA (87-89% hydrolyzed), phosphate buffered saline (PBS) tablets, tween 80, trifluoroacetic acid (TFA; >99%), and calcium chloride dihydrate (>99%) were obtained from Merck (USA). Dichloromethane (DCM; >99.5%), N,N-dimethylformamide (DMF; >99.5%), and acetone (>99.5%) were supplied by DaeJung (Korea). Acetonitrile (ACN; >99.9%) was purchased from J.T. Bakers (USA). PDMS (Sylgard 184) was obtained from Sewang Hitech Silicone (Korea). Alcaine® (0.5% proparacaine hydrochloride ophthalmic solution) and Maxidex® (0.1% dexamethasone ophthalmic suspension) were purchased from Alcon-Couvreur (Belgium).

### 2.2. Preparation of dexamethasone-loaded nanoparticles

I prepared dexamethasone-loaded nanoparticles via S/O/W emulsification, as shown in Figure 1 [24]. In short, 300 mg PLGA and 100 mg dexamethasone were both dissolved in 7 mL DCM. The resulting solution was then added to 8 mL of 1% w/v PVA solution, which was emulsified with a homogenizer (Sonic Dismembrator Model 500, Fisher Scientific, France) at 160 W for 10 min. The emulsion was transferred into 100 mL of 1% w/v PVA solution at room temperature and stirred at 400 rpm under vacuum (-10 psi) for 60 min for solvent evaporation. To

optimize the size of the nanoparticles, the nanoparticles were prepared with three different centrifugal filtration speed at 0, 200, or 500 rpm for 10 min. After the filtration, the precipitates were eliminated and only the suspension at the top was collected. The collected suspension was then washed with deionized (DI) water three times via centrifugation at 13,000 rpm for 10 min. Thereafter, the suspension was freeze-dried for 20 h to obtain dry dexamethasone-loaded nanoparticles. PLGA nanoparticles loaded with Nile red (NR/NP) were also prepared to evaluate the *in vivo* preocular retention properties. As such, 5 mg Nile Red and 500 mg PLGA were dissolved in 7 mL DCM for emulsification.

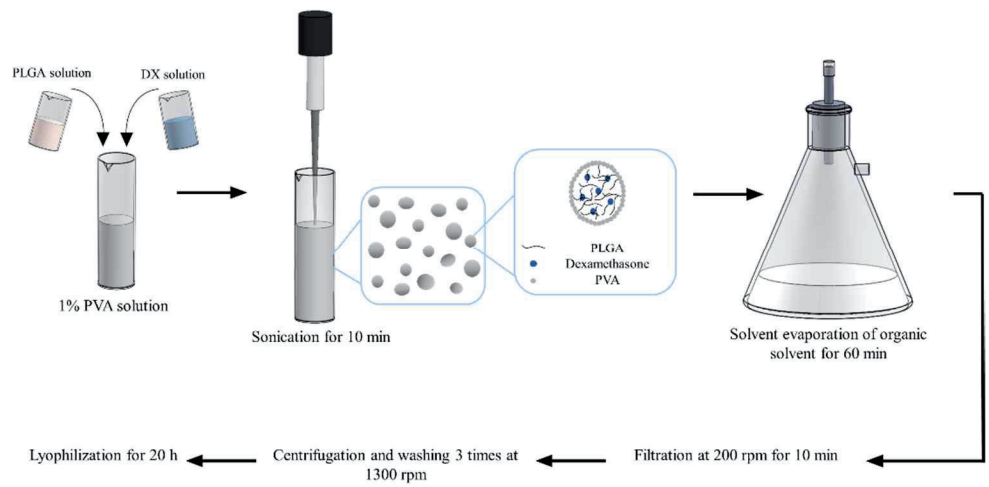


**Figure 1.** Illustrations of preparation of PLGA nanoparticles.

### 2.3. Characterizations of nanoparticles

The morphology of DX/NP was examined by SEM (JSM-7800F Prime, JEOL, Japan). Its size distribution and surface charge were determined using DLS (ELS-2000ZS, Otsuka Electronics, Japan) and a zetasizer (Nano ZS, Malvern, UK) with a particle suspension prepared in DI water [25]. To measure the dexamethasone loading amount, 4 mg of DX/NP was fully dissolved in 4 mL of N,N-dimethylformamide. The supernatant was then diluted with ACN in a 1:1 ratio. Drug concentration in each resulting solution was measured using high-performance liquid chromatography (HPLC; Agilent 1260 series, Agilent Technologies, USA) with a Diamonsil C18 column (5 μm, 150 x 4.6 mm). Column temperature and absorbance wavelength were set at 37 °C and 240 nm, respectively. Injection volume and flow rate were 20 μL and 1.5 mL/min, respectively. The mobile phase was comprised of 0.1% TFA and ACN mixed in a 65:35 ratio. Given with the loading amount of the DX/NP, the encapsulation efficiency (EE) was calculated by the following equation:

$$\text{EE}\ (\%) = \frac{\text{Amount of drug loaded in nanoparticles}}{\text{Initial amount of drug}}\ \text{x}\ 100 \quad (1)$$

To optimize the nanoparticles for the ocular drug delivery, the drug release profiles of all obtained nanoparticles were examined. 5 mg of each nanoparticles prepared with different centrifugal filtration were each suspended in 3 mL of pH 7.4 phosphate buffered saline (PBS) with 0.5% w/v Tween 80. Each suspension was transferred in a dialysis

membrane bag (SnakeSkinTM Dialysis Tubing, 10 kDa, Thermo Scientific, USA), and immersed in 50 mL of the medium. As incubated at 37 °C, at designated time, 5 mL of the release medium was collected and the same volume of fresh medium was replaced. The amount of dexamethasone in collected medium was measured using HPLC as described above. The optimized nanoparticles were named DX/NP and used for further experiments.

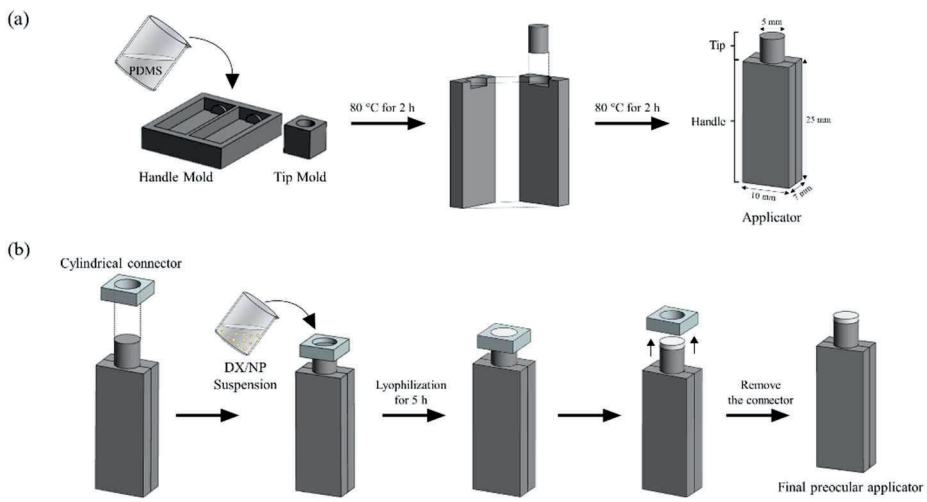


**Figure 2**. Illustrations of fabrication of the DX/NP loaded tablet on PDMS preocular applicator. (a) Fabrication of PDMS preocular applicator. (b) Tablet preparation on the tip of the applicator.

### 2.4. Preparation of dry tablet-loaded preocular applicators

A preocular applicator with a dry tablet on the tip was prepared, as depicted in Figure 2. To prepare a preocular applicator, two different molds were first made to fabricate a handle and tip (Fig. 2(a)). In the molds, a mixture of a PDMS base and its curing agent (10:1, v/v) was poured and cured slightly at 80 °C for 2 h. Then, each of the constituent pieces was assembled and cured further at 80 °C for another 2 h to allow for their bonding. In this work, I produced two different tablets, with media containing both PVA and alginate, and PVA alone, to yield DX/NP AL_TAB and DX/NP TAB, respectively. To prepare the DX/NP AL_TAB, 20 mg/mL DX/NP were suspended in a solution containing 0.1% w/v PVA and 2% w/v alginate. Above this alginate concentration, the solution became too viscous to properly distribute the DX/NP. To prepare the DX/NP TAB, 20 mg/mL DX/NP were suspended in a solution of 0.1% w/v PVA only. Twenty μL of the resulting suspension was then poured in the reservoir that was made by tightly fitting a cylindrical connector with open ends to a tip of the applicator (Fig. 2(b)). The whole piece was then rapidly frozen using liquid nitrogen, which was then lyophilized at 0.01 bar at – 80 °C for 5 h (FreeZone 6 Dryer system, Labconco, USA) [26, 27]. After carefully removing the connector, the preocular applicator loaded with the dry tablet formulation was completely prepared for the experiments. To evaluate the *in vivo* preocular retention properties, NR/NP was embedded instead of DX/NP to produce tablets of NR/NP AL_TAB and NR/NP TAB, prepared under the same condition employed for DX/NP AL_TAB and DX/NP TAB,

respectively.

### 2.5. Characterizations of dry tablets

The drug loading amount was measured by fully submerging the tablet at the applicator tip in 1 mL DMF. To completely extract the drug, the solution with the tablet was sonicated for 2 h and then centrifuged at 13,000 rpm to collect the supernatant, which was then diluted with ACN in a 1:1 ratio. The concentrations of the drug were measured using HPLC as described above.

The thicknesses of the tablets herein were measured using a caliper (ABSOLUTE Digimatic Caliper, Mitutoyo, Japan). To assess the particle distribution in the tablet, the NR/NP TAB and NR/NP AL_TAB were imaged with a fluorescence microscope (Leica DMI4000 B, Leica Microsystems, Germany). To examine the effect of alginate in tablet medium, DX/NP AL_TAB and DX/NP TAB were each immersed in 1 mL of the tear fluid mimicking medium, i.e., PBS containing $Ca^{2+}$ (10 mM, pH 7.4, $[Ca^{2+}]$ = 39.4 μg/mL) [28], for 10 min at 37 °C. The viscosity of the resulting solution was then measured using a rheometer (Advanced Rheometric Expansion System, Rheometric Scientific, USA), where gap separation, temperature, and shear rate were set at 0.8 mm, 25 °C, and 100 $s^{-1}$, respectively.

### 2.6. *In vitro* evaluations

#### 2.6.1. Drug release profiles of formulations

To examine the *in vitro* drug release profiles, 400 μg DX/NP, two different tablets containing the DX/NP (i.e., DX/NP TAB and DX/NP AL_TAB), and Maxidex®, all of which contained the same amount of about 35 μg dexamethasone were each placed in a dialysis membrane bag (SnakeSkinTM Dialysis Tubing, 10 kDa, Thermo Scientific, USA). The bag was then immersed in 5 mL pH 7.4 PBS containing 39.4 μg/mL $Ca^{2+}$ and 0.5% w/v Tween 80 to meet the sink condition of dexamethasone [29]. While being incubated at 37 °C, at scheduled times, 1 mL of the release medium was collected and the same volume of fresh medium was replaced. The amount of released dexamethasone was measured using HPLC as described above.

#### 2.6.2. Cytotoxicity study

The *in vitro* cytotoxicity of DX/NP was evaluated using HCECs (PCS-700-010, ATCC, USA). HCECs were grown in a corneal epithelial cell basal medium (PCS-700-030, ATCC, USA) with supplements (PCS-700-040, ATCC, USA) at 37 °C in a humidified environment with 5% $CO_2$. Prior to the assay, HCECs were seeded in a 96-well plate at $1x10^5$ cells/well and grown for 24 h. Subsequently, 100 μL of the DX/NP suspension, which was prepared in the cell growth medium at concentrations of 5, 10, 25, 50, 100, 250, 500, and 1000 μg/mL, was added to each well and incubated at 37 °C for 24 h. The medium was then completely removed and replaced with 100 μL of fresh medium.

Thereafter, 10 μL of an EZ-Cytox solution was added to each well and incubated at 37 °C for 2 h under dark conditions. Cell viability was measured using a microplate reader, with absorbance and reference wavelengths of 450 nm and 600 nm, respectively (VersaMax ELISA Microplate Reader; Molecular Devices, USA).

### 2.7. *In vivo* experiments

*In vivo* experiments were conducted with the healthy eyes of male New Zealand White rabbits (weight 2.1-2.5 kg). Rabbits were granted free access to food and water and were housed in a controlled environment: temperature; 21 ± 1 °C, humidity; 55 ± 1%, and light/dark cycle; 12 h/12 h. The *in vivo* experimental protocols were approved by the Institutional Animal Care and Use Committee at Seoul National University Hospital (IACUC No. 19-0133).

#### 2.7.1. Preocular retention study

The *in vivo* preocular retention properties of the nanoparticles were evaluated after topical administration of Nile red nanoparticles to the rabbit eye. With the NR/NP AL_TAB and NR/NP TAB, the *in vivo* profile of tablet disintegration was assessed. For this, the eye was imaged using a digital camera (Galaxy S10, Samsung, Korea) at 0 and 30 s after topically administrate the tablet to the rabbit eyes. The preocular retention properties were assessed as reported in previous studies, with slight modifications [30, 31]. Briefly, the Nile red-loaded tablets, NR/NP AL_TAB or NR/NP TAB, on the applicator tip was applied directly on the lower cul-de-sac of the rabbit's left eye. At scheduled times, the

rabbit eye was topically anesthetized by topical administration of a drop of Alcaine® and the entire preocular surface was thoroughly wiped with a surgical sponge (PVA spear; Sidapharm, Greek) to collect the remaining NR/NP. The sponge was then fully submerged in 5 mL DMF and sonicated for 2 h to fully extract the NR/NP. The amount of Nile red in the sample was measured using HPLC-mass spectroscopy (LC-MS) with a Polaris 5 C18-A (2.7 μm pore size, 4.6 x 150 mm) and the following conditions: column temperature; 30 °C, absorbance wavelength; 243 nm, injection volume; 20 μL, and flow rate; 0.45 mL/min. The mobile phase consisted of 0.1% formic acid and ACN in the ratio, 55:45. For statistics, at each time point, four animals (i.e., one left eye for each rabbit) were assigned for each formulation.

### 2.7.2. Pharmacokinetic study

To assess the *in vivo* ocular drug bioavailability, each of the three formulations (i.e., 35 μL Maxidex®, the DX/NP AL_TAB, and DX/NP TAB in the applicator) with the same dose of dexamethasone (c.a. 35 μg of dexamethasone) was directly administered onto the lower cul-de-sac of rabbit eyes. At scheduled times, the rabbit was anesthetized with a subcutaneous injection of a cocktail containing 20 mg/kg ketamine and 10 mg/kg xylazine. Thereafter, approximately 100 μL of aqueous humor (AH) was collected using a 31 G needle (BD Ultra-Fine II, Becton Dickinson and Company, USA). Drug concentration in AH was analyzed by LC-MS as described above. For statistics, three animals (i.e., the left eye of each rabbit) were assigned per time point for each formulation.

### 2.8. Statistical Analysis

Statistical analysis was performed using the amount of particles remaining on the preocular surface and drug concentration in AH by the Mann-Whitney U-test. A p-value $<0.05$ was considered to indicate statistical significance (SPSS version 22, IBM, USA).

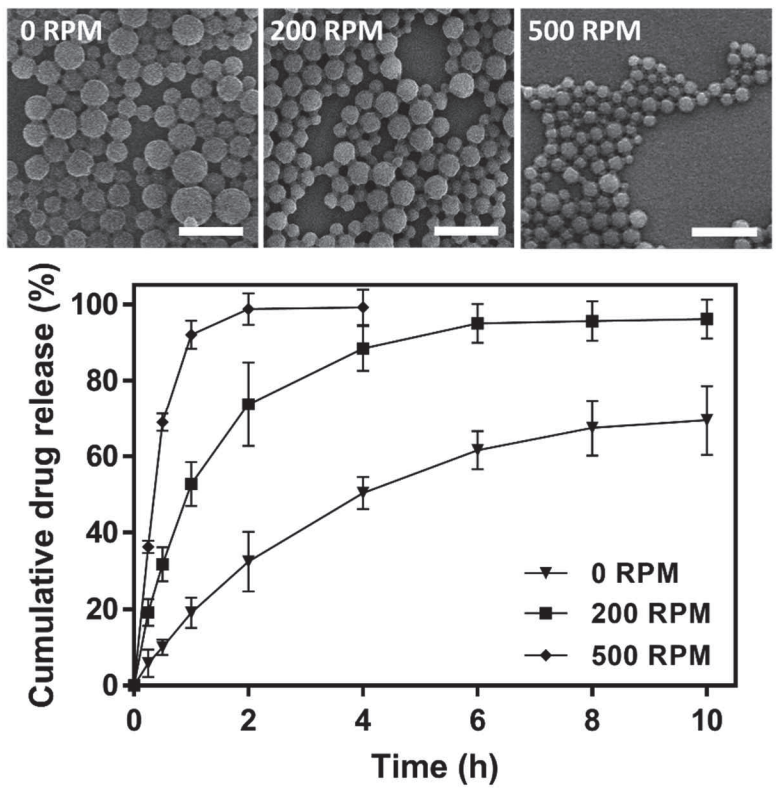


**Figure 3.** Representative scanning electron microscope image of nanoparticles filtered at 0 rpm (unfiltered), 200 rpm, and 500 rpm, and their *in vitro* drug release profiles.

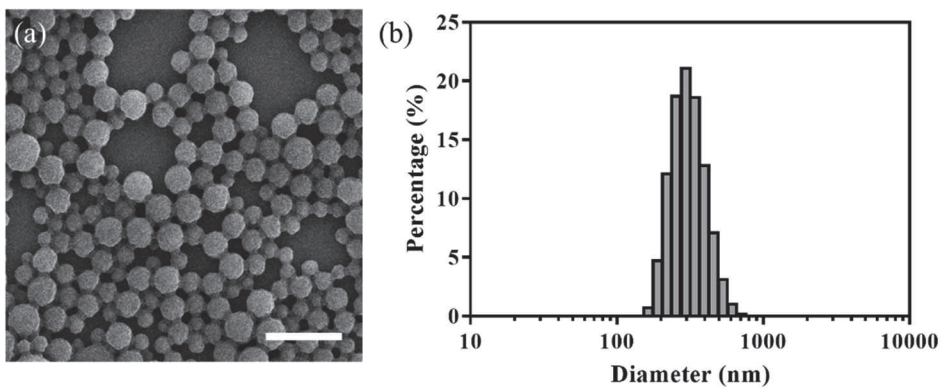


**Figure 4.** Representative scanning electron microscope image and particle size distribution of DX/NP. Scale bar = 1 μm. Polydispersity index = 0.056.

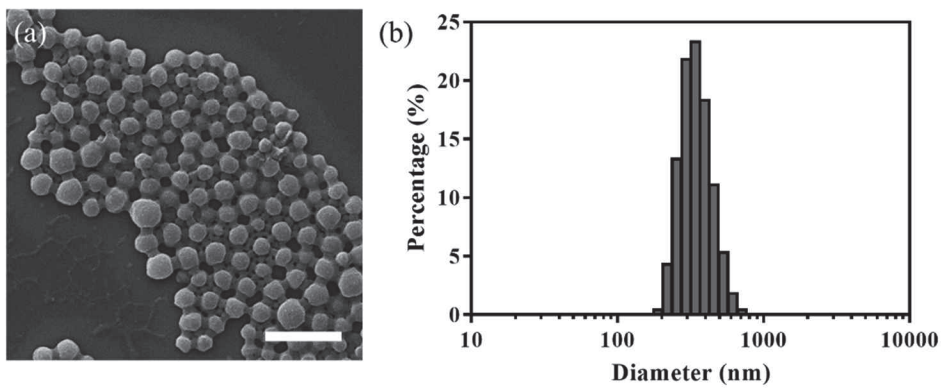


**Figure 5.** Representative scanning electron microscope image and particle size distribution of NR/NP. Scale bar = 1 μm. Polydispersity index = 0.158.

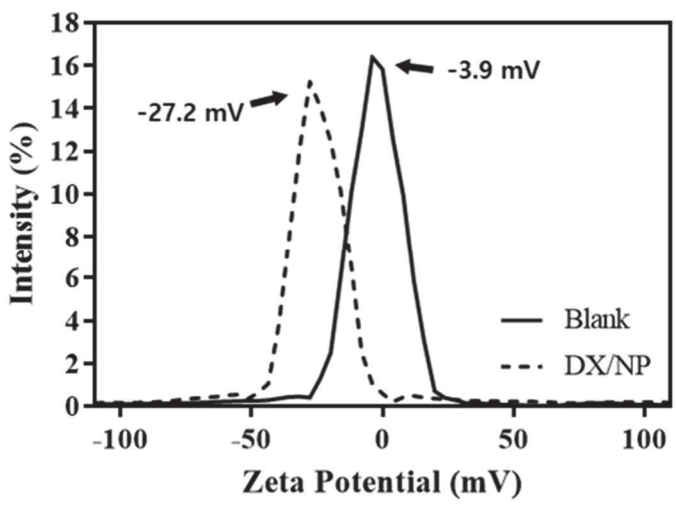


**Figure 6.** Zeta potentials of the blank PLGA nanoparticles and DX/NP.

## 3. Results

### 3.1 Characterizations dexamethasone-loaded nanoparticles

I prepared dexamethasone-loaded nanoparticles using the S/O/W emulsion method. To optimize the PLGA nanoparticles for the tablet formulation, three different particles were prepared with different centrifugal filtration speeds of 0 rpm (unfiltered), 200 rpm, and 500 rpm (i.e., unfiltered, 200 RPM, and 500 RPM). The nanoparticles were found to be in spherical shape, as shown in Figure 3. Unfiltered nanoparticle exhibited the diameter of 400-500 nm, whereas relatively large particles were eliminated with 200 RPM and 500 RPM, showing the diameters of 300-400 nm and 100-150 nm, respectively. The loading amount of unfiltered, 200 RPM, and 500 RPM were measured to be 344.51 ± 8.46, 85.45 ± 5.44, and 9.04 ± .041 μg/mg, respectively.

The drug release profiles of three nanoparticles were obtained to optimize nanoparticles for formation of ocular dry tablet. The drug amount in a tablet was designed to be 35 μg, which is a single-dose of commercialized dexamethasone suspension Maxidex®. However, because the unfiltered particles could load large amount of drug, only small amount of unfiltered particles could be loaded in a tablet, resulting in greater loss of drug in case of particle clearance from the preocular area. The unfiltered and 200 RPM showed sustained release for 10 h, while 500 RPM displayed 100% release at 4 h. The drug release profile of unfiltered showed longer controlled release. The 500 RPM also was not suitable due to its small drug loading amount, as too many particles were required to be loaded in a tablet to reach target drug amount.

Therefore, the 200 RPM was selected to be the optimal nanoparticle for this study as it loaded suitable dexamethasone amount for tablet formation. Further analysis were conducted with 200 RPM, hereafter called DX/NP. Particle diameter of DX/NP measured using DLS was 336.92 ± 5.56 nm (Fig. 4(b)) while encapsulation efficiency was 25.6%.

The size and morphology of NR/NP were determined to be similar to those of DX/NP (Fig. 5(a) and (b)). As shown in Figure 6, the zeta potential of the blank PLGA nanoparticles was measured to be - 3.9 mV, which was shifted to -27.2 mV with the DX/NP due to a negative charge of the encapsulated dexamethasone [32].

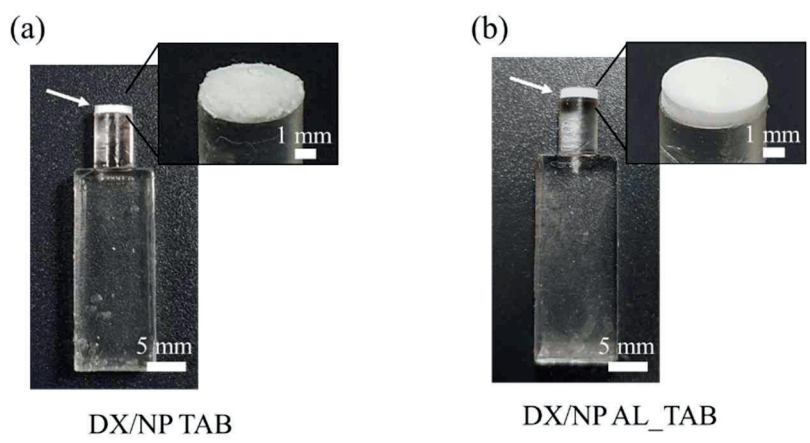


**Figure 7.** Representative optical images of the dry tablet formulation containing drug-loaded nanoparticles on the tip of the applicators. (a) DX/NP TAB. (b) DX/NP AL_TAB.

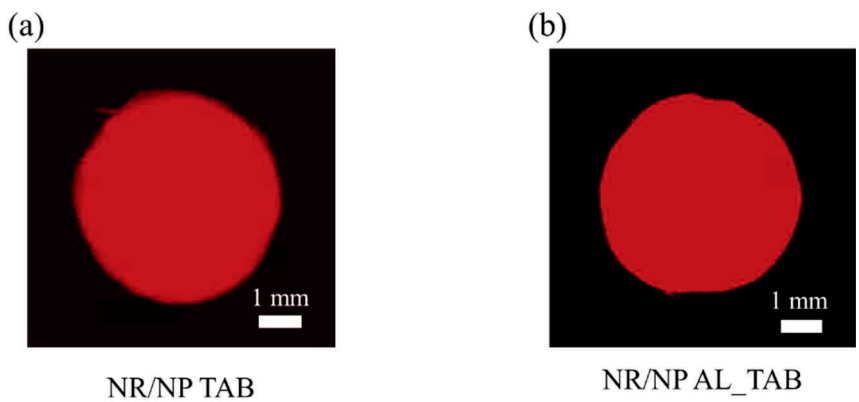


**Figure 8.** Representative fluorescence images of dry tablet formulation containing Nile Red-loaded nanoparticles. (a) NR/NP TAB. (b) NR/NP AL_TAB.

### 3.2 Characterizations of dry tablet formulation

The dry tablet formulation loaded on the tip of the applicator was prepared and characterized. Figure 7 shows the tablets prepared in the present experiment (i.e., the DX/NP TAB and DX/NP AL_TAB). The tablets displayed a cylindrical shape and were well deposited at the tip of applicator. As shown in Figure 8, evenly distributed fluorescent signal were exhibited throughout both NR/NP tablets, implying that the particles were homogeneously distributed within the tablet when prepared with this method. The same amount of DX/NP was embedded during tablet preparation with loading amount of 34.11 ± 0.48 and 34.89 ± 0.28 μg for DX/NP TAB and DX/NP AL_TAB, respectively. For the same reason, the thicknesses of the DX/NP TAB and DX/NP AL_TAB were also measured to be similar (1.02 ± 0.03 and 1.02 ± 0.06 mm, respectively). When tablets were dissolved in $Ca^{2+}$-containing PBS, the solution with DX/NP AL_TAB containing 400 μg alginate had a higher viscosity of 0.93 Pa s than that with DX/NP TAB without alginate of 0.01 Pa s.

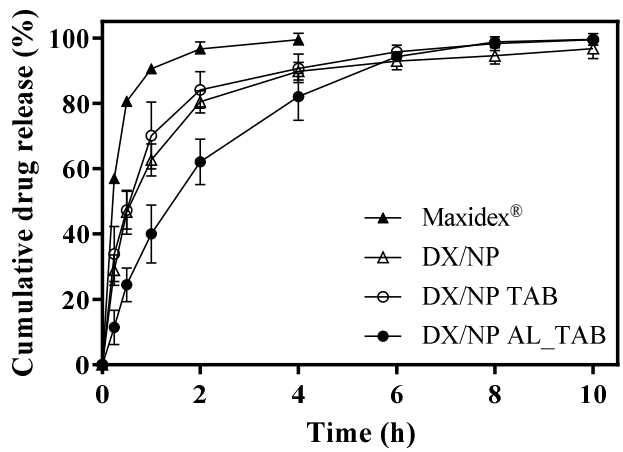


**Figure 9.** *In vitro* drug release profiles of Maxidex® (▲), DX/NP (△), DX/NP TAB (○), and DX/NP AL_TAB (●).

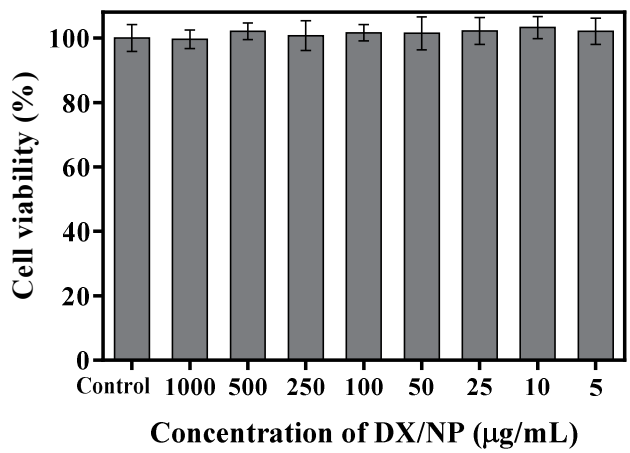


**Figure 10.** Cytotoxicity of DX/NP on human primary corneal epithelial cells.

### 3.3 *In vitro* experimental results

The *in vitro* drug release profiles of the DX/NP and both DX/NP tablets, shown in Figure 9, displayed controlled drug release for 10 h. Among the formulations, the release profiles did not differ much because the tablet medium were rapidly dissolved and almost immediately freed DX/NP. With DX/NP AL_TAB, the release of dexamethasone was slightly suppressed, which could be caused by viscosity increase of the medium in a dialysis membrane bag from the interaction between $Ca^{2+}$ in tear and alginate. A suspension of dexamethasone, Maxidex®, was exhibited a complete dissolution in 2 h. Cytotoxicity of the DX/NP was tested with human corneal epithelial cells. The nanoparticles exhibited greater than 90% of cell viability at all testing concentrations (Figure 10), suggesting that the DX/NP was not toxic to living cells.

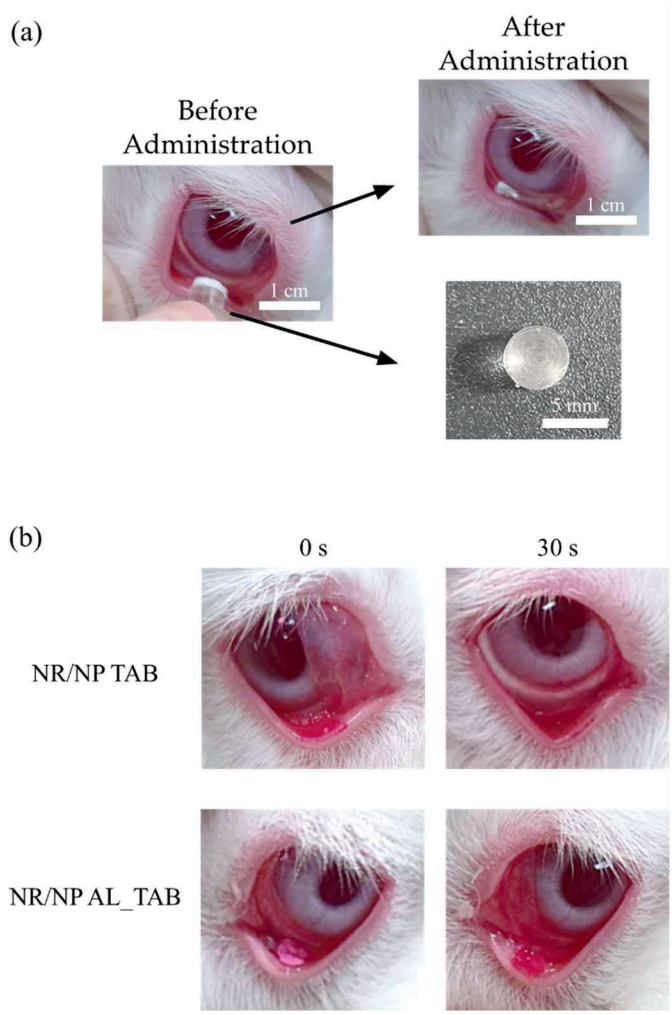


**Figure 11.** Representative images of the tablet application on the rabbit eyes. (a) Images of before and after topical administration of the DX/NP TAB and DX/NP AL_TAB using the applicator herein. (b) Representative disintegration profiles of NR/NP TAB and NR/NP AL_TAB topically administrated on the lower conjunctiva of rabbit eye.

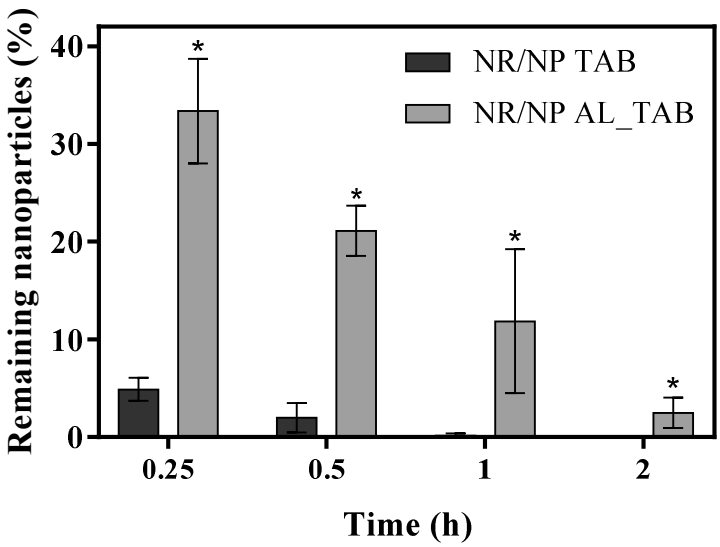


**Figure 12.** *In vivo* preocular retention profiles of NR/NP TAB and NR/NP AL_TAB after administrated on the lower conjunctiva of rabbit eye. * indicated statistical significance between the formulations at each time point ($p < 0.05$).

### 3.4 *In vivo* preocular retention evaluations

All tablets tested in the *in vivo* experiment were almost instantaneously fully detached and released from the applicator tip to the preocular surface when they were in contact with the lower cul-de-sac of rabbit eyes, as depicted in Figure 11(a). The tablets herein were disintegrated within 30 s by dissolution in lacrimal fluid when topically administered to rabbit eyes (Fig. 11(b)).

I employed NR/NP TAB and NR/NP AL_TAB, two different tablets with the Nile red-loaded nanoparticles, to examine the effect of alginate on the preocular residence time. As shown in Figure 12, the amount of nanoparticles remaining at the preocular surface increased by incorporating alginate in the tablet medium. For the tablet without alginate, relatively low percent of remaining particles was exhibited (i.e., 4.8 and 0.2% at 15 min and 1 h, respectively). However, 33 and 12% of particles remained at 15 min and 1 h, respectively, due to the presence of alginate in the tablet, and could still be detected until 2 h. As conventional eye drops would completely disappear within 5 min [33], such results suggest a greater improvement in preocular drug retention. For the DX/NP AL_TAB at 1 h, a higher variability was observed in particle retention. As the tablet medium instantaneously dissolved in lacrimal fluid, alginate in the dissolved medium interacted with $Ca^{2+}$ ions, leading increase of the tear viscosity and thus enhancement of retention at the preocular surface. However, as the tear turnover vary, continuous dilution of alginate also vary greatly depending on the subject. After 2 h, most of particles would be cleared from the preocular surface.

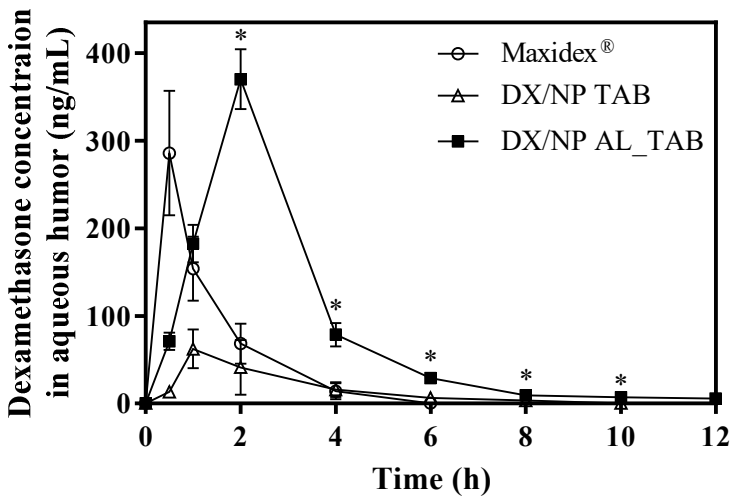


**Figure 13.** Dexamethasone concentration in the aqueous humor of rabbit eyes after topical administration of Maxidex® (○), DX/NP TAB (△), and DX/NP AL_TAB (■). * indicated statistical significance between the Maxidex® and DX/NP AL_TAB at each time point.

**Table 1.** Pharmacokinetic parameters of dexamethasone in the aqueous humor of rabbit eyes.

| **Formulations** | **$T_{max}$ (h)** | **$C_{max}$ ($ng \cdot mL^{-1}$)** | **AUC[a] ($ng \cdot h \cdot mL^{-1}$)** |
|---|---|---|---|
| Maxidex® | 0.5 | 285.93 | 388.51 |
| DX/NP TAB | 1 | 62.18 | 165.93 |
| DX/NP AL_TAB | 2 | 370.33 | 981.23 |

*[a] calculated using the trapezoidal rule.*

### 3.5 *In vivo* pharmacokinetic evaluations

The pharmacokinetic profiles of the tablet formulations were assessed and compared with that of Maxidex®, a commercially-available dexamethasone eye drops. Figure 13 and Table 1 display the drug concentration profiles in the aqueous humor and their pharmacokinetic parameters, respectively. At 30 min, Maxidex® had a maximum drug concentration ($C_{max}$) of 285.93 ng/mL; however, the drug concentration rapidly decreased to an undetectable level at 6 h after the administration. Interestingly, albeit formulated in a dry tablet, the DX/NP TAB without alginate presented a much lower drug bioavailability. In addition, area under the drug concentration-time curve (AUC) and $C_{max}$ for the DX/NP TAB were less than a half of those with Maxidex®. Such finding could be due to a lower amount of drug exposure at the eye surface with DX/NP TAB. Only a few amount of the drug within DX/NP was actually released during the early period of post-administration before the particles rapidly removed by tear fluid. On the other hand, 100% drug would be exposed on the eye surface right after a bolus administration with Maxidex®.

The DX/NP AL_TAB obtained the highest drug bioavailability. In fact, its AUC was more than 2.5-fold greater than that of Maxidex®. By incorporating alginate in the tablet, the viscosity of the tear fluid was increased, enabling a larger portion of drug-loaded particles to be retained in the preocular space for a longer time. During this period, the DX/NP would release and expose a significant amount of dexamethasone in a sustained manner, resulting gradual increase of the drug concentration in tear before being absorbed into the AH. As a result, $T_{max}$,

the time to reach maximum drug concentration, was shifted to a later point (i.e., 2 h) and the value of $C_{max}$ itself was further increased, compared to that of Maxidex®. Due to prolonged retention and sustained drug release, the clearance of the drug from the AH was delayed, resulting the drug concentration in AH statistically significantly higher than that of Maxidex® at 4 to 10 h ($p < 0.05$).

## 4. Discussion

Topical drug administration is considered to be an easy route for ophthalmic drug delivery. However, for conventional formulations of eye drops and suspensions, the additional liquid administration accelerates tear clearance to further lower drug bioavailability in the eye [34, 35]. To overcome this, a dry tablet loaded with polymeric particles had been proposed as topical drug delivery formulation. The tablet was prepared by compressing the medium of mannitol and drug-loaded microparticles composed of PLGA and a mucoadhesion promoter, PEG [36]. Thus, dissolution of tablet medium, mannitol, increased the tear viscosity allowing prolongation of interaction time between preocular mucin and PEG in particles. However, the compressed tablet with high density appeared to dissolve in lacrimal fluid in several minutes, during which undissolved tablet with relatively large size could cause eye irritation [36]. To overcome, the dry formulation in this study was prepared by lyophilization to be able to dissolve rapidly in tear fluid [9].

Herein, I proposed a rapidly-dissolving dry tablet formulation embedded with drug-loaded PLGA nanoparticles for topical drug delivery to the eye. The tablets were amorphous physical composites of long alginate polymer chains forming networks and nanoparticles fixed within the structure. When the tablet was delivered to the preocular surfaces and separated from the applicator, the tablet medium was dissolved almost immediately and freed the drug-loaded nanoparticles. Due to the presence of alginate in the tablet medium, tear viscosity increased due to the molecular interaction between alginate and calcium ion in tear.

Hampered clearance of tear led to a higher preocular retention of DX/NP (Figure 12). Residing longer in the preocular space, DX/NP could consistently release the dexamethasone to tear fluid, providing more time for drug to be adsorbed into the eye and ultimately enhancing ocular drug bioavailability (Figure 13).

In this study, I prepared the tablets by freeze-drying the DX/NP suspension in water-soluble polymer, allowing high porosity within the tablet for rapid dissolution in the tear tablet. The DX/NP was designed to be nanosized to prevent the irritation and discomfort on the sensitive eye surface [36]. Therefore, alginate content employed for the tablet herein was observed with no sign of eye irritation or discomfort during i*n vivo* experiments. By using the applicator, the tablet could be delivered without contamination, thereby hygienic application was achieved. Owing to its biocompatibility, PDMS was employed as material for the applicator, as applicator might tough the eye surface. More importantly, as hydrophobic PDMS possess a low surface release energy, the hydrophilic dry tablet could be instantaneously separated from the applicator and adhere onto the eye when wet with tear fluid as shown in Figure 11(a) [37]. In addition, since the tablet would be separated without loss, an accurate dosage of drug could be delivered to the eye using the dry tablet formulation.

The findings of this study may be further expanded to assess the pharmacodynamics profile of dexamethasone using an endotoxin-induced uveitis model, i.e., an animal model for acute uveitis in humans [38, 39]. In this animal model, the ocular inflammation in a rabbit model

could be induced by intravitreal injection of lipopolysaccharide endotoxin from *Salmonella typhimurium*. Thus, at scheduled times after our dry tablet is administered topically to the eye, the eye can be monitored using a slit lamp microscope to assess the severity of inflammation.

## 5. Conclusion

Herein, I have derived a topical ocular drug formulation consisting of a dry tablet containing viscosity enhancer alginate and drug-loaded PLGA nanoparticles to improve the bioavailability of drugs. In addition, for easy and hygienic administration, a preocular applicator was also designed to deliver the developed tablet formulation. The tablet comprising of hydrophilic polymers could be separated instantaneously from the PDMS applicator when applied on the eye because of hydrophobicity and low surface release energy of constituent applicator material.

After topical application, the tablet medium dissolved in tear fluid rapidly to release drug-loaded nanoparticles, while alginate in tablet interacted with calcium ion in the tear to enhance the tear viscosity. Consequently, the drug-loaded nanoparticles can prolong the time in ocular surface, and as doing so, particles release the drug in a sustained manner, eventually extending ocular drug availability. Therefore, I conclude that the combination of a dry tablet formulation with alginate medium, drug-loaded PLGA nanoparticles, and a preocular applicator is

a promising strategy to achieve topical accurate dose delivery of ophthalmic drugs to the eye, with extended drug availability.

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# Abstract in Korean


국문초록

안과 질환의 치료를 위해 사용되는 점안 약물은 일반적으로 용액 또는 현탁액의 형태로 국소 전달되는데, 전안부에서 눈물 순환이 매우 빠르게 이루어지기 때문에 투여된 약물이 빠르게 씻겨 나가, 5% 미만의 약물만이 안구 내 표적 조직까지 도달하는 것으로 알려져 있다. 정확한 용량의 약물을 무균 상태로 눈에 쉽게 전달하고 약물의 생체이용도를 향상시키기 위하여, 본 연구에서는 약물이 탑재된 나노 입자들이 빠르게 용해되는 알지네이트 (alginate) 미디움의 건식 타블렛과 간편한 투약을 위한 전안부용 어플리케이터가 결합된 시스템을 개발하였다.

건식 타블렛은 알지네이트와 약물이 탑재된 나노 입자가 섞인 현탁액을 동결 건조한 제형으로써, 미디움으로 사용된 다당류 폴리머 알지네이트는 눈물에 풍부하게 있는 칼슘과 가교 반응하여 눈물의 점도를 높여 나노 입자의 전안부 잔류 시간을 향상 시킬 수 있으며, 생체 적합한 고분자인 폴리락틱코글라이콜릭산 (PLGA)로 제작한 나노 입자에 코르티코스테로이드 항염증약인 덱사메타손을 탑재함으로써 약물의 서방출 효과를 가질 수 있도록 하였다. 또한, 건식 타블렛을 전안부 어플리케이터의 팁 부분에 위치시켜 핸들 부분을 잡고 투약 할 수 있도록 디자인하여 오염없이 정량의 약물을 전달할 수 있게 하였다.

약물이 탑재된 나노 입자는 solid-in-oil-in-water 에멀젼 기법으로 제조하여 85.45 $\mu$g/mg의 약물이 탑재되도록 하였으며, 나노 입자를

담지한 건식 타블렛은 *in vitro* 실험을 통해 10시간동안 약물이 서방출됨을 확인하였다. 토끼의 눈에 어플레케이터를 사용하여 국소 투약 했을 때, 건식 타블렛이 어플리케이터에서 완전히 분리되어 눈 표면에 자극없이 눈물에 용해되는 것을 관찰하였으며, 분리된 타블렛은 내부의 알지네이트로 인해 점도가 향상되는 효과로 인해 나노 입자가 2시간동안 눈 표면에 남아있음을 확인하였다. 생체 내 효능을 평가하기 위해, 토끼의 눈에 어플리케이터를 사용하여 건식 타블렛을 국소 투약한 후 방수 내에 흡수되는 약물의 농도를 시판되고 있는 덱사메타손 점안액인 Maxidex®와 비교하였을 때 약물생체이용도가 Maxidex®보다 2.5배이상 증가하였다.

본 연구를 통하여 나노 입자를 담지한 알지네이트 건식 타블렛과 전안부용 어플리케이터가 정확한 용량의 안과 약물을 위생적으로 투약 가능하게 하고, 전안부에서의 약물의 잔류 시간을 늘려 생체이용도를 향상 시킬 수 있음을 증명하였다. 그러므로, 기존의 잦은 투약과 부정확한 용량의 안과 약물 투약으로 인한 부작용을 방지하고 치료의 효과와 환자의 편의성을 모두 증진 시킬 수 있다는 점에서 큰 의미가 있음을 확인하였다.

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**주 요 어 :** 폴리락틱코글라이콜릭산, 나노입자, 알지네이트, 안과 약물 전달, 서방형 약물 방출, 덱사메타손

**학 번 :** 2018-24001

공학석사 학위논문

# STUDY ON DRY TABLET FORMULATION CONTAINING POLYMERIC NANOPARTICLES WITH A PREOCULAR APPLICATOR FOR OPHTHALMIC DRUG DELIVERY

전안부 약물 전달을 위한 어플리케이터에
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2020년 8월

서울대학교 대학원

공과대학 협동과정 바이오엔지니어링 전공

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지도교수 최 영 빈

이 논문을 공학석사 학위논문으로 제출함

2020년 7월

서울대학교 대학원

공과대학 협동과정 바이오엔지니어링 전공

류 우 미

류우미의 석사 학위논문을 인준함

2020년 6월

위 원 장 김 영 수 (인)

부 위 원 장 최 영 빈 (인)

위 원 이 정 찬 (인)

# STUDY ON DRY TABLET FORMULATION CONTAINING POLYMERIC NANOPARTICLES WITH A PREOCULAR APPLICATOR FOR OPHTHALMIC DRUG DELIVERY

BY

**WOO MI RYU**

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Bioengineering

INTERDICIPLINARY PROGRAM IN BIOENGINEERING

THE GRADUATE SCHOOL

SEOUL NATIONAL UNIVERSITY

JUNE 2020

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| Vice chairperson | *Young Bin Choy, Ph. D.* |
| Member | *Jung Chan Lee, Ph. D* |

## Abstract

# STUDY ON DRY TABLET FORMULATION CONTAINING POLYMERIC NANOPARTICLES WITH A PREOCULAR APPLICATOR FOR OPHTHALMIC DRUG DELIVERY

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Topical administration in a form of solution and suspension is a common route for ophthalmic drug delivery. However, due to rapid tear clearance, less than 5% of the administrated drug can reach the ocular interior target tissue. In order to deliver an accurate dosage of the drug and enhance ocular drug bioavailability, I propose a combined system of a rapidly-dissolving dry alginate tablet containing drug-loaded poly (lactic-co-glycolic acid) (PLGA) nanoparticles and an ease-of-use preocular applicator. The dry tablet herein was prepared via lyophilization of dexamethasone-loaded PLGA nanoparticle in the alginate medium on

the tip of the PDMS applicator.

The nanoparticles prepared by solid-in-oil-in-water emulsion were loaded with 85.45 μg/mg dexamethasone, and the dry tablet exhibited a controlled drug release for 10 h. To evaluate *in vivo* efficacy, after topical administration of the dry tablet to rabbit eye with the preocular applicator, dexamethasone concentration in the aqueous humor was measured and compared to that of Maxidex®, a commercially-available dexamethasone eye drops. When applied to the rabbit eye, the dry tablet was completely detached from the applicator and dissolved in tear on the eye surface. The nanoparticles remained on the preocular space up to 2 h due to enhancement of tear viscosity from the interaction between alginate in tablet and calcium ion in the tear, resulting in more than 2.5-fold increase in ocular drug bioavailability compared to that of Maxidex®.

Through this study, I envision that the preocular applicator combined with the dry alginate tablet containing drug-loaded PLGA nanoparticles can be a promising system for aseptically delivering an accurate dose of the ophthalmic drug with enhanced bioavailability.

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**Keywords** : PLGA, Nanoparticles, Dexamethasone, Alginate, Ophthalmic drug delivery, Sustained drug release,

**Student Number** : 2018-24001

# Table of Contents

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# 1. Introduction

## 1.1 Background knowledge

Topical administration in a form of solution and suspension is a widely preferred and common route for ophthalmic drug delivery for its ease of administration. However, due to rapid tear clearance and blinking, the entry of the drug into the interior of the eye is highly restricted [1, 2]. After topical instillation, a major portion of the drug is rapidly removed from the preocular surfaces due to lacrimation and tear turnover within a few minutes, leading to a short drug residence time [3]. Moreover, to reach the intraocular tissues, the remaining drug on the preocular surface should penetrate the stratified corneal tissue comprising of 5 to 7 layers of epithelial cells [4]. Ultimately, less than 5% of topically administrated is delivered to the intraocular target tissue, resulting in limited ocular drug bioavailability [5, 6].

To enhance ocular drug bioavailability, it is necessary to extend the precorneal drug residence time in the cul-de-sac to enable prolonged the drug adsorption. Ophthalmic drug carriers, such as micro and nanoparticles, have been suggested to prolong drug retention on the preocular space of the eye and release the drug in a sustained manner [3]. However, when delivered in a form of a suspension, the loaded drug could be released from its carrier when dispersed in the aqueous medium [7]. Furthermore, an additional fluid would expedite solution drainage into the nasolacrimal duct, promoting the clearance of drug-loaded particles [2, 8]. Therefore, dry tablet formulation has been suggested as an alternative strategy. The drug carrier would be formed in a dry tablet

formulation, which would be dissolved in tear and release the drug-loaded particles on the eye surfaces [9]. As the tablet medium dissolves the tear viscosity would increase and delay tear clearance, eventually improving the preocular residence time of the drug. Nevertheless, the administration of a dry tablet might irritate the sensitive eye causing inconvenience for patients. Besides, as the tablet would be fetched with the bare fingers for application, the eye would be exposed to a great risk of bacterial and fungal infections which might cause serious diseases such as corneal ulceration [10-12]. These complications demonstrated the need of a better tablet formulation and a proper applicator to deliver the drug hygienically.

## 1.2 Strategy

Herein, I proposed a combined system of a rapidly-dissolving dry tablet containing drug-loaded nanoparticles with an ease-of-use preocular applicator. The applicator was designed to consist of two sections, a handle and a tablet-loading tip. When administrated, the dry tablet on the applicator tip would touch the preocular surfaces to deliver the formulation while the handle is held. This would allow an easy and, more importantly, aseptic topical administration of the dry tablet.

To test the proposed system and strategy, I prepared a preocular applicator using the biocompatible polydimethylsiloxane (PDMS) in this study. The applicator was designed to be similar shape and dimension to that of the commercially-available, single-use applicator of artificial tear fluid for patients' familiarity [13]. The applicator consists of two parts: a tip where a dry tablet formulation is loaded and a handle where patient could hold while applying. For the sustained drug release formulation, I prepared poly(lactic-co-glycolic acid) (PLGA) nanoparticles to be loaded with dexamethasone, a widely used corticosteroid drug for the eye inflammation treatment [14, 15]. To produce the rapidly-dissolving tablet, the nanoparticles were dispersed in a solution containing a mixture of polyvinyl alcohol (PVA), and alginate, which was then freeze-dried on top of the applicator tip. PLGA has been widely used for polymeric drug delivery for its good biocompatibility and biodegradability to produce non-toxic by-products [16, 17]. PVA has been approved for clinical use in various ophthalmic drug products [18-20]. In this study, I also exploited highly-biocompatible alginate in the

tablet medium as a viscosity enhancer of tear fluids. Alginate is a polysaccharide derived polymer [21] that can be crosslinked in the presence of the multivalent cations to form a gel [22, 23]. Since the dry tablet was a physical composite of the alginate polymer chains with drug-loaded nanoparticles held within the structure, when delivered to the preocular surfaces, the tablet dissolved in the $Ca^{2+}$-abundant tear fluid, and the alginate could increase tear viscosity, thereby synergistically extending the preocular residence time of the drug-loaded nanoparticles.

Dexamethasone-loaded PLGA nanoparticles (DX/NP) were prepared by the solid-in-oil-in-water (S/O/W) emulsion method and characterized by scanning electron microscopy (SEM) and dynamic light scattering (DLS), which were employed to assess nanoparticle size and morphology. The DX/NP was examined for its cytotoxicity using human corneal epithelial cells (HCECs). To examine the effect of alginate on tear viscosity enhancement, two distinct tablet formulations containing DX/NP were prepared with two different tablet media containing both PVA and alginate (DX/NP AL_TAB) and only PVA (i.e., without alginate) (DX/NP TAB), respectively. For *in vivo* experiments, these tablet formulations were applied to rabbit eyes, and drug concentration in the aqueous humor was assessed and compared to the commercially-available ophthalmic dexamethasone medication, Maxidex®, (i.e., an aqueous suspension of dexamethasone itself).

## 2. Materials and Methods

### 2.1. Materials

PLGA (lactic acid:glycolic acid = 50:50; i.v. = 48,000) was purchased from Evonik Industry (Germany). Dexamethasone and Nile red were purchased from Tokyo Chemical Industry (Japan). PVA (87-89% hydrolyzed), phosphate buffered saline (PBS) tablets, tween 80, trifluoroacetic acid (TFA; >99%), and calcium chloride dihydrate (>99%) were obtained from Merck (USA). Dichloromethane (DCM; >99.5%), N,N-dimethylformamide (DMF; >99.5%), and acetone (>99.5%) were supplied by DaeJung (Korea). Acetonitrile (ACN; >99.9%) was purchased from J.T. Bakers (USA). PDMS (Sylgard 184) was obtained from Sewang Hitech Silicone (Korea). Alcaine® (0.5% proparacaine hydrochloride ophthalmic solution) and Maxidex® (0.1% dexamethasone ophthalmic suspension) were purchased from Alcon-Couvreur (Belgium).

### 2.2. Preparation of dexamethasone-loaded nanoparticles

I prepared dexamethasone-loaded nanoparticles via S/O/W emulsification, as shown in Figure 1 [24]. In short, 300 mg PLGA and 100 mg dexamethasone were both dissolved in 7 mL DCM. The resulting solution was then added to 8 mL of 1% w/v PVA solution, which was emulsified with a homogenizer (Sonic Dismembrator Model 500, Fisher Scientific, France) at 160 W for 10 min. The emulsion was transferred into 100 mL of 1% w/v PVA solution at room temperature and stirred at 400 rpm under vacuum (-10 psi) for 60 min for solvent evaporation. To

optimize the size of the nanoparticles, the nanoparticles were prepared with three different centrifugal filtration speed at 0, 200, or 500 rpm for 10 min. After the filtration, the precipitates were eliminated and only the suspension at the top was collected. The collected suspension was then washed with deionized (DI) water three times via centrifugation at 13,000 rpm for 10 min. Thereafter, the suspension was freeze-dried for 20 h to obtain dry dexamethasone-loaded nanoparticles. PLGA nanoparticles loaded with Nile red (NR/NP) were also prepared to evaluate the *in vivo* preocular retention properties. As such, 5 mg Nile Red and 500 mg PLGA were dissolved in 7 mL DCM for emulsification.

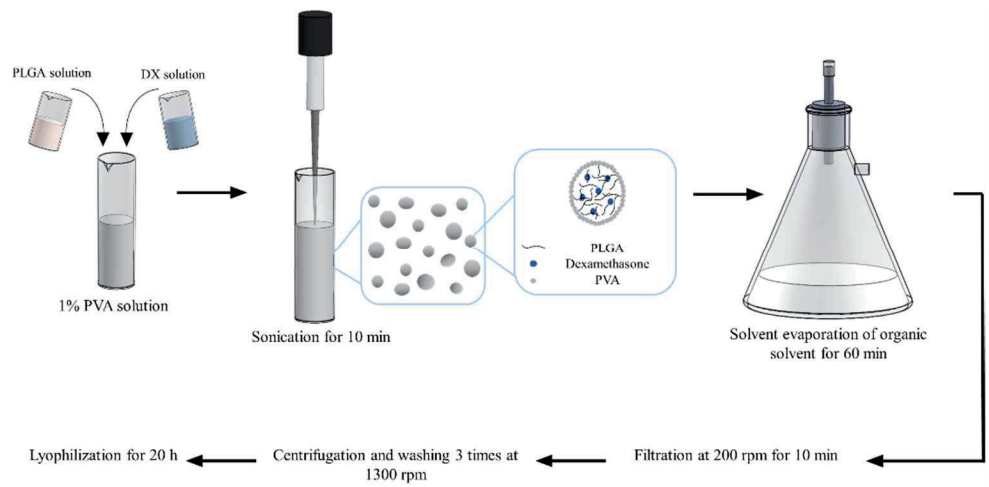


**Figure 1.** Illustrations of preparation of PLGA nanoparticles.

### 2.3. Characterizations of nanoparticles

The morphology of DX/NP was examined by SEM (JSM-7800F Prime, JEOL, Japan). Its size distribution and surface charge were determined using DLS (ELS-2000ZS, Otsuka Electronics, Japan) and a zetasizer (Nano ZS, Malvern, UK) with a particle suspension prepared in DI water [25]. To measure the dexamethasone loading amount, 4 mg of DX/NP was fully dissolved in 4 mL of N,N-dimethylformamide. The supernatant was then diluted with ACN in a 1:1 ratio. Drug concentration in each resulting solution was measured using high-performance liquid chromatography (HPLC; Agilent 1260 series, Agilent Technologies, USA) with a Diamonsil C18 column (5 μm, 150 x 4.6 mm). Column temperature and absorbance wavelength were set at 37 °C and 240 nm, respectively. Injection volume and flow rate were 20 μL and 1.5 mL/min, respectively. The mobile phase was comprised of 0.1% TFA and ACN mixed in a 65:35 ratio. Given with the loading amount of the DX/NP, the encapsulation efficiency (EE) was calculated by the following equation:

$$\text{EE}\ (\%) = \frac{\text{Amount of drug loaded in nanoparticles}}{\text{Initial amount of drug}}\ \text{x}\ 100 \quad (1)$$

To optimize the nanoparticles for the ocular drug delivery, the drug release profiles of all obtained nanoparticles were examined. 5 mg of each nanoparticles prepared with different centrifugal filtration were each suspended in 3 mL of pH 7.4 phosphate buffered saline (PBS) with 0.5% w/v Tween 80. Each suspension was transferred in a dialysis

membrane bag (SnakeSkinTM Dialysis Tubing, 10 kDa, Thermo Scientific, USA), and immersed in 50 mL of the medium. As incubated at 37 °C, at designated time, 5 mL of the release medium was collected and the same volume of fresh medium was replaced. The amount of dexamethasone in collected medium was measured using HPLC as described above. The optimized nanoparticles were named DX/NP and used for further experiments.

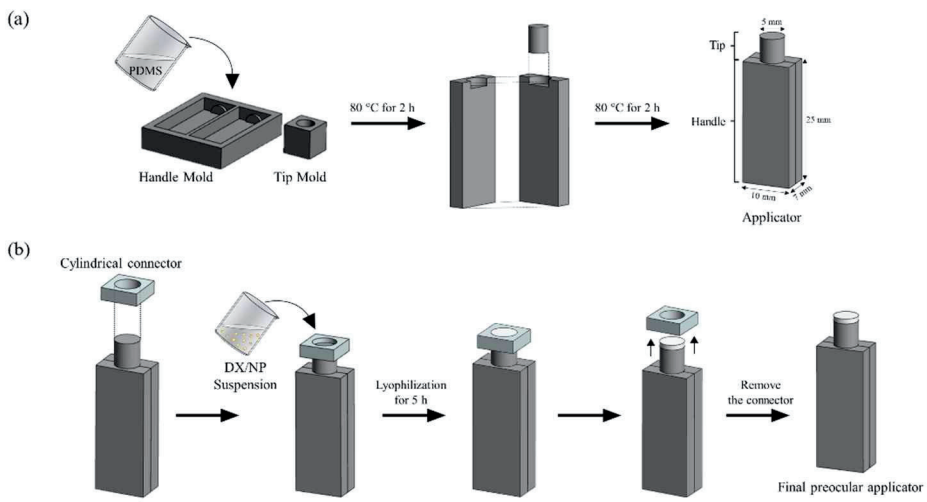


**Figure 2**. Illustrations of fabrication of the DX/NP loaded tablet on PDMS preocular applicator. (a) Fabrication of PDMS preocular applicator. (b) Tablet preparation on the tip of the applicator.

### 2.4. Preparation of dry tablet-loaded preocular applicators

A preocular applicator with a dry tablet on the tip was prepared, as depicted in Figure 2. To prepare a preocular applicator, two different molds were first made to fabricate a handle and tip (Fig. 2(a)). In the molds, a mixture of a PDMS base and its curing agent (10:1, v/v) was poured and cured slightly at 80 °C for 2 h. Then, each of the constituent pieces was assembled and cured further at 80 °C for another 2 h to allow for their bonding. In this work, I produced two different tablets, with media containing both PVA and alginate, and PVA alone, to yield DX/NP AL_TAB and DX/NP TAB, respectively. To prepare the DX/NP AL_TAB, 20 mg/mL DX/NP were suspended in a solution containing 0.1% w/v PVA and 2% w/v alginate. Above this alginate concentration, the solution became too viscous to properly distribute the DX/NP. To prepare the DX/NP TAB, 20 mg/mL DX/NP were suspended in a solution of 0.1% w/v PVA only. Twenty μL of the resulting suspension was then poured in the reservoir that was made by tightly fitting a cylindrical connector with open ends to a tip of the applicator (Fig. 2(b)). The whole piece was then rapidly frozen using liquid nitrogen, which was then lyophilized at 0.01 bar at – 80 °C for 5 h (FreeZone 6 Dryer system, Labconco, USA) [26, 27]. After carefully removing the connector, the preocular applicator loaded with the dry tablet formulation was completely prepared for the experiments. To evaluate the *in vivo* preocular retention properties, NR/NP was embedded instead of DX/NP to produce tablets of NR/NP AL_TAB and NR/NP TAB, prepared under the same condition employed for DX/NP AL_TAB and DX/NP TAB,

respectively.

### 2.5. Characterizations of dry tablets

The drug loading amount was measured by fully submerging the tablet at the applicator tip in 1 mL DMF. To completely extract the drug, the solution with the tablet was sonicated for 2 h and then centrifuged at 13,000 rpm to collect the supernatant, which was then diluted with ACN in a 1:1 ratio. The concentrations of the drug were measured using HPLC as described above.

The thicknesses of the tablets herein were measured using a caliper (ABSOLUTE Digimatic Caliper, Mitutoyo, Japan). To assess the particle distribution in the tablet, the NR/NP TAB and NR/NP AL_TAB were imaged with a fluorescence microscope (Leica DMI4000 B, Leica Microsystems, Germany). To examine the effect of alginate in tablet medium, DX/NP AL_TAB and DX/NP TAB were each immersed in 1 mL of the tear fluid mimicking medium, i.e., PBS containing $Ca^{2+}$ (10 mM, pH 7.4, $[Ca^{2+}]$ = 39.4 μg/mL) [28], for 10 min at 37 °C. The viscosity of the resulting solution was then measured using a rheometer (Advanced Rheometric Expansion System, Rheometric Scientific, USA), where gap separation, temperature, and shear rate were set at 0.8 mm, 25 °C, and 100 $s^{-1}$, respectively.

### 2.6. *In vitro* evaluations

#### 2.6.1. Drug release profiles of formulations

To examine the *in vitro* drug release profiles, 400 μg DX/NP, two different tablets containing the DX/NP (i.e., DX/NP TAB and DX/NP AL_TAB), and Maxidex®, all of which contained the same amount of about 35 μg dexamethasone were each placed in a dialysis membrane bag (SnakeSkinTM Dialysis Tubing, 10 kDa, Thermo Scientific, USA). The bag was then immersed in 5 mL pH 7.4 PBS containing 39.4 μg/mL $Ca^{2+}$ and 0.5% w/v Tween 80 to meet the sink condition of dexamethasone [29]. While being incubated at 37 °C, at scheduled times, 1 mL of the release medium was collected and the same volume of fresh medium was replaced. The amount of released dexamethasone was measured using HPLC as described above.

#### 2.6.2. Cytotoxicity study

The *in vitro* cytotoxicity of DX/NP was evaluated using HCECs (PCS-700-010, ATCC, USA). HCECs were grown in a corneal epithelial cell basal medium (PCS-700-030, ATCC, USA) with supplements (PCS-700-040, ATCC, USA) at 37 °C in a humidified environment with 5% $CO_2$. Prior to the assay, HCECs were seeded in a 96-well plate at $1x10^5$ cells/well and grown for 24 h. Subsequently, 100 μL of the DX/NP suspension, which was prepared in the cell growth medium at concentrations of 5, 10, 25, 50, 100, 250, 500, and 1000 μg/mL, was added to each well and incubated at 37 °C for 24 h. The medium was then completely removed and replaced with 100 μL of fresh medium.

Thereafter, 10 μL of an EZ-Cytox solution was added to each well and incubated at 37 °C for 2 h under dark conditions. Cell viability was measured using a microplate reader, with absorbance and reference wavelengths of 450 nm and 600 nm, respectively (VersaMax ELISA Microplate Reader; Molecular Devices, USA).

### 2.7. *In vivo* experiments

*In vivo* experiments were conducted with the healthy eyes of male New Zealand White rabbits (weight 2.1-2.5 kg). Rabbits were granted free access to food and water and were housed in a controlled environment: temperature; 21 ± 1 °C, humidity; 55 ± 1%, and light/dark cycle; 12 h/12 h. The *in vivo* experimental protocols were approved by the Institutional Animal Care and Use Committee at Seoul National University Hospital (IACUC No. 19-0133).

#### 2.7.1. Preocular retention study

The *in vivo* preocular retention properties of the nanoparticles were evaluated after topical administration of Nile red nanoparticles to the rabbit eye. With the NR/NP AL_TAB and NR/NP TAB, the *in vivo* profile of tablet disintegration was assessed. For this, the eye was imaged using a digital camera (Galaxy S10, Samsung, Korea) at 0 and 30 s after topically administrate the tablet to the rabbit eyes. The preocular retention properties were assessed as reported in previous studies, with slight modifications [30, 31]. Briefly, the Nile red-loaded tablets, NR/NP AL_TAB or NR/NP TAB, on the applicator tip was applied directly on the lower cul-de-sac of the rabbit's left eye. At scheduled times, the

rabbit eye was topically anesthetized by topical administration of a drop of Alcaine® and the entire preocular surface was thoroughly wiped with a surgical sponge (PVA spear; Sidapharm, Greek) to collect the remaining NR/NP. The sponge was then fully submerged in 5 mL DMF and sonicated for 2 h to fully extract the NR/NP. The amount of Nile red in the sample was measured using HPLC-mass spectroscopy (LC-MS) with a Polaris 5 C18-A (2.7 μm pore size, 4.6 x 150 mm) and the following conditions: column temperature; 30 °C, absorbance wavelength; 243 nm, injection volume; 20 μL, and flow rate; 0.45 mL/min. The mobile phase consisted of 0.1% formic acid and ACN in the ratio, 55:45. For statistics, at each time point, four animals (i.e., one left eye for each rabbit) were assigned for each formulation.

### 2.7.2. Pharmacokinetic study

To assess the *in vivo* ocular drug bioavailability, each of the three formulations (i.e., 35 μL Maxidex®, the DX/NP AL_TAB, and DX/NP TAB in the applicator) with the same dose of dexamethasone (c.a. 35 μg of dexamethasone) was directly administered onto the lower cul-de-sac of rabbit eyes. At scheduled times, the rabbit was anesthetized with a subcutaneous injection of a cocktail containing 20 mg/kg ketamine and 10 mg/kg xylazine. Thereafter, approximately 100 μL of aqueous humor (AH) was collected using a 31 G needle (BD Ultra-Fine II, Becton Dickinson and Company, USA). Drug concentration in AH was analyzed by LC-MS as described above. For statistics, three animals (i.e., the left eye of each rabbit) were assigned per time point for each formulation.

### 2.8. Statistical Analysis

Statistical analysis was performed using the amount of particles remaining on the preocular surface and drug concentration in AH by the Mann-Whitney U-test. A p-value $<0.05$ was considered to indicate statistical significance (SPSS version 22, IBM, USA).

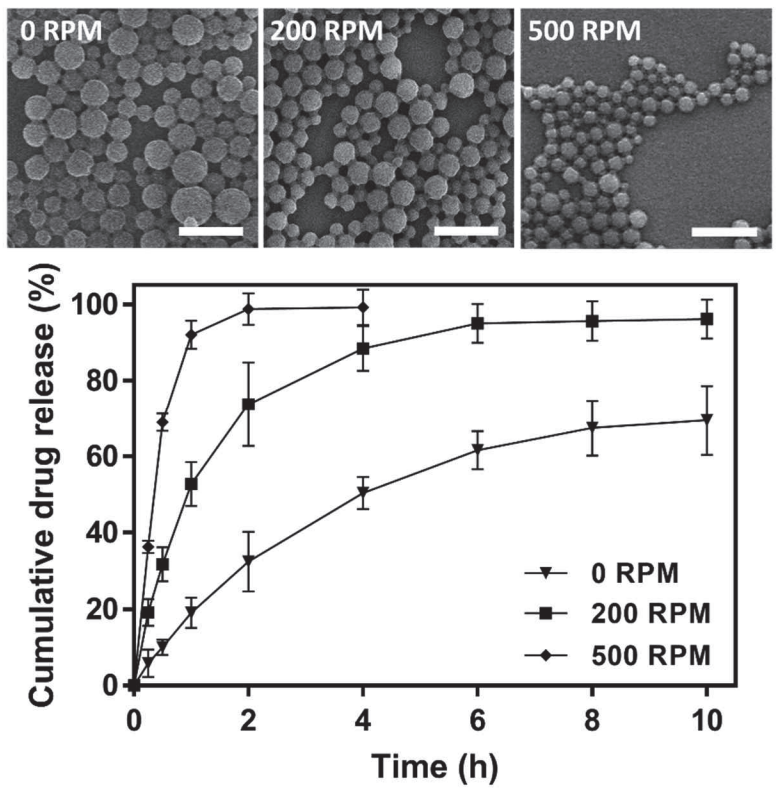


**Figure 3.** Representative scanning electron microscope image of nanoparticles filtered at 0 rpm (unfiltered), 200 rpm, and 500 rpm, and their *in vitro* drug release profiles.

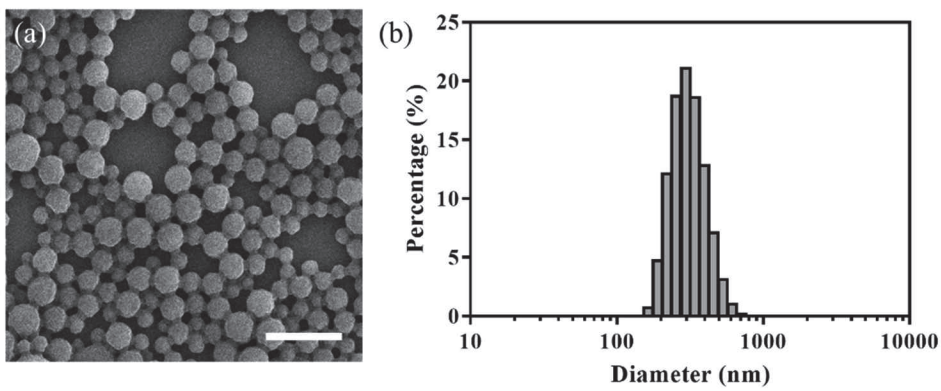


**Figure 4.** Representative scanning electron microscope image and particle size distribution of DX/NP. Scale bar = 1 μm. Polydispersity index = 0.056.

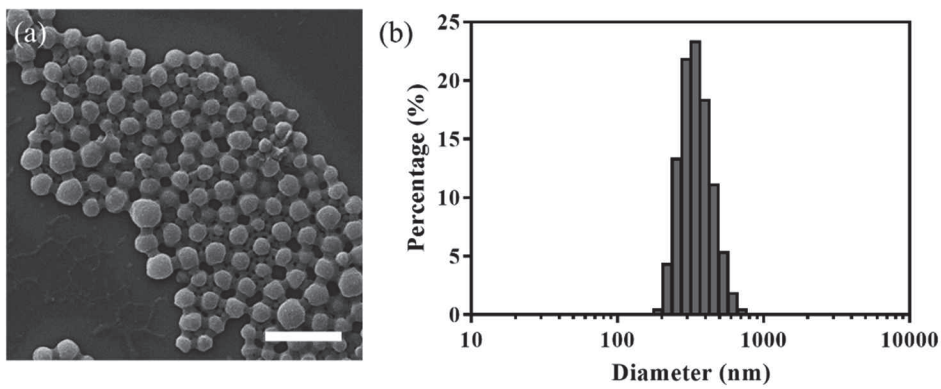


**Figure 5.** Representative scanning electron microscope image and particle size distribution of NR/NP. Scale bar = 1 μm. Polydispersity index = 0.158.

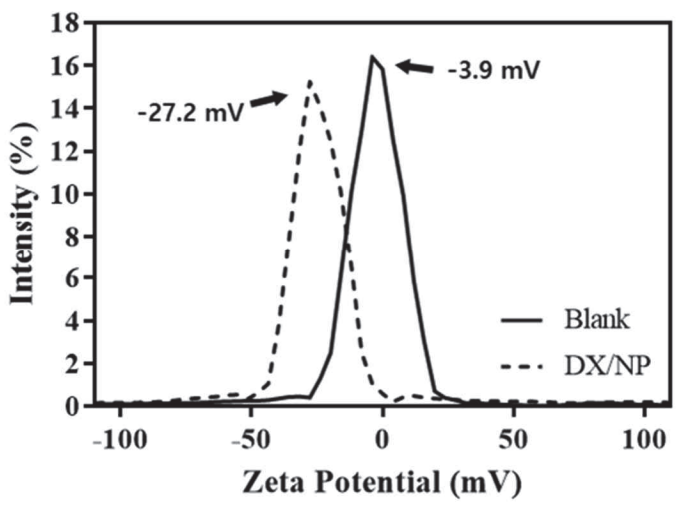


**Figure 6.** Zeta potentials of the blank PLGA nanoparticles and DX/NP.

## 3. Results

### 3.1 Characterizations dexamethasone-loaded nanoparticles

I prepared dexamethasone-loaded nanoparticles using the S/O/W emulsion method. To optimize the PLGA nanoparticles for the tablet formulation, three different particles were prepared with different centrifugal filtration speeds of 0 rpm (unfiltered), 200 rpm, and 500 rpm (i.e., unfiltered, 200 RPM, and 500 RPM). The nanoparticles were found to be in spherical shape, as shown in Figure 3. Unfiltered nanoparticle exhibited the diameter of 400-500 nm, whereas relatively large particles were eliminated with 200 RPM and 500 RPM, showing the diameters of 300-400 nm and 100-150 nm, respectively. The loading amount of unfiltered, 200 RPM, and 500 RPM were measured to be 344.51 ± 8.46, 85.45 ± 5.44, and 9.04 ± .041 μg/mg, respectively.

The drug release profiles of three nanoparticles were obtained to optimize nanoparticles for formation of ocular dry tablet. The drug amount in a tablet was designed to be 35 μg, which is a single-dose of commercialized dexamethasone suspension Maxidex®. However, because the unfiltered particles could load large amount of drug, only small amount of unfiltered particles could be loaded in a tablet, resulting in greater loss of drug in case of particle clearance from the preocular area. The unfiltered and 200 RPM showed sustained release for 10 h, while 500 RPM displayed 100% release at 4 h. The drug release profile of unfiltered showed longer controlled release. The 500 RPM also was not suitable due to its small drug loading amount, as too many particles were required to be loaded in a tablet to reach target drug amount.

Therefore, the 200 RPM was selected to be the optimal nanoparticle for this study as it loaded suitable dexamethasone amount for tablet formation. Further analysis were conducted with 200 RPM, hereafter called DX/NP. Particle diameter of DX/NP measured using DLS was 336.92 ± 5.56 nm (Fig. 4(b)) while encapsulation efficiency was 25.6%.

The size and morphology of NR/NP were determined to be similar to those of DX/NP (Fig. 5(a) and (b)). As shown in Figure 6, the zeta potential of the blank PLGA nanoparticles was measured to be - 3.9 mV, which was shifted to -27.2 mV with the DX/NP due to a negative charge of the encapsulated dexamethasone [32].

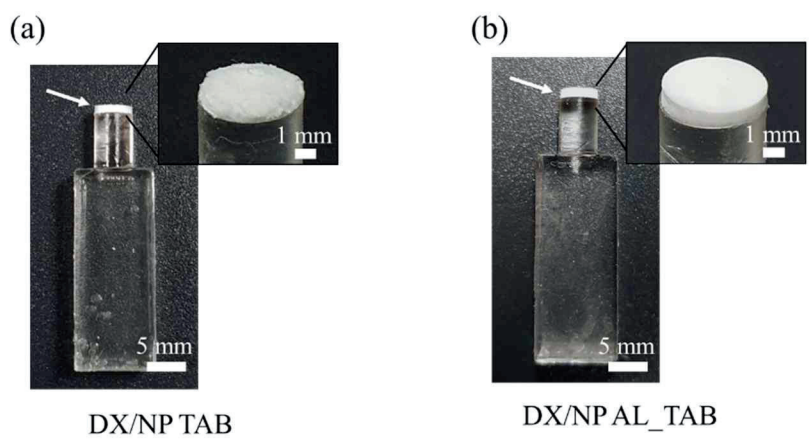


**Figure 7.** Representative optical images of the dry tablet formulation containing drug-loaded nanoparticles on the tip of the applicators. (a) DX/NP TAB. (b) DX/NP AL_TAB.

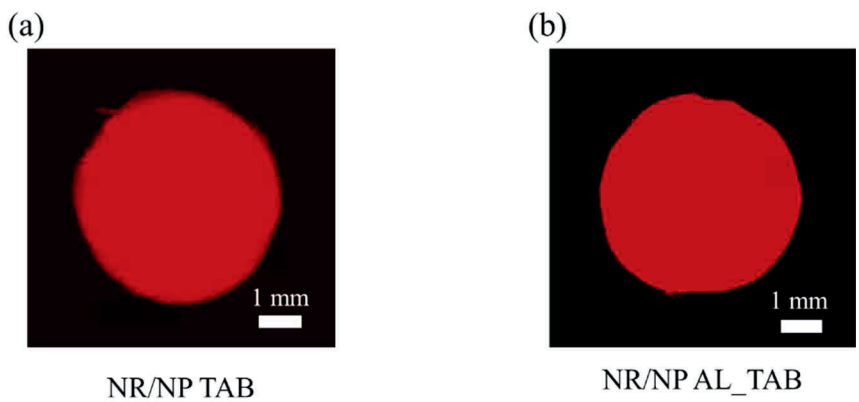


**Figure 8.** Representative fluorescence images of dry tablet formulation containing Nile Red-loaded nanoparticles. (a) NR/NP TAB. (b) NR/NP AL_TAB.

### 3.2 Characterizations of dry tablet formulation

The dry tablet formulation loaded on the tip of the applicator was prepared and characterized. Figure 7 shows the tablets prepared in the present experiment (i.e., the DX/NP TAB and DX/NP AL_TAB). The tablets displayed a cylindrical shape and were well deposited at the tip of applicator. As shown in Figure 8, evenly distributed fluorescent signal were exhibited throughout both NR/NP tablets, implying that the particles were homogeneously distributed within the tablet when prepared with this method. The same amount of DX/NP was embedded during tablet preparation with loading amount of 34.11 ± 0.48 and 34.89 ± 0.28 μg for DX/NP TAB and DX/NP AL_TAB, respectively. For the same reason, the thicknesses of the DX/NP TAB and DX/NP AL_TAB were also measured to be similar (1.02 ± 0.03 and 1.02 ± 0.06 mm, respectively). When tablets were dissolved in $Ca^{2+}$-containing PBS, the solution with DX/NP AL_TAB containing 400 μg alginate had a higher viscosity of 0.93 Pa s than that with DX/NP TAB without alginate of 0.01 Pa s.

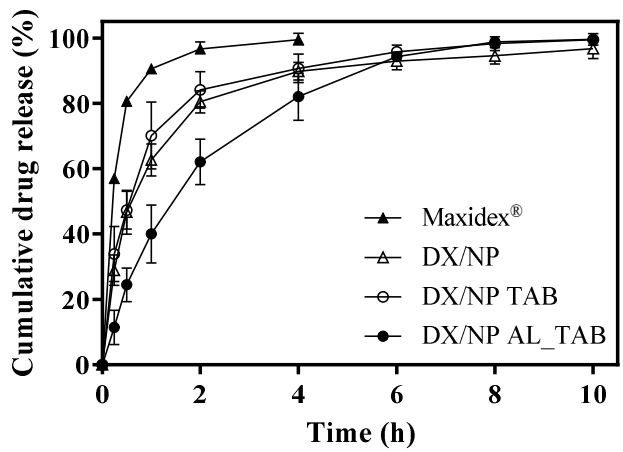


**Figure 9.** *In vitro* drug release profiles of Maxidex® (▲), DX/NP (△), DX/NP TAB (○), and DX/NP AL_TAB (●).

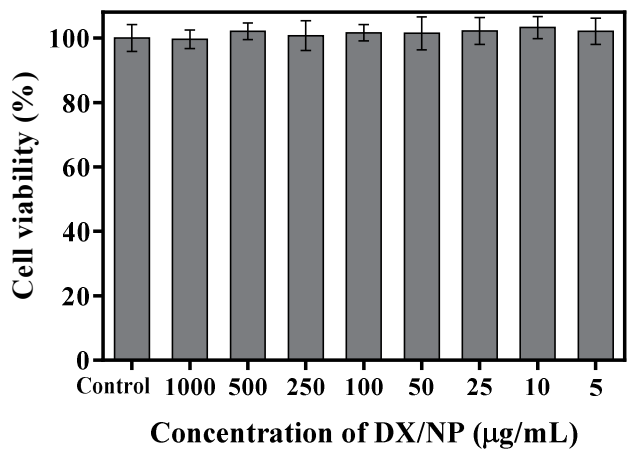


**Figure 10.** Cytotoxicity of DX/NP on human primary corneal epithelial cells.

### 3.3 *In vitro* experimental results

The *in vitro* drug release profiles of the DX/NP and both DX/NP tablets, shown in Figure 9, displayed controlled drug release for 10 h. Among the formulations, the release profiles did not differ much because the tablet medium were rapidly dissolved and almost immediately freed DX/NP. With DX/NP AL_TAB, the release of dexamethasone was slightly suppressed, which could be caused by viscosity increase of the medium in a dialysis membrane bag from the interaction between $Ca^{2+}$ in tear and alginate. A suspension of dexamethasone, Maxidex®, was exhibited a complete dissolution in 2 h. Cytotoxicity of the DX/NP was tested with human corneal epithelial cells. The nanoparticles exhibited greater than 90% of cell viability at all testing concentrations (Figure 10), suggesting that the DX/NP was not toxic to living cells.

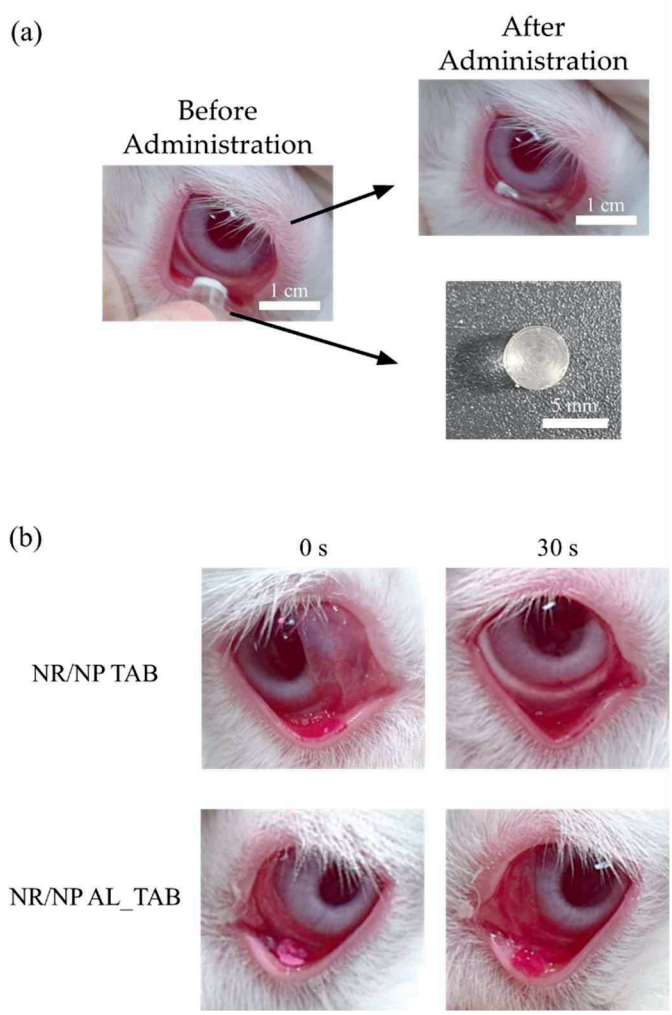


**Figure 11.** Representative images of the tablet application on the rabbit eyes. (a) Images of before and after topical administration of the DX/NP TAB and DX/NP AL_TAB using the applicator herein. (b) Representative disintegration profiles of NR/NP TAB and NR/NP AL_TAB topically administrated on the lower conjunctiva of rabbit eye.

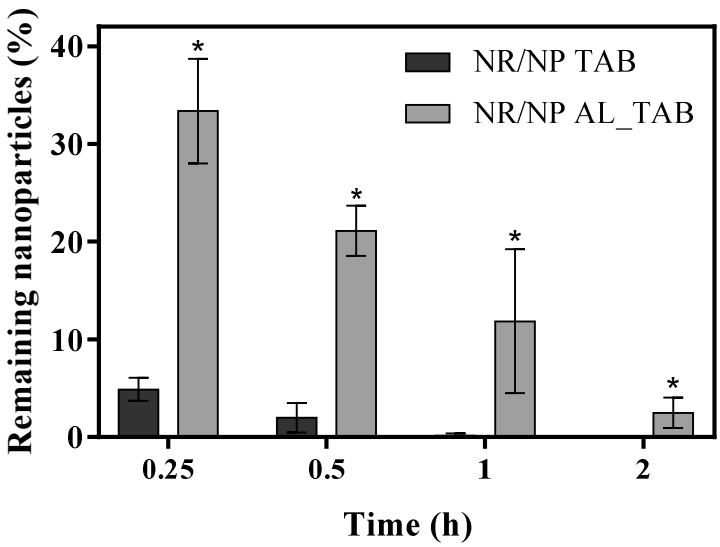


**Figure 12.** *In vivo* preocular retention profiles of NR/NP TAB and NR/NP AL_TAB after administrated on the lower conjunctiva of rabbit eye. * indicated statistical significance between the formulations at each time point ($p < 0.05$).

### 3.4 *In vivo* preocular retention evaluations

All tablets tested in the *in vivo* experiment were almost instantaneously fully detached and released from the applicator tip to the preocular surface when they were in contact with the lower cul-de-sac of rabbit eyes, as depicted in Figure 11(a). The tablets herein were disintegrated within 30 s by dissolution in lacrimal fluid when topically administered to rabbit eyes (Fig. 11(b)).

I employed NR/NP TAB and NR/NP AL_TAB, two different tablets with the Nile red-loaded nanoparticles, to examine the effect of alginate on the preocular residence time. As shown in Figure 12, the amount of nanoparticles remaining at the preocular surface increased by incorporating alginate in the tablet medium. For the tablet without alginate, relatively low percent of remaining particles was exhibited (i.e., 4.8 and 0.2% at 15 min and 1 h, respectively). However, 33 and 12% of particles remained at 15 min and 1 h, respectively, due to the presence of alginate in the tablet, and could still be detected until 2 h. As conventional eye drops would completely disappear within 5 min [33], such results suggest a greater improvement in preocular drug retention. For the DX/NP AL_TAB at 1 h, a higher variability was observed in particle retention. As the tablet medium instantaneously dissolved in lacrimal fluid, alginate in the dissolved medium interacted with $Ca^{2+}$ ions, leading increase of the tear viscosity and thus enhancement of retention at the preocular surface. However, as the tear turnover vary, continuous dilution of alginate also vary greatly depending on the subject. After 2 h, most of particles would be cleared from the preocular surface.

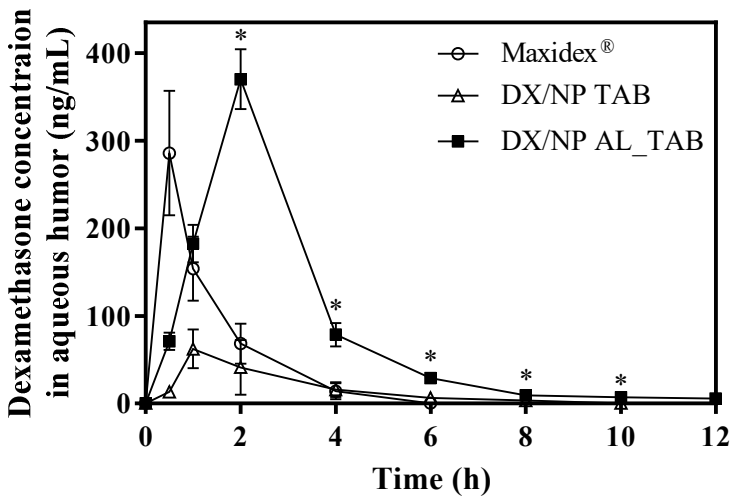


**Figure 13.** Dexamethasone concentration in the aqueous humor of rabbit eyes after topical administration of Maxidex® (○), DX/NP TAB (△), and DX/NP AL_TAB (■). * indicated statistical significance between the Maxidex® and DX/NP AL_TAB at each time point.

**Table 1.** Pharmacokinetic parameters of dexamethasone in the aqueous humor of rabbit eyes.

| **Formulations** | **$T_{max}$ (h)** | **$C_{max}$ ($ng \cdot mL^{-1}$)** | **AUC[a] ($ng \cdot h \cdot mL^{-1}$)** |
|---|---|---|---|
| Maxidex® | 0.5 | 285.93 | 388.51 |
| DX/NP TAB | 1 | 62.18 | 165.93 |
| DX/NP AL_TAB | 2 | 370.33 | 981.23 |

*[a] calculated using the trapezoidal rule.*

### 3.5 *In vivo* pharmacokinetic evaluations

The pharmacokinetic profiles of the tablet formulations were assessed and compared with that of Maxidex®, a commercially-available dexamethasone eye drops. Figure 13 and Table 1 display the drug concentration profiles in the aqueous humor and their pharmacokinetic parameters, respectively. At 30 min, Maxidex® had a maximum drug concentration ($C_{max}$) of 285.93 ng/mL; however, the drug concentration rapidly decreased to an undetectable level at 6 h after the administration. Interestingly, albeit formulated in a dry tablet, the DX/NP TAB without alginate presented a much lower drug bioavailability. In addition, area under the drug concentration-time curve (AUC) and $C_{max}$ for the DX/NP TAB were less than a half of those with Maxidex®. Such finding could be due to a lower amount of drug exposure at the eye surface with DX/NP TAB. Only a few amount of the drug within DX/NP was actually released during the early period of post-administration before the particles rapidly removed by tear fluid. On the other hand, 100% drug would be exposed on the eye surface right after a bolus administration with Maxidex®.

The DX/NP AL_TAB obtained the highest drug bioavailability. In fact, its AUC was more than 2.5-fold greater than that of Maxidex®. By incorporating alginate in the tablet, the viscosity of the tear fluid was increased, enabling a larger portion of drug-loaded particles to be retained in the preocular space for a longer time. During this period, the DX/NP would release and expose a significant amount of dexamethasone in a sustained manner, resulting gradual increase of the drug concentration in tear before being absorbed into the AH. As a result, $T_{max}$,

the time to reach maximum drug concentration, was shifted to a later point (i.e., 2 h) and the value of $C_{max}$ itself was further increased, compared to that of Maxidex®. Due to prolonged retention and sustained drug release, the clearance of the drug from the AH was delayed, resulting the drug concentration in AH statistically significantly higher than that of Maxidex® at 4 to 10 h ($p < 0.05$).

## 4. Discussion

Topical drug administration is considered to be an easy route for ophthalmic drug delivery. However, for conventional formulations of eye drops and suspensions, the additional liquid administration accelerates tear clearance to further lower drug bioavailability in the eye [34, 35]. To overcome this, a dry tablet loaded with polymeric particles had been proposed as topical drug delivery formulation. The tablet was prepared by compressing the medium of mannitol and drug-loaded microparticles composed of PLGA and a mucoadhesion promoter, PEG [36]. Thus, dissolution of tablet medium, mannitol, increased the tear viscosity allowing prolongation of interaction time between preocular mucin and PEG in particles. However, the compressed tablet with high density appeared to dissolve in lacrimal fluid in several minutes, during which undissolved tablet with relatively large size could cause eye irritation [36]. To overcome, the dry formulation in this study was prepared by lyophilization to be able to dissolve rapidly in tear fluid [9].

Herein, I proposed a rapidly-dissolving dry tablet formulation embedded with drug-loaded PLGA nanoparticles for topical drug delivery to the eye. The tablets were amorphous physical composites of long alginate polymer chains forming networks and nanoparticles fixed within the structure. When the tablet was delivered to the preocular surfaces and separated from the applicator, the tablet medium was dissolved almost immediately and freed the drug-loaded nanoparticles. Due to the presence of alginate in the tablet medium, tear viscosity increased due to the molecular interaction between alginate and calcium ion in tear.

Hampered clearance of tear led to a higher preocular retention of DX/NP (Figure 12). Residing longer in the preocular space, DX/NP could consistently release the dexamethasone to tear fluid, providing more time for drug to be adsorbed into the eye and ultimately enhancing ocular drug bioavailability (Figure 13).

In this study, I prepared the tablets by freeze-drying the DX/NP suspension in water-soluble polymer, allowing high porosity within the tablet for rapid dissolution in the tear tablet. The DX/NP was designed to be nanosized to prevent the irritation and discomfort on the sensitive eye surface [36]. Therefore, alginate content employed for the tablet herein was observed with no sign of eye irritation or discomfort during i*n vivo* experiments. By using the applicator, the tablet could be delivered without contamination, thereby hygienic application was achieved. Owing to its biocompatibility, PDMS was employed as material for the applicator, as applicator might tough the eye surface. More importantly, as hydrophobic PDMS possess a low surface release energy, the hydrophilic dry tablet could be instantaneously separated from the applicator and adhere onto the eye when wet with tear fluid as shown in Figure 11(a) [37]. In addition, since the tablet would be separated without loss, an accurate dosage of drug could be delivered to the eye using the dry tablet formulation.

The findings of this study may be further expanded to assess the pharmacodynamics profile of dexamethasone using an endotoxin-induced uveitis model, i.e., an animal model for acute uveitis in humans [38, 39]. In this animal model, the ocular inflammation in a rabbit model

could be induced by intravitreal injection of lipopolysaccharide endotoxin from *Salmonella typhimurium*. Thus, at scheduled times after our dry tablet is administered topically to the eye, the eye can be monitored using a slit lamp microscope to assess the severity of inflammation.

## 5. Conclusion

Herein, I have derived a topical ocular drug formulation consisting of a dry tablet containing viscosity enhancer alginate and drug-loaded PLGA nanoparticles to improve the bioavailability of drugs. In addition, for easy and hygienic administration, a preocular applicator was also designed to deliver the developed tablet formulation. The tablet comprising of hydrophilic polymers could be separated instantaneously from the PDMS applicator when applied on the eye because of hydrophobicity and low surface release energy of constituent applicator material.

After topical application, the tablet medium dissolved in tear fluid rapidly to release drug-loaded nanoparticles, while alginate in tablet interacted with calcium ion in the tear to enhance the tear viscosity. Consequently, the drug-loaded nanoparticles can prolong the time in ocular surface, and as doing so, particles release the drug in a sustained manner, eventually extending ocular drug availability. Therefore, I conclude that the combination of a dry tablet formulation with alginate medium, drug-loaded PLGA nanoparticles, and a preocular applicator is

a promising strategy to achieve topical accurate dose delivery of ophthalmic drugs to the eye, with extended drug availability.

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# Abstract in Korean


국문초록

안과 질환의 치료를 위해 사용되는 점안 약물은 일반적으로 용액 또는 현탁액의 형태로 국소 전달되는데, 전안부에서 눈물 순환이 매우 빠르게 이루어지기 때문에 투여된 약물이 빠르게 씻겨 나가, 5% 미만의 약물만이 안구 내 표적 조직까지 도달하는 것으로 알려져 있다. 정확한 용량의 약물을 무균 상태로 눈에 쉽게 전달하고 약물의 생체이용도를 향상시키기 위하여, 본 연구에서는 약물이 탑재된 나노 입자들이 빠르게 용해되는 알지네이트 (alginate) 미디움의 건식 타블렛과 간편한 투약을 위한 전안부용 어플리케이터가 결합된 시스템을 개발하였다.

건식 타블렛은 알지네이트와 약물이 탑재된 나노 입자가 섞인 현탁액을 동결 건조한 제형으로써, 미디움으로 사용된 다당류 폴리머 알지네이트는 눈물에 풍부하게 있는 칼슘과 가교 반응하여 눈물의 점도를 높여 나노 입자의 전안부 잔류 시간을 향상 시킬 수 있으며, 생체 적합한 고분자인 폴리락틱코글라이콜릭산 (PLGA)로 제작한 나노 입자에 코르티코스테로이드 항염증약인 덱사메타손을 탑재함으로써 약물의 서방출 효과를 가질 수 있도록 하였다. 또한, 건식 타블렛을 전안부 어플리케이터의 팁 부분에 위치시켜 핸들 부분을 잡고 투약 할 수 있도록 디자인하여 오염없이 정량의 약물을 전달할 수 있게 하였다.

약물이 탑재된 나노 입자는 solid-in-oil-in-water 에멀젼 기법으로 제조하여 85.45 $\mu$g/mg의 약물이 탑재되도록 하였으며, 나노 입자를

담지한 건식 타블렛은 *in vitro* 실험을 통해 10시간동안 약물이 서방출됨을 확인하였다. 토끼의 눈에 어플레케이터를 사용하여 국소 투약 했을 때, 건식 타블렛이 어플리케이터에서 완전히 분리되어 눈 표면에 자극없이 눈물에 용해되는 것을 관찰하였으며, 분리된 타블렛은 내부의 알지네이트로 인해 점도가 향상되는 효과로 인해 나노 입자가 2시간동안 눈 표면에 남아있음을 확인하였다. 생체 내 효능을 평가하기 위해, 토끼의 눈에 어플리케이터를 사용하여 건식 타블렛을 국소 투약한 후 방수 내에 흡수되는 약물의 농도를 시판되고 있는 덱사메타손 점안액인 Maxidex®와 비교하였을 때 약물생체이용도가 Maxidex®보다 2.5배이상 증가하였다.

본 연구를 통하여 나노 입자를 담지한 알지네이트 건식 타블렛과 전안부용 어플리케이터가 정확한 용량의 안과 약물을 위생적으로 투약 가능하게 하고, 전안부에서의 약물의 잔류 시간을 늘려 생체이용도를 향상 시킬 수 있음을 증명하였다. 그러므로, 기존의 잦은 투약과 부정확한 용량의 안과 약물 투약으로 인한 부작용을 방지하고 치료의 효과와 환자의 편의성을 모두 증진 시킬 수 있다는 점에서 큰 의미가 있음을 확인하였다.

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