Chiang Mai J. Sci. 2013; 40(4) : 656-668
http://it.science.cmu.ac.th/ejournal/
Contributed Paper

Development of a Stable Latanoprost Solution for Use as Eye Drops

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Received: 7 December 2012
Accepted: 23 February 2013

ABSTRACT

The aim of this study was to develop latanoprost as a sterile eye drop solution using hydroxypropyl-β-cyclodextrin (HP-β-CD) as a solubilizing and stabilizing agent. Latanoprost eye drops were prepared according to the requirements for eye drop preparations. The inclusion complex of latanoprost/HP-β-CD was characterized by NMR spectroscopy. The efficacy of latanoprost eye drops prepared by using the complex with HP-β-CD was evaluated by measuring the intraocular pressure in a rabbit model. The stability of latanoprost eye drops was determined after storage at 5°C and 25°C/60%RH. The NMR results show that latanoprost was incorporated into the cavity of cyclodextrin. The dosage form of the new eye drop solution was stable at 25°C/60%RH for at least 6 months. The decrease of intraocular pressure observed was similar to that for Xalatan®.

Keywords: latanoprost, hydroxypropyl-β-cyclodextrin, intraocular pressure reduction, glaucoma

1. INTRODUCTION

Latanoprost is the $F_{2\alpha}$ analogue of prostaglandins used for the treatment of open angle glaucoma. Its chemical name is isopropyl-(Z)-7[(1R, 2R, 3R, 5S) 3, 5-dihydroxy-2-[(3R)-3-hydroxy-5-phenylpentyl] cyclopentyl]-5-heptenoate [1] and its molecular weight is 432.58 g/mol. The chemical structure is shown in Figure 1. It is a selective agonist of the prostaglandin F prostanoid receptor that reduces the intraocular pressure and increases the outflow of the aqueous humor. The main mechanism of action of this agent is an increased uveoscleral outflow [2]. Latanoprost is a prodrug that is well absorbed *via* the cornea, and is then activated by hydrolysis to the active form of latanoprost acid (see Figure 1). Latanoprost is a colorless or slightly yellow oily viscous liquid and practically insoluble in water [3]. The solubility of latanoprost was reported to be 12.9 μg/mL in water [4] but latanoprost eye drops under the

trade name of Xalatan® was manufactured by Pfizer Inc. (USA) contains 50 μg/mL of latanoprost in an aqueous preparation. The manufacturer recommends that Xalatan® to be stored in the refrigerator.

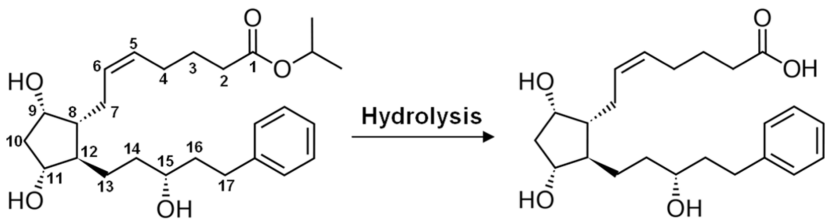

Figure 1. Chemical structure of latanoprost and its active metabolite-latanoprost acid.

The development of latanoprost as an aqueous preparation is important since it may then act more quickly and have better biological activity. Latanoprost has been developed as an eye-drop preparation by forming a complex micelle [5]. In that form it can be stored at room temperature to improve its usability and solubility. Ocular drug excipients are able to enhance the solubility of eye-drops by using cosolvents such as PEG300 and glycerol [6], a surfactant/ chitosan micelle system [7], a complex micelle system (non-ionic surfactants, polyethylene glycol monostearate 25 and polyethylene glycol monostearate 40) [8] and cyclodextrin as solubilizing agents [9-11].

Cyclodextrins are a group of structurally related natural products formed during bacterial digestion of cellulose. These cyclic oligosaccharides consist of (α-1, 4)-linked α-D-glucopyranose units having a lipophilic central cavity and a hydrophilic outer surface [9]. In aqueous solutions cyclodextrins are able to form inclusion complexes with many drugs by taking up a drug molecule or more frequently some lipophilic moiety of the molecule, into its central cavity. Cyclodextrin derivatives used in ophthalmology include the hydroxypropyl-β-cyclodextrin (HP-β-CD) for ocular drug delivery [10, 11]. Due to the low solubility of latanoprost in water, cyclodextrins has therefore been chosen to enhance its solubility [3, 12] and to formulate as an aqueous solution and maintain its stability [10-11]. HP-β-CD is not only highly soluble in water (>600 mg/mL) but also has low toxicity and is a common additive in eye preparations. The advantages of HP-β-CD in latanoprost eye drops are in enhancing drug stability. In addition, cyclodextrin may enhance corneal permeability [13].

The aim of this study was to prepare and investigate an inclusion complex of latanoprost with HP-β-CD as an eye-drop preparation for improving latanoprost solubility and stability.

2. MATERIALS AND METHODS

2.1 Materials

Latanoprost (lot no. PCDINT01909) was obtained from Precise Chemi Pharma Put. Ltd. (India). HP-β-CD or Cavasol® W7 HP Pharma (lot no. 73B033) was from ISP Pharmaceuticals, USA. Sodium hyaluronate (lot no. A04A) was from Shiseido Co., Ltd., Japan. All other excipients were pharmaceutical grades and other reagents were reagent grades. Fluorescein sodium was obtained from BDH Limited (England) and xylazine hydrochloride was from Farvet, Netherlands. Pentobarbital was obtained from Ceva, France. Alpha-chymotrypsin was from Merck, Germany. Xalatan® (lot no. S08720) was used as a

reference formulation. A Tonometer (Tonovet® Type TV01, Denmark) was employed to measure intraocular pressure.

2.2 Animals

Male New Zealand white rabbits, hybrid strain weighing about 3 kg were purchased from the Department of Animal Science, Faculty of Agriculture, Kasetsart University, Thailand. All animal experiments were reviewed and approved by the Office of the National Research Council of Thailand (approved no.PS-54006). Rabbits were individually housed in stainless-steel cages and in a 12 h light/dark cycle in a controlled room temperature, and were allowed free access to food and tap water for a minimum of a week before the experiments.

2.3 The One-dimensional ^{1}H, ^{13}C NMR Spectrum Experiment

Latanoprost and HP-β-CD were dissolved in CD_3OD and D_2O respectively. Complexes were prepared by dissolving HP-β-CD and latanoprost (same molar ratio in the eye drop preparations) in purified water until a clear solution was obtained. The dry complexed powder was reconstituted in D_2O. The ^{1}H-NMR and ^{13}C-NMR spectra were performed on a Fourier Transform NMR Spectrometer 500 MHz, Model UNITY (Varian, Germany). ^{1}H-NMR chemical shifts were referenced to an internal standard TMS; for the ^{13}C-NMR spectra, the resonance line of $CDCl_3$ at 77.00 ppm was used as a reference.

2.4 Preparation of Model Latanoprost Eye Drop Formulations

The formulation was designed as a clear ophthalmic solution, free from particulate matter. The ingredients and sodium chloride equivalent values are shown in Tables 1 and 2. The formulations were adjusted to a pH 6.7 with an osmolarity of 294 mOsmol/L. This is able to comfort the eyes [14]. Benzalkonium chloride in a solution of 50% was added as a preservative. HP-β-CD was used as a solubilizing agent for the lipophilic drug at an optimized concentration of 0.359 %w/v. The finished product specifications were set as standard guidelines for an eye drop preparation. The details of manufacturing followed the standard method for a sterile eye preparation. This product was filtered through a 0.45 μm pre-filter membrane, and then sterilized by filtration through a 0.2 μm polysulfone membrane using an aseptic technique. Three pilot batches manufacturing (10 L) for the stability study were prepared and dispensed into a 2.5 mL natural color LDPE eye drop bottle with an LDPE inner plug and PP screw on a pilfer proof cap.

Table 1. Formulation for the latanoprost eye drops used in this study.

Ingredients	Composition (mg)
Latanoprost	0.125
Hydroxypropyl-β-cyclodextrin	8.975
Monobasic sodium phosphate (monohydrate)	17.500
Dibasic sodium phosphate (anhydrous)	15.000
Sodium hyaluronate	4.500
Sodium chloride	10.000
Benzalkonium chloride (50% solution)	1.000
Water for injection qs to	2.5 mL

Table 2. The amount of sodium chloride equivalent of ingredients in the formulation.

Ingredients	Equivalent to sodium chloride (mg in 1 mL of water)
Latanoprost	insignificant
Hydroxypropyl-β-cyclodextrin	0.15
Monobasic sodium phosphate (monohydrate)	2.03
Dibasic sodium phosphate (anhydrous)	2.52
Sodium hyaluronate	0.02
Benzalkonium chloride (50% solution)	0.03

Sodium chloride should be added in the formulation = 9 mg - 4.75 mg = 4.25 mg in 1 mL of water.

2.5 Analysis of Latanoprost and Validation Method

The latanoprost concentration of the eye drops was determined by high performance liquid chromatographic (HPLC) system (Ultimate, USA). A HPLC system consisted of solvent delivery pump (SR-3000), injector with an injection volume of 20 μL (WPS 3000SL), a UV-detector at a wavelength of 210 nm and a reversed phase column (C18, 250×4.6 mm) were kept at 25°C. The mobile phase consisted of acetonitrile: 0.05 M phosphate buffer (60:40 v/v) and adjusted to pH 3.0 and a flow rate was maintained at 1.0 mL/min. The system met the requirements according to USP [15] and had a tailing factor of a standard solution chromatograph of not more than 2.0 and a %RSD standard response of 6 replicate injections of not more than 2.0%.

The method was validated for 8 parameters: specificity, range, linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), and robustness. In addition the systems suitability parameter was also calculated. To demonstrate specificity in the presence of other excipients used in the formulation, latanoprost was spiked (approximately 25 μg/mL) into the drug product. A chromatogram was run, and observed result and compared with the standard solution to confirm the same retention time and also that the product used was capable of separating the analyte peak from its excipients. To evaluate the linearity, the standard solutions corresponding to 50-150 μg/mL of latanoprost concentration were prepared. The slope, intercept and correlation coefficient were obtained from the linear regression analysis. To determine the repeatability and intermediate precision, six replicated samples of latanoprost were analyzed three times within the same day (intra-day) and three other days (inter-day variation). The LOD and LOQ values were calculated as the signal-to-noise ratio of 3:1 and 10:1 respectively.

2.6 Sterility Test

The stability test of latanoprost eye drops was performed as method described under sterility tests <71> specified in USP [15].

2.7 Stability Study

The accelerated and long-term stability studies were performed following the ASEAN Harmonized on stability of drug products guidance [16]. Three difference product lots were kept at the following storage condition and sampling times were taken into account. Those conditions were 5°C and 25°C with 60% relative humidity for 1, 3, and 6 months, respectively.

The manufacturer of Xalatan® claims that

"Once opened the container may be stored at room temperature for up to 30°C for 6 weeks". It means that the products meet the specification after opening eye bottle. Therefore in-use stability was performed for test the specification especially the sterility of the products. The protocol was developed to simultaneously simulate the real life application of eye drops by sampling latanoprost eye drops and storing them at 30°C, then the eye drop bottles were opened and the content used for testing samples daily. Sufficient amounts of eye drops remained for testing every week for 6 weeks.

2.8 Eye Irritation Test

This test method followed the Acute Eye Irritation/Corrosion method no. 405 in the OECD Guidelines for animal Testing of Chemicals [17]. The eye irritation was performed by using 3 rabbits in this experiment. Both eyes of each rabbit provisionally selected for testing were examined within 24 h before testing started. Only one drop of fluorescein sodium ophthalmic solution (2%w/v) was dropped into both eyes of the test rabbits. After 1 minute the rabbits eyes were washed with normal saline solution and the eye irritation determined using a hand slit lamp. Animals showing eye irritation, ocular defects, or pre-existing corneal injury were not used. For testing the latanoprost eye drops, a dose of 0.1 mL was used. The duration of the observation period was sufficient to fully evaluate the magnitude and reversibility of the observed effects. The eyes were examined at 1, 24, 48, and 72 h after the test sample was applied. To determine the reversibility of the effects of eye irritation, the animals were observed for 21 days post administration of the latanoprost fluid. If reversibility of the eye irritation was seen before 21 days, the experiment was stopped at that time. The grades of ocular reactions (conjunctivae, cornea and iris) were recorded at each examination (for details see grading as described in the full reference). Any other lesions in the eye (e.g. pannus, staining) or adverse systemic effects were reported.

2.9 Intraocular Pressure Reduction in Rabbits

The 18 rabbits were fasted 12-16 h before experiments and separated into 3 groups (6 rabbits per group) which restrained in a box-type fixation. The intraocular pressure of each rabbit was measured using the tonometer while they were conscious. Animals were anaesthetized using 2 mg/kg of xylazine hydrochloride intramuscularly followed by an intravenous injection of 25 mg/kg of pentobarbital. Fifty mL (10 U) of α-chymotrypsin was injected into the posterior ocular chamber of both eyes using a 30-gauge needle for reduction of intraocular pressure in the eyes [18-19]. After injection, rabbit's eyes were treated with chloramphenicol and betamethasone eye drops twice a day for 10 days. After 21 days, the intraocular pressures of the rabbits were measured and recorded for the experiment when the intraocular pressure exceeded 25 mmHg. In this study, 3 groups of rabbits were administered with 50 μL of a 0.9% sodium chloride solution, 50 μL of latanoprost eye drops and 50 μL of Xalatan® eye drops, respectively. The intraocular pressures of both eyes were measured immediately prior to administration of the drug and 1, 2 and 3 h after drug administration, respectively. The difference between the multiple groups was assessed by the student's *t*-test. Values of p less than 0.05 were considered to be significantly different.

3. RESULTS

3.1 Interaction of Latanoprost with Cyclodextrins

^{1}H, ^{13}C NMR were employed to investigate the free latanoprost and complex latanoprost formation. The atomic numbering of latanoprost is shown in Figure 1. Latanoprost was analyzed in D_2O and the complex was analyzed in CD_3OD. Table 3 shows that the chemical shifts of free latanoprost are in good agreement with a previous report [20]. The chemical shift displacements on the solutions containing latanoprost/HP-β-CD complex are also listed in Table 3.

Table 3 Proton and carbon chemical shifts of free latanoprost and a solution containing the latanoprost/hydroxypropyl-β-cyclodextrin complex.

Atom number	Chemical shift (δ) ppm			
	Free latanoprost		Latanoprost complex	
	^{13}C	^{1}H	^{13}C	^{1}H
1	175.019	---	*	–––
2	34.917	2.262 (t, *J* = 14.87 Hz, 2 H)	*	**
3	26.083	1.244 (m, 2 H)	*	**
4	27.416	2.107 (m, 2 H)	*	**
5	129.313	5.390 (m, 1 H)	*	**
6	126.665	5.520 (m, 1 H)	*	**
7	27.585	2.083, (m, 1 H)	*	**
		2.236 (m, 1 H)		
8	51.608	1.392 (m, 1 H)	62.291	1.009
9	73.836	4.061 (bdd, *J* = 4.63, 4.63, 1 H)	74.322	4.630
10	43.821	1.502 (dd, *J* = , 2 H)	57.698	1.019
11	78.444	3.834 (bdd, J = 7.56, 8.05, 1 H)	78.428	3.786
12	52.443	1.329 (m, 1 H)	66.788	1.009
13	30.130	1.551 (m, 1 H)	*	**
		1.662 (m, 1 H)		
14	36.247	1.707 (m, 1 H)	*	**
		1.768 (m, 1 H)		
15	72.052	3.548 (m, 1 H)	*	**
16	40.422	1.976 (m, 1 H)	*	**
17	33.075	2.624 (ddd, *J* = 9.76, 9.51, 9.76 Hz, 1 H)	*	**
		2.766 (ddd, *J* = 9.76, 1.46, 9.76 Hz, 1 H)		
2-propyl (CH-O)	68.895	4.941 (h, *J* = 37.56 Hz 1 H)	*	**
2-propyl (2 x CH_3)	22.098	1.192 (d, *J* = 7.31 Hz, 6 H)	*	**
ipso	143.744	---	*	---
2 x ortho	130.108	7.190 (bd, *J* = 8.29 Hz, 2 H)	*	*
2 x meta	130.818	7.232 (bt, *J* = 14.87 Hz, 2 H)	*	*
para	129.405	7.123 (bt, *J* = 17.07 Hz, 1 H)	*	*

* Signals disappeared

** Affected proton chemical shift

The ^{13}C NMR spectra showed a 2-propyl (-$CH(CH_3)_2$) peak at 22.098 ppm and benzene ring peaks at 129.405, 130.108, 130.818, 143.744 ppm with an attached proton of about 7 ppm. This group is the non-polar which formed a complex within the cavity of cyclodextrin.

The chemical shift of the ^{13}C and ^{1}H of the latanoprost was affected by the cyclodextrin environment. The signal of the C-atom number C_1-C_7 and C_{13}-C_{17} disappeared. This refers to the side chain of latanoprost (including 2-propyl and the benzene ring). The most significant changes of the latanoprost complex proton chemical shifts were observed for the CH, CH_2 proton of C_1-C_7, C_{13}-C_{17}, and CH_3 of the 2-propyl end and also the CH of the benzene ring. All signals disappeared from the NMR spectra. This determined that there was an insertion

of the latanoprost side chain into the HP-β-CD. In addition, the dihydroxy cyclopentane protons (OH, CH, and CH_2 at C_8-C_{12}) exhibited minor changes in chemical shift downfield of the complexation with HP-β-CD. However, the inclusion of the dihydroxy cyclopentane moiety into the HP-β-CD cavity was not complete, and there was only a partial insertion of the latanoprost into the cavity of the cyclodextrin.

We concluded that the latanoprost molecules interacted with HP-β-CD as illustrated in the proposed scheme (Figure 2). This shows that the OH group of cyclopentane is outside the cavity and the benzene ring and dimethyl side chain are deeply enclosed within the cavity. It is highly possible that the hydroxyl group is restricted by hydrogen bonding on the rim of the cavity or with the aqueous solution.

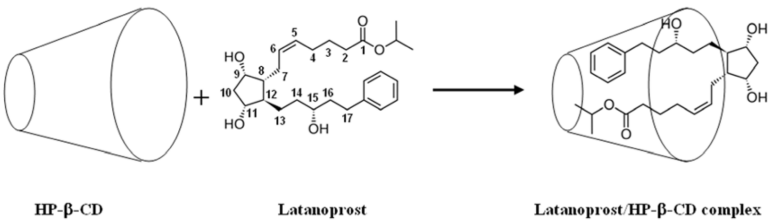

Figure 2. Proposed scheme for the inclusion complex of latanoprost inside hydroxypropyl-β-cyclodextrin.

3.2 Formulation Development of Latanoprost Eye Drops

Latanoprost does not dissolve in water or phosphate buffer solution when prepared at a concentration of 50 μg/mL. After HP-β-CD was used as a solubilizing agent, a clear solution was obtained.

The latanoprost complexed with HP-β-CD formulations included a phosphate buffering agent adjusted to a pH of 6.7 by using monobasic sodium phosphate monohydrate and anhydrous dibasic sodium phosphate. The amount of the buffering agent was calculated by the Henderson-Hasselbalch equation according to equation (1).

$$pH = pKa + \log \frac{[salt]}{[acid]} \quad (1)$$

$$= 6.72$$

This formulation (Table 1) was developed for its tonicity that was calculated by the sodium chloride equivalent method by using the E-value. The E-value of latanoprost does not appear in any literature so the theoretical osmolarity was calculated according to equation (2) [14].

$$\frac{mOsmol}{L} = \frac{g}{L} \times \frac{mols}{g} \times \frac{osmol}{g} \times \frac{1000\,mOsmol}{osmol} \quad (2)$$

$$= 0.12 \text{ mOsmol/L}$$

Due to the low concentration of latanoprost in the eye drop formulation its osmolarity value was low resulting in a low contribution to the calculated sodium chloride equivalent value. The excipients equivalent to amount of sodium chloride (mg) which calculated from equation (2) is shown in Table 2. All excipients equivalent to total amount of sodium chloride is 4.75 mg. This is not sufficient to produce isotonicity of eye drop formulation (0.9% w/v of sodium chloride). Therefore we added sodium chloride to about 0.4% w/v for isotonic adjustment and the osmolarity was re-evaluated experimentally.

The composition of the formula includes: 50 μg/mL latanoprost, 3.59 mg/mL HP-β-CD as solubilizing agent, 7 mg/mL monobasic sodium phosphate monohydrate and 6 mg/mL of anhydrous dibasic sodium phosphate as buffering agent, 1.8 mg/mL sodium hyaluronate for adjustment

of viscosity, 4 mg/mL sodium chloride for isotonic adjustment, 0.2 mg/mL benzalkonium chloride as preservative and water for injection added to 1 mL. The amount of the working formula (per 1 bottle) is presented in Table 1. The calculated value of osmolarity is close to the obtained value (294 vs 275 mOsmol/L).

3.3 Method Validation

The method for validation of the latanoprost assay was performed because latanoprost is not officially in any pharmacopoeia. The latanoprost chromatogram revealed a specificity of the method. The retention time of latanoprost was about 4 min and the peak was not interfered with any excipient in eye drop formulations. The linear plots between concentrations and peak areas produced a good correlation with $r^2 = 1$. The system precision was determined from the results of six replicate injections. The %RSD of the assay for latanoprost within one day was not more than 2% (0.22 - 0.82%) and %RSD between 3 days was 0.62%. The recovery experiment to study the accuracy was carried out by the spiking placebo method. A known quantity of drug corresponding to 75, 100, and 125% of the label claim of the drug were added. The %recovery of all concentration was 99.3%. The results indicate that the method is highly accurate for determination of latanoprost. The results of the other validation parameters were well within the acceptance criteria [15]. For results of the validation, the assay method was ensured during the stability study.

3.4 Stability Study

A stability study of an ophthalmic solution of latanoprost was performed and the result is shown in Table 4. The solution was found to be clear and found to be free from particulate matter. The assay content of latanoprost was between 98.9 and 102.7 %LA after storage at 5°C for a period of 6 months. The latanoprost content after storage at 25°C was between 99.4 and 101.7 %LA for a period of 6 months. The latanoprost content was stable at 5°C and room temperature (the content changes were less than 2% of the initial). The pH and viscosity of the products also remained constant throughout the period of the stability studies. Determination of the pH and viscosity of the latanoprost eye drops were about 6.7 and 15.5 cps, respectively and these did not change after storage for 6 months at both 5°C and 25°C. The osmolarity of the latanoprost eye drops for both storage conditions was 274-280 mOsmol/L. In addition, the solution remained sterile after storage for 6 months. The results indicate that this formulation is stable in the packaging of the eye drops.

The in-use stability study results are shown in Table 5. The assay results during the study period (6 weeks) were 100.3 and 99.2 %LA. The pH value was constant at 6.7 and the viscosity slightly increased by about 15 to 17 cps. This product was sterile after repeated opening the eye drops during the in-use study.

Table 4. Stability results of latanoprost eye drops (mean ± SD, n = 6) from 3 different batches.

Test	Stability condition	Months			
		0	1	3	6
Appearance	5°C	Clear, colorless liquid, free from visible particles			
	25°C/60%RH	Clear, colorless liquid, free from visible particles			
Assay (90-110% LA)	5°C	101.6 ± 1.35	98.9 ± 1.55	99.5 ± 1.05	102.7 ± 0.76
	25°C/60%RH	101.6 ± 1.35	99.4 ± 1.95	101.7 ± 1.49	100.7 ± 0.89
pH (6.0-8.0)	5°C	6.7 ± 0.00	6.7 ± 0.00	6.7 ± 0.00	6.7 ± 0.06
	25°C/60%RH	6.7 ± 0.00	6.6 ± 0.06	6.7 ± 0.06	6.6 ± 0.00
Viscosity (10-20 cps.)	5°C	15.5 ± 0.56	15.3 ± 0.46	15.6 ± 0.36	15.5 ± 0.44
	25°C/60%RH	15.5 ± 0.56	15.2 ± 0.51	15.5 ± 0.50	15.3 ± 0.68
Osmolarity (270-328 mOsmol/L)	5°C	275 ± 6.08	274 ± 3.21	275 ± 4.16	280 ± 8.50
	25°C/60%RH	275 ± 6.08	277 ± 4.16	279 ± 6.80	278 ± 6.43

Table 5. In-use stability results of latanoprost eye drops (3 difference batches). Mean ± SD, n= 6

Test	Stored at 30°C with drops removed from the eye bottles every				
	Initial	2nd week	4th day-week	5th week	6th week
Appearance	Clear, colorless liquid, free from visible particles				
Assay (90-110% LA)	100.3 ± 1.20	97.2 ± 1.40	97.9 ± 1.35	99.2 ± 1.70	99.2 ± 1.55
pH (6.0-8.0)	6.71 ± 0.00	6.71 ± 0.00	6.67 ± 0.06	6.70 ± 0.00	6.71 ± 0.06
Viscosity (10-20 cps.)	15.38 ± 0.65	14.98 ± 0.45	16.65 ± 0.56	16.96 ± 0.66	16.94 ± 0.58
Sterility	Sterile	Sterile	Sterile	Sterile	Sterile

3.5 Eye Irritation Test and Intraocular Pressure Reduction

The eye irritation test of the latanoprost eye drops was determined by observing the rabbit's eyes. No eye irritation of any rabbit was found after 72 h.

The intraocular pressure reduction after administration of normal saline as control, latanoprost eye drops and Xalatan® eye drops that were free from HP-β-CD formulation are shown in Figure 3. The intraocular pressure after administration of normal saline solution did not change after 1 h and decreased to 0.33±0.33 and 1.16±0.87 mmHg after 2 and 3 h, respectively. The intraocular pressure reduction after administration of latanoprost eye drops decreased to 6.00±2.62, 7.83±2.52, 8.83±2.49 mmHg after 1, 2 and 3 h, respectively. The intraocular pressure reduction after administration of Xalatan® eye drops decreased to 6.66±1.11, 8.00±1.86, 9.50±1.76 mmHg after 1, 2 and 3 h, respectively. The statistics showed that latanoprost eye drops and Xalatan® eye drops significantly changed the intraocular pressure in comparison with normal saline ($p < 0.01$).

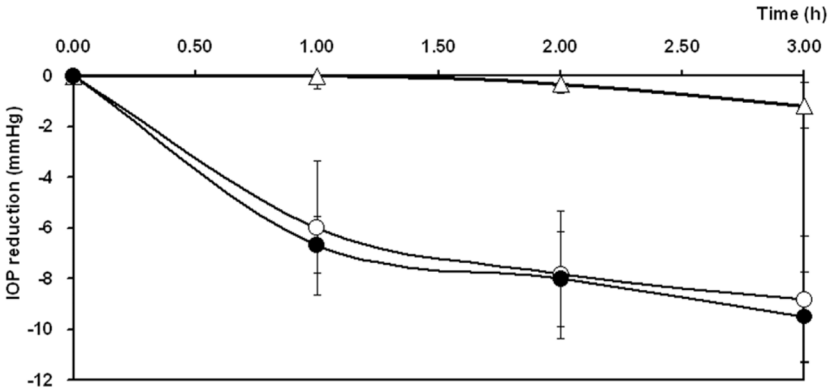

Figure 3. Intraocular pressure (IOP) changes in rabbits (n = 6) after administration of 0.9 % NaCl (△), Latanoprost eye drops (O) and Xalatan® eye drops (●). The data represent the changes in IOP from baseline values (mean ± SE, n = 6).

4. DISCUSSION

Although increasing solubility of latanoprost by introducing alcohol is possible, it is not practical in eyes' preparations. The solubility of latanoprost in aqueous solution is limited (12.9 and 50 μg/mL in water and PBS pH 7.2, respectively). In-house experiment revealed that latanoprost did not completely dissolve in water (pH 7.0) or PBS (pH 7.2) when prepared at a concentration of 50 μg/mL. The clear solution was obtained after adding the HP-β-CD as solubilizing agent at wide range of pH (5.0 to 7.2).

The inclusion of latanoprost into HP-β-CD was formed as host-guest complex. The lipophilic cavity of the cyclodextrins provides solubility of latanoprost while the hydrophilic surface contributes to its solubility in water. The proposed schematic representation of latanoprost complex with cyclodextrin is presented in Figure 2. The two side chains of latanoprost (benzene ring and 2-propyl) were completely incorporated into the cavity of the cylodextrin because of its hydrophobic properties. The dihydroxy cyclopentane stays outside the cyclodextrin cavity because of the hydrophilic properties of its hydroxyl group. Complexes between cyclodextrins and numerous guest molecules have been studied by the NMR method [21-22]. Changes in the chemical shifts are employed to identify interactions between cyclodextrins and drug molecules and to determine binding constants. The solubility of latanoprost was enhanced as a result of it forming a complex with cyclodextrin.

The latanoprost solution eye drop formulation was adjusted to pH 6.7 because latanoprost was more susceptible from hydrolysis at less than pH 4 and at more than pH 6.7 [23]. In addition, the previous work reported that latanoprost ophthalmic at pH 5-6 was more stable than that at pH 7.0 [24]. Therefore formulation adjustment to pH 6.7 was done. Benzalkonium chloride was employed as preservative in this study that is similar to other ophthalmic formulations [25]. Ochiai *et al.* [26] found that latanoprost-stabilizing micelles were not influenced by the presence of benzalkonium chloride 0.02%. The viscosity increasing agent in eye drop formulation needs to retain drug in the eyes. Normally, hydroxypropylmethylcellulose (HPMC) was chosen as viscosity increasing agent due to its safety. In addition, chitosan can be used for increasing viscosity in artificial tear [27]. Sodium hyaluronate was used in this formulation for adjustment of the viscosity [28]. The results indicated that sodium

hyaluronate solution was better tolerated than that of the hydroxyethylcellulose solution. The concentration of sodium hyaluronate was 0.18% according to the concentration for dry eye treatment [29]. The viscosity of latanoprost eye drops was about 15 cps from the addition of sodium hyaluronate. Eye drops were able to be dropped out of the bottle but be able to retain in the eyes for their pharmacological action.

The osmolarity of the products did not change significantly versus time (0-6 months) and temperature (5°C and 25°C). Latanoprost eye drops conformed within their specifications. In addition, the complex formation increased the stability of latanoprost by the insertion of easily hydrolyzed part into the cavity of the HP-β-CD.

After the eye-drop bottles were opened, all stability parameters were within acceptable range including their sterility. This packaging of latanoprost eye drops containing 2.5 mL (about 80 drops) was sufficient for 6 weeks treatment.

After injection of alpha-chymotrypsin into rabbits' eyes for 21 days, it induced their intraocular pressure in all rabbits by more than 25 mmHg. The ocular hypertensive rabbit model prepared by intravitreal injection may cause inflammation in the eye and may block the trabecular meshwork resulting in an irregular release of aqueous humor. The effect of latanoprost (both the new developed formulation and Xalatan®) lowered the intraocular pressure compared with normal saline solution. The HP-β-CD complex formation with latanoprost did not change the efficacy of latanoprost in its formulation when compared with Xalatan®. Both products decreased the intraocular pressure by about 10 mmHg within 3 h. The normal value of the intraocular pressure in humans is between 10 and 20 mmHg (average is 15.5 mmHg). The latanoprost/HP-β-CD eye drops did not alter the efficacy of latanoprost because latanoprost was incorporated in the cavity and did not change the form of this drug such as ionization and salt formation. The latanoprost eye drops reduced the intraocular pressure after drug administration to a normal value (about 15 mmHg) within 3 h (intraocular pressure decreased from 25 mmHg to 15 mmHg or reduced by about 10 mmHg from the baseline). There were no differences in intraocular pressure after being treated with latanoprost eye drops and Xalatan® ($p>0.05$). From the specification of the finished product, the stability study and the efficacy of the eye drops we can claim that this developed formulation using HP-β-CD as complexing agent is suitable for an eye drop preparation of latanoprost.

Gonzalez *et al.* [10] compared clinical and stability studies of latanoprost with cyclodextrin-containing eye drops (not known formulation) and Xalatan® eye drops. The results showed intraocular pressure reduction in human was about 9 mmHg in both groups and exhibited similar adverse effects.

5. CONCLUSION

The formation of the complex between latanoprost and HP-β-CD was confirmed by ^{1}H-NMR and ^{13}C-NMR. When latanoprost HP-β-CD inclusion complex was developed as a sterile eye drop solution, the formulation was a clear solution and suitable for use as eye drops. This product was stable when stored at 5°C and 25°C for at least 6 months.

ACKNOWLEDGEMENTS

This work was supported in part by (1) the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, (2) Graduate School, Prince of

Songkla University, (3) Nanotec-PSU Excellence Center on Drug Delivery System, Faculty of Pharmaceutical Sciences, Prince of Songkla University. The author would like to thanks Interthai Pharmaceutical Manufacturing Limited (IPML) for support all of chemicals and facilities. We thank to Thailand Institute of Scientific and Technological Research (TISTR) for animal experimental part. The author would like to thank Dr. Brian Hodgson for assistance with the English.

DECLARATION OF INTEREST

The author declares no conflict of interest.

REFERENCES

[1] Rele, R.V., Mhatre V.V., Parab, J.M. and Warkar, C.B., Simultaneous RP HPLC determination of latanoprost and timolol maleate in combined pharmaceutical dosage form, *J. Chem. Pharm. Res.,* 2011; **3(1):** 138-144.

[2] Widomski, P., Baran, P., Goĺębiewski, P., Surowiec, I. and Bielejewska, A., Validated liquid chromatographic method for analysis of the isomers of latanoprost, *Acta Pharm*., 2008; **20(2):** 157-164.

[3] Jimenez-Bayardo, A., Tornero-Montaňo, J.R., Lopez-Sanchez, M.I. and Cruz-Olmos, E. 2011. US Patent No. US2011/0105605 A1 (2011).

[4] Drug bank database. Available on internet at http://www.drugbank.ca/drug/DB00654.

[5] Ochiai, A., Iida, K., Takabe, H., Kawamura, E., Sato, Y., Kato, Y., Ohkuma, M. and Danjo, K., Formulation design of latanoprost eye drops to improve the stability at room temperature, *J. Pharm. Sci. Technol.*, 2010; **70**: 324-332.

[6] Jithan, A.V., Mohan, C.K. and Vimaladevi, M., Development and evaluation of a chloramphenicol hypertonic ophthalmic solution, *Indian J. Pharm. Sci.*, 2008; **70(1)**: 66-70.

[7] Pepić, I., Hafner, A., Lovrić, J., Pirkić, B. and Filipović-Grčić, J., A nonionic surfactant/chitosan micelle system in an innovative eye drop formulation, *J. Pharm. Sci.*, 2010; **99(10)**: 4317-4325.

[8] Ochiai, A. and Danjo, K., The stabilization mechanism of latanoprost, *Int. J. Pharm.*, 2011; **410**: 23-30

[9] Brewster, M.E. and Loftsson, T., Cyclodextrin as pharmaceutical solubilizers, *Adv. Drug Deliv. Rev.*, 2007; **59**: 645-666.

[10] Gonzalez, J.R., Baiza-Duran, L., Quintann-Haa, J., Tornero-Montano, R., Castaneda-Hernandez, G., Ortiz, M., Alarcon-Oceguera, F., Beltran-Loustaunau, M., Cortez-Gastelum, M., Garciduenas-Mejia, J., Gomez-Bastar, P., Jimenez-Roman, J., Korder-Ortega, V., Paczka-Zapata, J., Torres-Segura, M. and Velasco-Gallegos, G., Comparison of the stability, efficacy, and adverse effect profile of the innovator 0.005% latanoprost ophthalmic solution and a novel cyclodextrin-containing formulation, *J. Clin. Pharmacol.*, 2007; **47**: 121-126.

[11] Bayado, J. and Guadalajara, A., Method of preparing a latanoprost ophthalmic solution and solution thus produced, EP Patent no. EP1759702A1, 2007.

[12] Ono, N., Rytting, J. and Pipkin, J., Effects of sulfobutylether β-cyclodextrin (Captisol®) on the solubility and stability of latanoprost in aqueous formulations, *The AAPS J.* 2007; 002571.

[13] Rajewski, R.A. and Stella, V.J., Pharmaceutical applications of

cyclodextrin 2. *In vivo* drug delivery, *J. Pharm. Sci.*, 1996; **85(11):** 1142-1169.

[14] Troy, D.B., *Remington: The Science and Practice of Pharmacy*, 21st edition, Lippincott Williams & Wilkins, 2005.

[15] US Pharmacopeial Convention, USP33-NF28, Rockville, MD, 2010.

[16] ACCSQ-PPWG meeting, Philippines, ASEAN guideline on stability study of drug product. 22 February 2005.

[17] OECD/OCDE, OECD Guideline for the testing of chemicals, acute eye irritation/corrosion, 24 April 2002.

[18] Konno, T., Maruichi, M., Takai, S., Oku, H., Sugiyama, T., Uchibori, T., Nagai, A., Kogi, K., Ikeda, T. and Miyazaki, M., Effect of chymase on intraocular pressure in rabbits, *Eur. J. Pharmacol.*, 2005; **524:** 132-137.

[19] Orihashi, M., Shima, Y., Tsuneki, H. and Kimura, I., Potent reduction in intraocular pressure by nipranidol plus latanoprost in ocular hypertensive rabbits, *Biol. Pharm. Bull.*, 2005; **28(1):** 65-68.

[20] Martynow, J.G., Jóźwik, J., Szelejewski, W., Achmatowicz, O., Kutner, A., Wiśniewski, K, Winiarski, J., Zegrocka-Stendel, O. and Gołębiewski, P., A new synthetic approach to high-purity (15R)-latanopros*, Eur. J. Org. Chem.*, 2007; 689-703.

[21] Fernades, C.M., Carvalho, R.A., Costa, S.P. and Veiga, F.J.B., Multimodel molecular encapsulation of nicardipine hydrochloride by β-cyclodextrin, hydroxypropyl-β-cyclodextrin and triacyl-β-cyclodextrin in solution, Structure studies by ^{1}H NMR and ROESY experiments, *Eur. J. Pharm. Sci.*, 2003; **18**: 285-296.

[22] Misuik, W. and Zalewska, M., Spectroscopic investigations on the inclusion interaction between hydroxypropyl-b-cyclodextrin and bupropion, *J. Mol. Liq.*, 2011; **159**: 220-225.

[23] Kimura, A., Asada, H., Tuchimoto, N. and Shimazaki, A., Stabilization of latanoprost ophthalmic solution at room temperature, *The AAPS J.* 2004; 000873.

[24] Sakai, Y., Yasueda, S. and Ohtori, A., Stability of latanoprost in an ophthalmic lipid emulsion using polyvinyl alcohol, *Int. J. Pharm.*, 2005; **305**: 176-179.

[25] Rowe, R.C., Sheskey, P.J., and Quinn, M.E., *Handbook of Pharmaceutical Excipients,* 6th edition. Pharmaceutical Press, London, UK, 2009.

[26] Ochiai, A., Ohkuma, M. and Danjo, K., Investigation of surfactant suitable for stabilizing of latanoprost, *Int. J. Pharm.*, 2012; **436:** 732-737.

[27] Leesawat, P., Vearnsilp, K. Yanasarn, N. and Thanawattanawanich, P., Artificial tear formulation from chitosan, *Chiang Mai J. Sci.*, 2005; **32(3):** 501-505.

[28] Ludwig, A. and Van, O.M., Evaluation of sodium hyaluronate as viscous vehicle for eye drops, *J. Pharm. Belg.*, 1989; **44(6):** 391-397.

[29] Vogel, R., Crockett, R.S., Oden, N., Laliberte, T.W. and Molina, L., Demonstration of efficacy in the treatment of dry eye disease with 0.18% sodium hyaluronate ophthalmic solution, *Am. J. Opthalmol.*, 2010; **149(4):** 594-601.