DEVELOPMENT OF SUSTAIN RELEASE POVIDONE IODINE OPHTHALMIC DROP THROUGH NOVEL *IN-SITU* GEL FORMULATION

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ABSTRACT

We have developed long-acting povidone iodine ophthalmic drops for the treatment of active infections of the conjunctiva and cornea by bacteria, mycobacteria, virus, fungus, or amoebic causes. The lead compound, IVIEW-1201 is an extended release *in-situ* gel povidone iodine composition which exhibits *sol-to-gel* phase transitions when instilling into the eye. The effective concentration of povidone iodine is maintained by the equilibrium between solution and the gel bound components resulting in a long lasting anti-viral, antimicrobial, and less irritation effect.

Keywords: povidone iodine, *in-situ* gel, sustain release, conjunctivitis

1 INTRODUCTION

The Acute conjunctivitis ("pink eye") is one of the most common and most contagious ocular infections seen in the United States, Japan and Europe. There are 5.9 million cases of infectious conjunctivitis annually in the United States and approximately 5.4 million cases in the EU annually. Approximately 50 percent of infectious conjunctivitis cases have a viral etiology, and 65 to 90 percent of these are caused by adenovirus. Viral conjunctivitis is highly infectious and transmissible, causing lost work and school days as well as increased healthcare costs and risks from unnecessary antibiotic prescriptions.

Povidone-iodine is a commercially available iodophor routinely used in ophthalmology and general surgery. Povidone-iodine solutions have been proven effective before (5% solution) [1,2] and after ocular surgery (1.25%) [3,4], at birth (2.5%), [5] and for active infections (1.25%). [6] PVP-I is the only agent known to prevent post-op endophthalmitis. Solutions of PVP-I are toxic to viruses (including HIV), fungi, parasites and bacteria with no known development of resistance. It is well described in the literature that aqueous PVP-I solutions exhibit greater antiseptic efficacy at lower concentrations. [7] Furthermore, these lower concentrations are less irritating to the eyes, ears and skin. A 0.6% PVP-I in combination with dexamethasone formulation is advancing into Phase III clinical trials by Shire in March 2017. [8, 9] However, a sustain release formulation of PVP-I is never been reported due its strong acidity and easily soluble in water.

Ophthalmic topical drug delivery is one of the important methods of application, but the existence of cornea barrier, tear dilution and lacrimal passage drainage effect limit the treatments and bioavailability of many topical ophthalmic preparations. An ideal ophthalmic formulation should be administrated in eye drop form, without causing blurred vision or irritation. This problem can be overcome using *in-situ* gel-forming drug delivery systems prepared from polymers that exhibit *sol-to-gel* phase transitions due to a change in a specific physicochemical parameter in the cul-de-sac. [10] *In-situ* forming gel (gel forming solution) uses special polymer materials' environment-sensitive nature, it keeps liquid state before administration, after administration, its viscosity increase dramatically due to physiological factors, forming a semi-solid state of the gel in administration place. So it stays a long time in the mucosal surface and releases drug slowly, leading to better efficacy. *In-situ* gel is easy to prepare, easy to use with dose accuracy and is comfortable to the eye. Thus, *in-situ* gel becomes a potentially novel formulation in ophthalmology. There are already several related products on the market, such as ion-sensitive Timolol maleate Ophthalmic Gel forming solution product Timoptic-XE® developed by Merck and Alcon.

We are developing long-acting povidone iodine (PVP-I) ophthalmic drops for the treatment of active infections of the conjunctiva and cornea by bacteria, mycobacteria, virus, fungus, or amoebic causes. There is currently no broadly effective therapy that treats all causes of infection and nothing is approved for the treatment of viral conjunctivitis. This represents a massive unmet need in ophthalmology. The lead compound, IVIEW-1201, is a novel *in-situ* gel PVP-I formulation where the effective concentration of PVP-I is maintained by the equilibrium between solution PVP-I and the gel bound components resulting in a long lasting, less toxic pharmacological effect. [11,12] IVIEW-1201 has two distinct features: first, it is effective against both bacteria and viruses and; second, there has been no documented case of resistance. It solves the three biggest problems in the treatment of eye infections: spectrum, resistance and long-acting.

2 EXPERIMENTATION

2.1 Selection of Gel Matrix

Deacetylated gellan gum, an anionic deacetylated extracellular polysaccharide secreted by *Pseudomonas Elodea*, are tetra-saccharide repeating units formed by polymerization of one molecule of α -L-rhamnose, one molecule of β -D-glucuronic and two molecules of β -D-glucoses. Deacetylated gellan gum has temperature-dependent and cation-induced gelation properties and a certain concentration of deacetylated gellan gum solution can form a moderate viscosity and strong water-holding gel with the cations in the tears. Ophthalmological composition of the type which undergoes liquid-gel phase transitionis shown in literature.[13]

Preparation of deacetylated gellan gum (DGG) (Kelcogel-Cg-La gellan gum, food grade gellan gum, CAS: 71010-52-1: E418, particle size: ~42 mesh (355 μm), purchased from CPKelco) solution: dissolve appropriate deacetylated gellan gum in deionized water and stir at 80°C water bath for 1 hour, cool to room temperature, stand until the material is fully swollen. Prepare 0.1% to 1.0% (w/w) concentration solution respectively. Preparation of simulated tear fluid (STF): $NaHCO_3$ 2.18g; NaCl 6.78g; $CaCl_2$•2H2O 0.084g; KCl 1.38g; dissolve in 1000 ml deionized water. Mix deacetylated gellan gum solution and simulated tear fluid by ratio 40:7. Measure the viscosity of deacetylated gellan gum solution before and after mixing with stimulated tear fluid via a rotary rheometer at 25°C and 34°C respectively.

2.2 *In-situ* gel eye drops formulation optimization

As shown in Fig. 1, we surprisingly found that after addition of povidone iodine, a few specific concentrations of deacetylated gellan gum-based solutions could form gel *in-situ* (e.g., a formulation containing 0.45% (w/w) deacetylated gellan gum), and the gel would change into the liquid form after adjusting to the surrounding pH. For solutions/formulations containing 0.3%, 0.35%, 0.4% (w/w) deacetylated gellan gum, their viscosities under the simulated physiological conditions were greater than those under non-physiological conditions, exhibiting typical *in-situ* gelling ability.

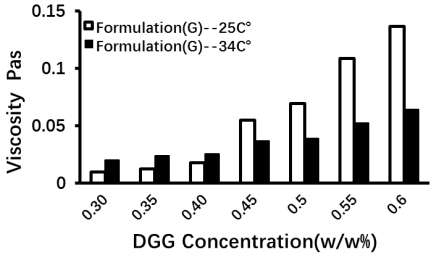

Fig. 1 The viscosity change of a DGG solution, containing povidone iodine and mannitol, at the room temperature (25 °C) and under simulated physiological conditions (mixing with STF by ratio 40:7, 34 °C)

NaCl was selected as osmotic pressure regulator. As DGG had an ionic sensitivity characteristic, we considered adding a small amount of NaCl in the formulation, so it did not form a gel while under storage condition, but gel formation would be triggered by mixing with a small amount of tear fluid in conjunctival sac. Formulations containing PVP-I *in-situ* gel and NaCl of different concentrations were prepared according to Table 3. PVP-I *in-situ* gel eye drops and 0.3% NaCl showed a weak gel state after standing for a period of time. The formulations would become liquid of low viscosity immediately after shaking slightly, making them idea candidates for gelling.

Table 1 Gel-forming observation of the formulations of PVP-I *in-situ* gel eye drops, containing different concentrations of NaCl

Concentration of NaCl (%, w/w)	Characteristics
0.1	Liquid, no particles
0.2	Liquid, no particles
0.3	Weak gel state after 24 hours, become low viscosity liquid immediately after gentle shaking, no particles
0.4	Become hard gel after standing, partial broken gel particles after shaking
0.5	Become hard gel after standing, partial broken gel particles after repeatedly shaking
0.6	Become hard gel after standing, partial broken gel particles after repeatedly shaking
0.7	Become hard gel, partial broken gel particles after vigorously shaking
0.8	Become hard gel immediately, partial broken gel particles after vigorously shaking
0.9	Become hard gel immediately, hard to shake

2.3 Evaluation of *in-situ* gel eye drops in vitro

2.3.1 In vitro dissolution

PVP-I *in-situ* gel eye drops was prepared according to the formulations set out in Table 2. 2g sample was measured precisely (about 2ml) and then added into a vial of 22 mm outer diameter, followed by addition of 350 μl simulated tear fluid (STF) and mixing quickly. The mixture was covered with a stopper and weighed precisely and recorded. Placed samples into an air shaker (34.5°C, 120 r/min), balanced for 10 min, and added simulated tear fluid (pre-heated to 34.5°C, 2ml) along the side-wall slowly, took

out all of the release medium at a different point in time, weighed quickly and recorded. 10 minute rebalance was needed after each shaking; took out the release medium before adding fresh STF (pre-heated to 34.5 °C); repeated this process until gel was dissolved completely. Draw gel dissolution time curve by plotting the total amount of gel dissolution vs time (n=3).

Table 2 Formulations of PVP-I *in-situ* gel eye drops

Formulation (G)	DGG (w/w)	PVP-I (w/w)	NaCl (w/w)	pH
	0.2%	0.6%	0.3%	5.0-5.5
	0.3%			
	0.4%			

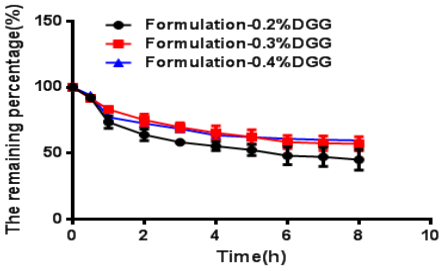

Fig. 2 The dissolution curve of PVP-I *in-situ* gel eye drops and DGG at different concentrations in STF

As results shown in Fig. 2, PVP-I *in-situ* gel eye drops containg 0.2% DGG (w/w) showed a good ability to retard tear erosion. There was still about 40% of gel base that was not dissolved after 8 hours of simulated tear fluid flushing. With the increase of concentration of deacetylated gellan gum, the dissolution of PVP-I *in-situ* gel eye drops became even slower, which effectively prolonged the residence time of PVP-I in the eye.

2.3.2 In vitro release

Took 2 ml PVP-I *in-situ* gel eye drops or 2 ml PVP-I normal saline solution, placed in a 14 KDa dialysis bag, added into 50 ml simulated tear fluid with pre-warmed to 34.5°C, shook samples via air shaker at 120 rpm, took out the release medium STF every 30 minutes, and added fresh release medium (pre-warmed to 34.5°C) quickly. Determined available iodine concentration by sodium thiosulfate titration (n=3), and calculated its accumulative release amount.

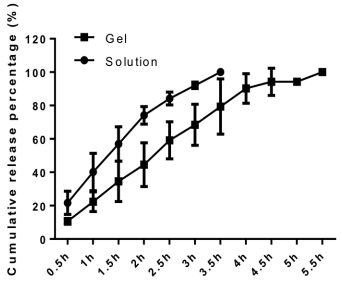

Fig. 3 In vitro cumulative release curve of PVP-I eye drops and PVP-I *in-situ* gel eye drops (n=3)

As results shown in Fig. 3, PVP-I *in-situ* gel eye drops had a significantly sustained-release character comparing with conventional povidone iodine eye drops, and extended PVP-I release steadily for about 5 hours.

2.4 Povidone iodine *in-situ* gel eye drops ophthalmic retention study

Placed 1 ml normal PVP-I saline and PVP-I *in-situ* gel eye drops in brown EP tube, added 0.5% fluorescein sodium respectively. Chose a healthy New Zealand rabbit, and made its head fixed. Dropped 50 µl fluorescent labeled PVP-I normal saline solution into its left eye and made it close passively for 10s. Observed fluorescence condition of left eyes at 0 min, 2 min, 4 min, 6 min, 8 min and 10 min via slit lamp; dropped 50 µl PVP-I *in-situ* gel eye drops into its right eye and made it close passively for 10 seconds. Observed fluorescence conditions of the right eyes at 0 min, 2 min, 5 min, 10 min, 20 min 30 min, 40 min, 50 min and 60 min with slit lamp.

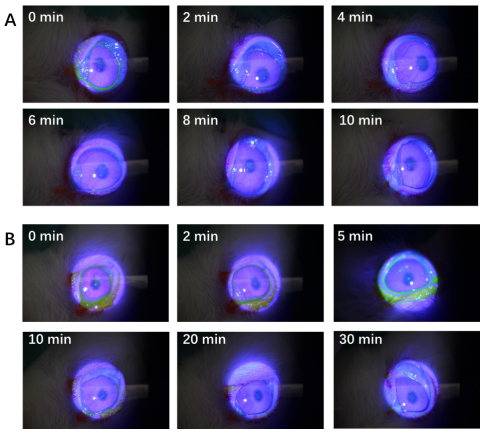

Fig. 4 The fluorescence photographs of the retention of PVP-I solution and PVP-I *in-situ* gel eye drops in rabbit eyes

As results shown in Fig. 4, conventional PVP-I eye drops was quickly eliminated after administration, and was retained for only 4 min in rabbit conjunctival sac. By contrast, the elimination rate of PVP-I *in-situ* gel eye drops was slowed down significantly after administration, and it could be retained in rabbit conjunctival sac for more than 20 min. The results showed that PVP-I *in-situ* gel eye drops extended povidone iodine efficacious time in eyes significantly longer.

3 DISCUSSIONS

Deacetylated gellan gum has temperature-dependent and cation-induced gelation properties, and a certain concentration of deacetylated gellan gum solution can form

a moderate viscosity and strong water-holding gel with the cations in the tears. [13] Merck's Timoptic-XE®, a long-acting ophthalmic timolol maleate formulation, has been shown to improve ocular bioavailability and reduce the frequency of drug administration. Comparing non-gelled polymer solutions with Timoptic-XE®, it was discovered that the gelation mechanism is an important factor for improved efficacy. Comparing with the traditional ophthalmic preparation, the ion-sensitive *in-situ* gel has the obvious advantages, such as long residence time in cornea, thus improved bioavailability; good histocompatibility and dosing accuracy; ability to stay in flowing liquid state before use, thus easy to fill, and easy for industrial production.

Gellan gum concentrations of 0.5% to 1% (w/w) are required for *in-situ* gel formation in all marketed products containing gellan gum. Moreover, since gellan gum is ion-sensitive, the inorganic salt such as sodium chloride cannot be added as osmotic pressure regulator in its formulation. We described here an *in-situ* gel-formation ophthalmic formulation containing povidone iodine ("PVP-I"). PVP-I is a polymer drug with significantly different physical and chemical properties, such as strong acidity, water-solubility, ion complex equilibrium, comparing to all reported small molecule drugs, which potentially affect gellan gum's gel-forming ability. However, we have surprisingly discovered that povidone iodine's addition into polysaccharide natural polymer materials such as deacetylated gellan gum, reduces the required gellan gum concentration in order for gel-formation significantly. Gellan gum's concentration can be less than 0.5% (w/w) in compositions containing PVP-I when mixing with the simulated tear to form a gel. Although gellan gum has an ion-sensitive property, its viscosity does not increase at physiological temperature (34°C) due to the dilution of simulated tear after mixing with simulated tear. Therefore, gellan gum itself cannot form *in-situ* gel in the eye upon instillation. However, we surprisingly found that the viscosity of the gellan gum solution containing PVP-I is significantly increased at physiological temperature (34°C) which shows a typical in-situ gel property after mixing with simulated tear.

Moreover, we surprisingly discovered that adding an appropriate concentration of sodium chloride as osmotic pressure regulator into compositions containing povidone-iodine and gellan gum will cause a significant thixotropy of the formulation. The composition will transform into a semi-solid gel state after sitting still for a few hours, but it can quickly turn into a free-flowing fluid with a gentle shake of the container. In addition, the addition of appropriate concentration of sodium chloride in PVP-I and gellan gum compositions makes the composition more sensitive to tear ions to form gel when mixing with tears. The composition not only extends PVP-I's retention time in the conjunctival sac with slower dissolution and extended release of the drug, but also can reduce povidone iodine's irritation to the eye. The stability of such extended release *in-situ* gel PVP-I composition is improved over its corresponding solution formulation, making it more suitable for clinical applications.

4 ACKNOWLEDGEMENTS

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