

In-vivo Evaluation Methods for Preparation of Ophthalmic *In-situ* Gel

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ABSTRACT

The eye is a vital component of the human sensory system, but various things can pose a threat to its cleanliness and well-being. An *in-situ* ophthalmic gel is one of the preparations developed to optimize the pharmacological activity in the eye. This formulation is intended to extend the drug's duration in the eye. An assessment is necessary to guarantee its occurrence and mitigate any adverse repercussions. The literature search conducted in this evaluation focused on an *in-vivo* study, enabling the collection of various pharmacokinetic data, including C_{max}, T_{max}, area under curve (AUC), and mean residence time (MRT). The polymer is an essential element in this inquiry, so the selection must be accurate according to the results of the literature review. The most effective method is high-performance liquid chromatography (HPLC), and the most effective polymer is a composite polymer formed by combining different types of polymers, such as Pluronic F127 and carbopol, pectin and thiolate, poloxamer and carbopol, and gellan gum and hydroxyethylcellulose. Aside from the polymer mixture, the poloxamer solution has the potential to produce highly positive results.

Keywords: *In-situ* gel, Poloxamer, Carbopol, Gellan gum, *In-vivo*.

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INTRODUCTION

It is not uncommon for people to suffer from eye diseases, and they can strike anyone at any time. When there is a disturbance or disease in the eye, it will be very distressing, and if it is not followed up on, it can be fatal to human life. That is because the eye is one of the senses that plays a vital role in human existence. Red eyes, itching, dizziness, visual impairment, and blindness are all examples of symptoms that the eyes can experience. As a result, it is essential to maintain the health of your eyes by undergoing routine checkups. This is vital in order to diagnose and treat eye illnesses as quickly as possible.^{1,2} When the cornea, which is the lymphatic membrane, is completely clear and located in the middle (symmetrical) between the two eyes, when the white part of the eye is completely white, when the pupil really looks black, clear, and there is a light reflection, and when there is light, the pupil shrinks, when the eyelids are able to open and close properly, when the eyes are good and directed when there are no secretions or dirt in the eye, and when there are no wrinkles in the eyes, then the eye is considered to be healthy or free of disturbances.³

Eye drops (oculoguttae), eye ointments (oculenta), eye washers (colyria), and unique means of administration such as lamellas and eye spray are all examples of pharmaceuticals that fall under the category of ophthalmic medications. Furthermore, the depot formulation can be utilized for the treatment of eyes that are either unharmed or injured. Pharmaceutical substances that have a local therapeutic impact, ophthalmic medications are utilized in the treatment of eye conditions. It is possible for pharmaceutical eye preparations to consist of ointments and solutions, both of which are required to adhere to stringent sterility standards. The eye medicine solution is a formulation that is sterile and devoid of particles, and it was developed specifically for use in the affected eye. A number of factors must be taken into consideration during the manufacturing process of ophthalmic medicinal solutions. These include the toxicity of the active components, the level of isotonicity, the utilization of multiple stabilizers, the absence of appropriate preservatives, the sterilizing process, and the appropriate packaging.⁴

Ophthalmic gel preparations *in-situ* are preparations that are initially provided in liquid form and subsequently converted

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to the gel phase depending on particular triggers such as pH, temperature, and the presence of ions. These preparations are administered *in-situ*. Conventional eye preparations, which include solutions, ointments, and suspensions, have a few drawbacks, including a limited amount of time that they come into contact with the cornea and the effect of blurring vision while they are being used. For the purpose of addressing the lack of traditional ophthalmic supplies that has arisen as a result of the utilization of lubricants and the prolonged contact times with the cornea, gel preparations have been made available. A number of *in-situ* assessments of ophthalmic gels have been carried out in order to guarantee that the preparation is up to par with the necessary standards and is risk-free for application.⁵

In-vivo is a term that describes procedures or treatments that are carried out on living organisms, such as animals or people. The process entails analyzing the effects, efficacy, or safety of a drug or treatment in a biological system that is authentic. The *in-vivo* method required the creation of corneal ulcers in rabbits, which were then treated with nano formulas and conventional eye drops that were administered.⁶

Method

The methodology employed involved doing a comprehensive literature review utilizing online databases such as Google Scholar and PubMed, covering the period from 2013 to 2023. The search was performed utilizing the keywords “*in-vivo*” and “*in-situ* gel ophthalmic.” Subsequently, the search results were evaluated, and a meticulous analysis was conducted to compare different sources and extract precise information. Subsequently, the results of the analysis are inputted into a unified table and organized based on the methodology and the results.

On the literature search carried out obtained 11 articles that can be included in the review this time of a total of 437 other articles that appear in the search online. The results are described as in Figure 1.

DISCUSSION

The process for evaluating the manufacture of *in-situ* ophthalmic gel *in-vivo* was obtained based on the findings of the literature search that was carried out, and the results of this evaluation are presented in Figure 1. Based on the findings of the searches that were conducted, the procedures that have been utilized in the evaluation of the creation of *in-situ* ophthalmic gel *in-vivo* are outlined in Figure 1.⁶⁻¹⁶

Polymers and mechanism details are distinct for each technique, which results in various outcomes. Different mechanisms are utilized in each piece of literature, with high-performance liquid chromatography (HPLC) being the predominant method for analyzing the data. In the field of analytical chemistry, high-performance liquid chromatography, often known as HPLC, is a technique that is commonly utilized to separate, identify, and quantify particular components that are present in mixtures. Numerous industries, including the food industry, the chemical industry, the pharmaceutical industry, and environmental analysis, make frequent use of

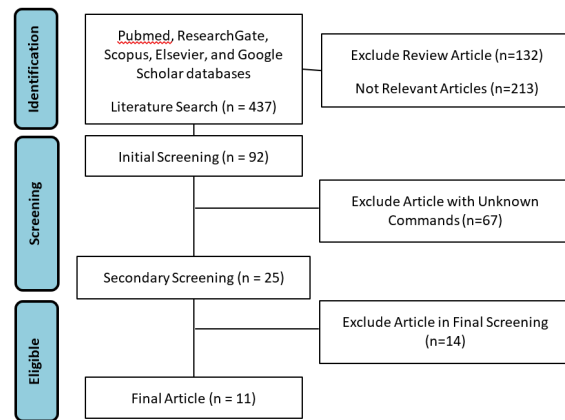


Figure 1: Flowchart for study selection

it. Analysis of medicines and metabolites in biological fluids, particularly plasma, serum, or urine, can also be performed with high-performance liquid chromatography (HPLC) in *in-vivo* research. The approach was confirmed to possess accuracy, precision, sensitivity, reliability, reproducibility, and efficiency.^{17,18}

In the other research, a fluorimeter microplate was used in the analysis of the results. By assessing medications and metabolites in biological fluids, notably plasma, serum, or urine, a microplate fluorimeter can be utilized to measure *in-vivo* research. This can be accomplished by measuring biochemicals. For the purpose of providing reliable and reproducible biological tests, microplates are an essential component. It is essential to select the most appropriate microplate in order to achieve reliable assay performance. Microplate well number, well shape, and material are three of the most important criteria to consider when selecting a microplate. In fluorescence experiments, fluorescein, cyanine 3, cyanine 5, green fluorescent protein (GFP), rhodamine, Texas Red, coumarin, and other fluorophores are utilized. Other fluorophores may also be utilized.¹⁹

Other studies also used Scion Image software to analyze the results. Scion Image is a piece of software that has the capability to perform image analysis in a wide range of *in-vivo* research projects. Scion Image is a publicly available computer software that has been adapted from the NIH Image software developed by the National Institutes of Health in the United States (Scion Corporation, 2000-2001). The main utilization is within the realm of biological image analysis. The Scion Image program specifically focuses on the examination of ferulic acid levels in plant matter and the quantitative analysis of herbal raw materials using thin-layer chromatography (TLC).²⁰ The approach that makes use of high-performance liquid chromatography (HPLC) is, in general, the method that is utilized the most frequently; nonetheless, this method necessitates the sacrifice of experimental animals. A corneal operation must be performed on a test animal in order to fulfill the requirements of the Scion image test protocol. One of the approaches that does not require the animal to be sacrificed is

Table 1: Description of the polymers

<i>Polymer</i>	<i>Polymer type</i>	<i>Description</i>	<i>Ref.</i>
Thermosensitive gel	Synthetic polymers	Thermosensitive gels are capable of performing rapid gelation, shape maintenance, and immunity, but tend to be quickly eliminated in physiological media. With higher gel concentration, the gel disintegrates slower, making it harder to remove from the lesion.	24
Gellan Gum	Biopolymer	Gellan gum can be readily transformed into a thermally stable, see-through gel. The gum gel is biocompatible and suitable for tissue injection. Gellan gum hydrogels have the ability to facilitate the growth of hydraulic chondrocytes. Injecting gellan gum hydrogels is effective in enclosing and providing support for human joint chondrocytes, while also promoting the production of ECM components.	25
A combination of the polymer Pluronic F127 and the carbopol	Mixture Polymer	The carbopol/Pluronic F127 mix can make the gel stronger in physiological conditions and change phases to create a strong gel that can survive the force of cutting in the eye and may help the drug last longer. This mixture holds the drug better than separate polymer solutions, which shows that it can be used as an <i>in-situ</i> gel formation method to deliver drugs to the eye.	26
Pectin and thiolate	Mixture Polymer	Thiolate pectin possesses mucoadhesive qualities that are very appealing, which enable it to extend the amount of time that the delivery system remains at the location where it is applied. This combination has been promising for improved drug delivery and therapeutic effectiveness.	27
Combination of Poloxamer and Carbopol	Mixture Polymer	This mixture protects the skin on the face from injury. When compared to employing poloxamer or carbopol alone, this combination has been found to enable controlled diffusion release of the puerarin medication for longer periods of time and increase drug retention in the precorneal area.	28
Gum gellan and hydroxyethyl cellulose	Mixture Polymer	Gellan gum and hydroxyethylcellulose are designed to increase drug retention and enhance drug delivery to the eye. This combination has demonstrated the ability to provide novel drug delivery methods for the eyes that are more therapeutically effective.	15
Reacted tamarind seed xyloglucan (RXG)	Biopolymer	RXG (reacted tamarind seed xyloglucan) demonstrates mucoadhesive and thermoresponsive <i>in-situ</i> gelling properties. RXG is an appealing polymer for a variety of drug delivery applications due to these features.	16
Poloxamer solution	Synthetic Polymers	Poloxamers can generate an <i>in-situ</i> gel system that increases the viscosity of a formulation when the temperature in the pre-corneal region changes. This characteristic extends the period of medication interaction on the eye's surface and slows drug removal, potentially enhancing bioavailability and therapeutic effects.	29
Poly(NIPAAm-MAA-VP)	Synthetic Polymers	Thermo-responsive polymers like Poly (NIPAAm-MAA-VP) have been explored for use in an <i>in-situ</i> gel system for eye medicine delivery. The system boosts medication retention and ocular delivery. Poly's sol-gel transitions in response to ocular temperature enhance drug contact periods and increase therapeutic efficacy, making it a suitable choice for such applications.	6

the use of a microplate fluorimeter. In this method, the animal is only required to measure the intensity of fluorescence of the gel preparation *in-situ* on the eye in comparison to the control. Overall, HPLC is still the best method to do considering the accuracy and clarity of the results obtained. This test can be supplemented by a tonometer examination to determine the IOP. The following results were obtained from the article search: Cmax, tmax, AUC (Area Under Curve), IOP (Intraocular Pressure), and MRT (Mean Residence Time). With non-partition model analysis, several pharmacokinetic parameters such as tmax, Cmax, and AUC are derived from a graph etched between concentrations (ng/ml) in water humor and time (h). Maximum concentrations detected in water humor (mmHg), time tmax required to reach maximum concentration, AUC of the area below the concentration curve to time, IOP to determine the ability of the tested formulation to lower intraocular pressure, and MRI to determine the time spent in the eye.^{9,14}

In ophthalmic studies, polymers are utilized for the purpose of drug delivery and sustained release in the treatment of eye ailments. They provide a number of benefits, including regulated medication release, decreased systemic toxicity, and enhanced patient comfort during the treatment process. These include synthetic polymers, biopolymers, and polymer mixes, all of which are examples of polymers that are utilized in ophthalmic research. The term “synthetic polymer” refers to a type of polymer that arises from the process of chemical synthesis. Linear polymers and branched/graft polymers are the two groups that can be further identified and classified. An example of a polymer that is obtained from natural sources is a biopolymer, which can be composed of polysaccharides or proteins. A polymer mixture is a mixture of two or more different types of polymer that can be blended to achieve certain features. These properties include the manipulation of the physical-chemical, mechanical, and biological properties of a system.²¹⁻²³ According to the findings of the literature

search, the following polymers are utilized for *in-vivo* testing of ophthalmic gel preparation, showed in Table 1:

Based on the data presented in Table 1, the most efficient polymer is a polymer of a mixed polymer type where the combinations complement each other and improve the performance of the polymer. There is also a poloxamer solution of a synthetic polymer that has been tested in the delivery of eye medicines.

CONCLUSIONS AND AUTHOR PERSPECTIVE

According to the reviewed literature, the most effective method is the high-performance liquid chromatography (HPLC) method, based on the acquired analysis results. This approach allows us to obtain C_{max}, t_{max}, AUC, and MRT data simultaneously. An IOP check with a tonometer can be used to enhance this information. Furthermore, the choice of polymers employed will significantly influence the outcome of the test. The maximal polymer is a composite polymer formed by combining different types of polymers, such as Pluronic F127 and carbopol, pectin and thiolate, poloxamer and carbopol, and gum gellan and hydroxyethylcellulose. Besides the polymer mixture, the poloxamer solution also holds significant potential for yielding highly favorable outcomes.

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