ISSN PRINT 2319 1775 Online 2320 7876
Research paper© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11, Iss 05, 2022

IN-SITU GEL FORMING OPHTHALMIC FORMULATIONS OF PARASYMPATHOMIMETIC DRUG

Neelam Jain*, Neeraj Jain[1], Satyendra Mishra[2], Alka[3], Rani Kumari[4]

*Faculty of Pharmacy, Oriental University, Indore-453555, M.P., India.
[1]Teerthankar Mahaveer College of Pharmacy, TMU, Moradabad- 244001, U.P., India.
[2]Kailash Institute of Pharmacy and Management, Gida, Gorakhpur-273209, U.P., India.
[3]Amity Institute of Pharmacy, Amity University, Lucknow campus, Uttar Pradesh, India.
[4]Faculty of Pharmaceutical sciences Rama University, Mandhana, Kanpur-209217, U.P., India.

***Corresponding author name:** Neelam Jain
***Corresponding author address:** Faculty of Pharmacy, Oriental University, Indore-453555, M.P., India.

Email id: neelamnj02@gmail.com
Contact no: +91-8982753610

ABSTRACT

In situ gels are systems which are applied as solutions or suspensions and are capable of undergoing rapid sol-to-gel transformation triggered by external stimulus such as temperature, pH etc. on instillation. The aim of the present investigation is to prepare and evaluate *in situ* gel-forming ophthalmic drug delivery system of parasympathomimetic drug carbachol, commonly known as carbamylcholine. Locust bean gum, an ophthalmic gel forming mucoadhesive polymer was chosen as polymer which undergoes instantaneous gel formation. Carbopol 934 was further incorporated as a viscosity enhancer in order to achieve the desired consistency so as to facilitate sustained drug release. The developed formulations were evaluated for clarity, pH measurement, gelling capacity, spreadability and *in-vitro* drug permeation study. Thus, *in-situ* gel based systems containing gums can be a valuable approach for ophthalmic drug delivery when compared to conventional systems.
KEYWORDS: Ocular delivery, *In-situ* gel, Gelling capacity, Locust bean gum, Carbachol.

INTRODUCTION

One of the most intriguing difficulties facing pharmaceutical scientists is medication delivery to the eye. The eye is protected by a number of intricate defence systems, making it challenging to establish an effective concentration of the medication inside the target area of the eye. This makes the successful delivery of pharmaceuticals into the eye exceedingly difficult. To get through the eye's defences without enduring long-term tissue damage is the formulator's problem. There is still acceptance for conventional ophthalmic dose forms such solutions, suspensions, ointments, etc. Due to inadequate bioavailability, decreased tear

ISSN PRINT 2319 1775 Online 2320 7876
Research paper© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11, Iss 05, 2022

formation, the impermeability of the corneal epithelium, non-productive absorption, and brief residence times that cause impaired vision, these dosage forms are no longer sufficient to treat major ocular illnesses including glaucoma. The need for more effective ocular delivery methods is driven by the development of newer, more sensitive diagnostic procedures and treatment substances [1].

An increased level of intraocular pressure (IOP) in glaucoma is a medical feature that may gradually impair vision. A major cause of blindness in the globe is glaucoma. By 2020, 80 million individuals are predicted to be impacted, up from the current projection of 40 million. A progressive condition known as open-angle glaucoma causes damage to the optic nerve. A progressive increase in intraocular pressure causes slow optic nerve damage, which leads to eventual complete blindness. Most ophthalmologists still start treating glaucoma patients with carbachol [2].

The parasympathomimetic drug carbachol, commonly known as carbamylcholine, has a direct action and stimulates the muscarinic and nicotinic receptors. It is frequently applied to open-angle glaucoma therapy. It causes the iris sphincter muscle to contract and go into miosis by acting on the M3 subtype of muscarinic receptor. This procedure speeds up the pace at which aqueous fluid drains from the eye, lowering intraocular pressure (Johnson et al., 1993). Since topical aqueous ophthalmic medicines like carbachol eye drops must be used often and only achieve limited ocular bioavailability (2-4 percent), glaucoma therapy needs to be improved (minimal precorneal residence time) [3]. Unfortunately, the majority of the medication does not reach the eyes; instead, it is lost through physiological drainage (non-productive loss). The systemic side effects are a result of this loss.

In-situ gel that gelates after administration as a result of physico-chemical processes taking place in the eye. By creating *in-situ* gel, one can enhance pre-corneal residence period and improve medication absorption. Before being administered within the body, the *in-situ* gel is a drug delivery system that is in the form of a sol, but it transforms into a gel when physiological circumstances change. On the cornea's surface, *in-situ* phase change takes place. Therefore, this kind of formulation offers advantages over both solutions and gels; they may increase the formulation's retention period as well as the drug's accuracy and simplicity of administration [4].

Increased drug accessibility to aqueous humour can result from increased drug penetration through the cornea as a result of increased drug contact time with the eye's surface (Increased precorneal residence time). This can be achieved by using a bioadhesive polymer, which keeps the medication from draining from the eye by building a non-covalent connection with the surface of the eye over an extended period of time. As a result, less medication will need to be used to provide the desired therapeutic impact. The frequency of systemic adverse effects will decrease with a dosage reduction. These technologies greatly enhanced the drug's ocular bioavailability, which may increase patient compliance [5]. Therefore, this study presents the development of ocular *in-situ* gel of carbachol for the treatment of glaucoma.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11, Iss 05, 2022

MATERIALS AND METHODS

Materials

M/s Zydus Cadila Health Care Ltd. graciously accepted the carbachol as a gift sample (Ahmedabad, India). Loba Chemie Pvt. Ltd. supplied the locust bean gum (LBG) and carbopol 934. (Mumbai, India). Magnesium chloride, sodium chloride, calcium chloride dihydrate, potassium dihydrogen phosphate, disodium hydrogen phosphate, acetone, methanol, chloroform, boric acid, sodium hydroxide, sodium bicarbonate, potassium chloride, glacial acetic acid, and benzalkonium chloride were bought from S.D. Fine chemicals (Mumbai, India). Throughout the investigation, double-distilled water was used.

Formulation of *in-situ* gel

As shown in Table 1, the chosen medication was added into the polymeric gel basis at a concentration of 0.3% w/w. The drug-containing suspension was removed from the aqueous medium by ultracentrifugation at 15000 rpm at 4^0C, and it was then slowly introduced by vortex in the sterile blank gel beneath a cabinet with laminar air flow. With the addition of sodium chloride (0.9% w/v), the solution was made isotonic. Then, as a preservative, benzalkonium chloride (0.02% v/v) was added. Continued vortexing was used to create an *in-situ* homogeneous gel, which was then sonicated to remove any bubbles. The produced gels were put into amber-colored glass vials and kept chilled between 4 and 8^0C.

Table 1: Composition for *in-situ* gelling system

Gel formulations	Drug (g)	Locust bean gum (g)	Carbopol 934 (g)	Benzalkonium chloride (% v/v)	Sodium chloride (% w/v)
G1	0.3	0.3	0.1	0.02	0.9
G2	0.3	0.3	0.1	0.02	0.9
G3	0.3	0.3	0.1	0.02	0.9
G4	0.3	0.2	0.2	0.02	0.9
G5	0.3	0.2	0.2	0.02	0.9
G6	0.3	0.2	0.2	0.02	0.9

Evaluation of *in-situ* gel formulations

Physical appearance

Clarity, colour, homogeneity, the presence of foreign particles, and the creation of any aggregates (lumps) of polymers were all checked on the manufactured gel by visual inspection against a black and white backdrop [6].

Measurement of pH

In 25 ml of purified water, 2.5 g of gel were precisely weighed and mixed. The pH of the gel dispersion was then determined at room temperature using a digital pH metre. The pH metre was calibrated before to measurement, and measurements were obtained by dipping the glass rod into the gel compositions [7].

In-vitro gelation study

By adding a drop of the formulation to a test tube that was sealed with paraffin and contained 2ml of freshly made simulated tear fluid (STF), pH 7.4, an *in-vitro* gelation investigation of

ISSN PRINT 2319 1775 Online 2320 7876
Research paper© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11, Iss 05, 2022

gel formulations was conducted. This test tube was positioned in the water bath at a constant temperature of 35^0C–10^0C. Based on the stiffness of the gel that forms and the length of time that it stays that way, the solution's ability to gel was assessed [7].

Spreadability

Two standard-sized glass slides were chosen. One of the slides was covered with 1 g of gel. The other slide was sandwiched between the two slides for its whole length of 5 cm, sitting on top of the formulations. The formulation between the two slides was uniformly squeezed to form a thin layer by applying 100 g of weight on the upper slide. The excess formulation that was sticking to the slides was scraped off once the weight was removed. The formulation was put on one of the fixed slides. With one end attached to a string to which load could be given with the aid of a straightforward pulley and a pan, the second movable slide was put over it. When a 30 g weight was placed on the pan, the amount of time it took for the upper slide to move 5.0 cm and separate from the lower slide under the influence of the weight was recorded. The following formula was used to determine spreadability [8]:

$$S = M.L / T$$

Where, S is spreadability, M is the weight of the top slide (grammes), L is the length of the glass slide (cm), and T is the time it takes for the slides to entirely separate from one another (sec). This research was done in triplicate.

Isotonicity study

To achieve hypertonic (3% w/v), hypotonic (0.2%), and isotonic (0.9%) concentrations, solutions of NaCl at three distinct concentrations were produced. There were four clear slides made. They carried the designations test, hypertonic (HT), hypotonic (HP), and isotonic (IS) (T). To prevent blood from coagulating, a drop of heparin solution (1 percent w/v) was added to the centre of each slide along with a little drop of blood.Each test solution (G1, G2, G3, G4, G5, and G6) was added as a drop to the corresponding slide. To observe the morphology of RBCs, the contents were mixed using the edge of the cover slip and viewed through a microscope at a 45X magnification. If a preparation is isotonic, neither the entry of water from the injected solution (hypotonic) nor the exit of water from the cell (hypertonic) will affect the structure of the cell [9].

***In-vitro* drug permeation study**

In-vitro drug release studies were conducted using Franz diffusion cells. To simulate *in-vivo* conditions like the corneal epithelial barrier, a piece of ordinary cellophane membrane was employed. It was placed over the open end of a glass tube that had a rubber band around it to make it watertight. The tube was kept in a thermostatically controlled water bath that was kept at 37°C while submerged in a 150 ml beaker containing 50 ml of synthetic tear fluid (pH 7.4). (receiver compartment). Over the cellophane membrane, one gram of gel formulation was added to the release tube (donor compartment).The reservoir compartment, which contained 50 ml of simulated tear fluid (pH 7.4) and was continually agitated at a speed of 100 rpm to mimic blinking, was in touch with the whole surface of the membrane. 5 ml aliquots of the release medium were taken out for analysis at regular intervals for up to 8 hours, and they were replaced with an equivalent volume of the same release medium at the same temperature to keep the volume constant. Using a UV-spectrophotometer at 530 nm, *in-*

ISSN PRINT 2319 1775 Online 2320 7876

Research paper© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11, Iss 05, 2022

vitro drug permeation from in-situ gel was spectrophotometrically examined. For all gel formulations, the results were collated, and a graph was drawn showing the cumulative percentage of drug permeation over time. The study was conducted three times [10].

RESULTS AND DISCUSSION

Evaluation of *in-situ* gel formulations

Physical appearance

As indicated in Table 2, it was revealed through the investigation that all of the gel formulations (G1, G2, G3, G4, G5, and G6) appeared translucent, demonstrating the lack of foreign particles.

Measurement of pH

All gel formulations (G1, G2, G3, G4, G5, and G6) had their pH values measured in triplicate, and Table 2 displays the mean values with standard deviation for each formulation. It was discovered to be between 6.6±0.35, 6.8±1.22, 6.5±1.07, 6.7±0.15, 6.4±0.64, and 6.5±0.03 in accordance with the established ocular range. All of the formulations are expected to be non-irritating when administered topically [11].

In-vitro gelation study

According to *in-vitro* gelation investigations, formulations G2 immediately exhibit stiff gelation that lasts for a long time, whereas G1 and G3 exhibit immediate gelation that lasts for 2–3 hours, as shown in Table 2. The viscosity of the gel formulation is indicated by the gel strength. It was found that gel strength increased along with an increase in polymer concentration.

Spreadability

The spreadability of the generated *in-situ* gel formulations (G1 to G6) was tested in triplicate, and the mean values with standard deviation are provided in Table 2. The produced gel's spreadability (G1 to G6) ranged from 1.41±0.72 g/cm/s to 2.35±0.25 g/cm/s. It was found that when the concentration of polymers increased, the formulation's spreadability reduced as a result of its increased viscosity [12]. When compared to other formulations, Formulation G2 has a lower viscosity and a higher spreadability (2.35±0.25 g.cm/s).

Table 2: Evaluation of the gel formulations (Mean± SD, n=3)

Gel formulations	Appearance	pH	Gelation capacity	Spreadability (g.cm/s)
G1	Translucent	6.6±0.35	++	1.41±0.72
G2	Translucent	6.8±1.22	+++	2.35±0.25
G3	Translucent	6.5±1.07	++	2.16±0.27
G4	Translucent	6.7±0.15	++	2.07±1.02
G5	Translucent	6.4±0.64	++	1.98±0.45
G6	Translucent	6.5±0.03	++	2.01±0.06

Where, +++ is immediate stiff gelation and ++ is immediate gelation.

IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

ISSN PRINT 2319 1775 Online 2320 7876

Research paper© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11, Iss 05, 2022

Isotonicity study

The prepared carbachol entrapped gel formulation under test did not exhibit any alteration in the morphology (swelling or shrinking) of blood cells. The gel formulation G2 were isotonic as indicated in Figure 1.

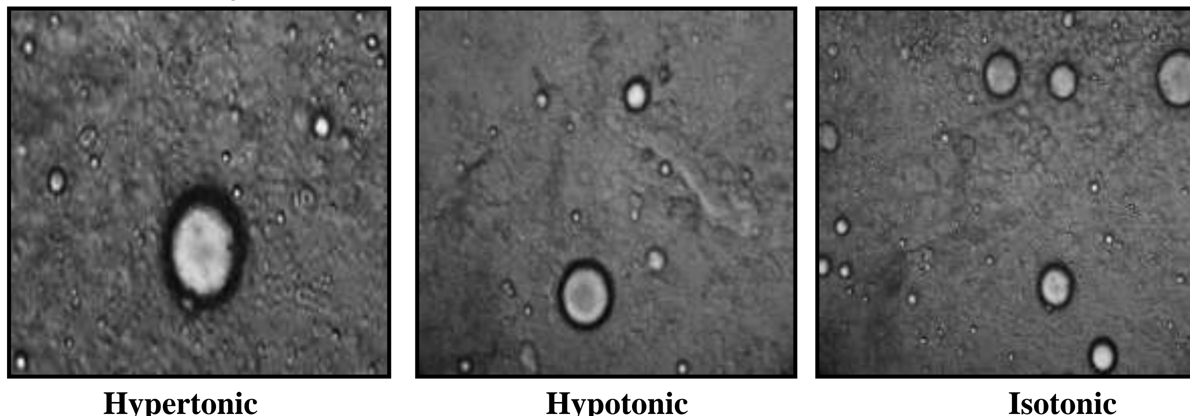

Figure 1: Photomicroscopy of gel formulation (G2) after isotonicity testing

***In-vitro* drug permeation study**

The *in-vitro* permeation profiles of carbachol-entrapped gels made with locust bean gum and carbopol 934 in simulated tear fluid of pH 7.4 is shown in Figure 2. After 8 hours, the total amount of medication that migrated from the gel formulation ranged from 50.13±0.81 to 66.23±0.22%. G2 had the slowest drug release (50.13±0.81%) of all the formulations because of its high gelling capacity. The presence of bioadhesive polymer, which keeps the formulation in touch with the eye for a long time, is responsible for these findings. This means that the gel system in which the drug is entrapped limits the drug's release because of the gel's polymer matrix, which improves the gel's consistency and limits drug release.

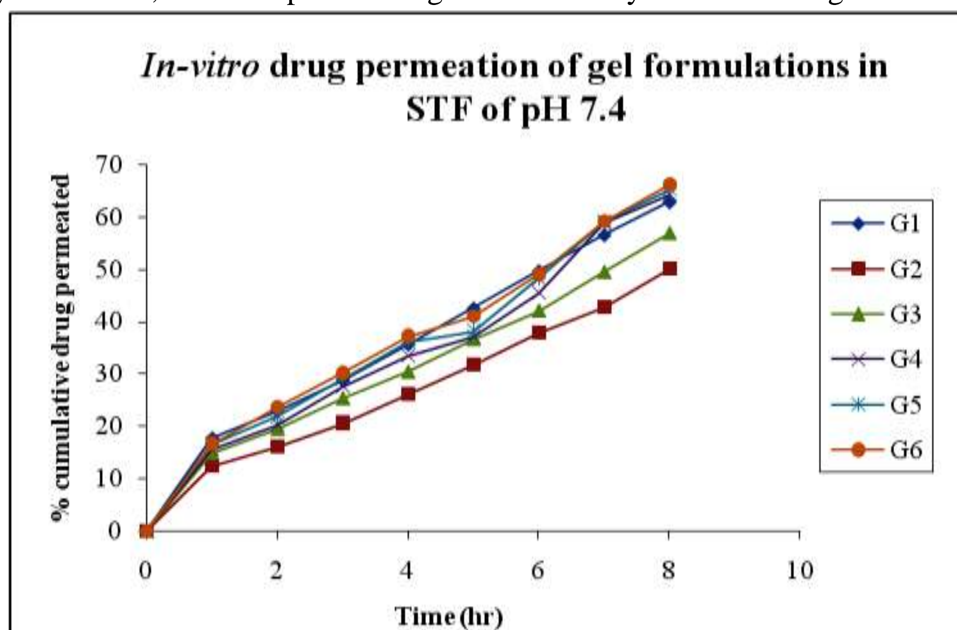

Figure 2: *In-vitro* permeation study of gel formulations (G1 to G6)

Research paper© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11, Iss 05, 2022

CONCLUSION

It was a fruitful exploratory investigation for creating carbachol gel system. A miotic substance called carbachol that is used to treat glaucoma was successfully put into a gel system. The new gel formulations showed superior characteristics to other dosage forms, suggesting a workable substitute to traditional eye drops. The newly created gel formulation is more bioavailable and effective at extending precorneal residence duration. These findings point to gels containing bioadhesive polymers as potential ocular carriers for the precise administration of carbachol for the treatment of glaucoma.

REFERENCES

1. Nair AB, Shah J, Jacob S, Al-Dhubiab BE, Sreeharsha N, Morsy MA. Experimental design, formulation and in vivo evaluation of a novel topical in situ gel system to treat ocular infections. PLOS ONE. 2021; 16: e0248857.
2. Kotreka UK, Davis VL, Adeyeye MC. Development of topical ophthalmic In Situ gel-forming estradiol delivery system intended for the prevention of age-related cataracts. PLOS ONE. 2017; 12: e0172306.
3. Ting Shen Zijian Yang. In-vivo and in-vitro Evaluation of in situ gel formulation of pemirolast potassium in allergic conjunctivitis. Drug Design, Development and Therapy. 2021; 15: 2099–2107.
4. Umay M, Güven M, Sami B. Behiye Ş, Yasemin Y. Development and in vitro/in vivo evaluation of thermo-sensitive in situ gelling systems for ocular allergy. Braz J Pharm Sci. 2019; 55: 1-11.
5. Mandal S, Manjunath KMJ, Thimmasetty, Prabhushankar GL, Geetha MS. Formulation and evaluation of an in situ gel-forming ophthalmic formulation of moxifloxacin hydrochloride. International Journal of Pharmaceutical Investigation. 2012; 2: 78-82.
6. Jain A, Deveda P, Vyas N. Development of antifungal emulsion based gel for topical fungal infections. Int J Pharm Res Dev. 2011; 2: 18–25.
7. Jain N, Verma A, Jain N. Anti-glaucomatic niosomal gel: a brief review. World J Pharm Pharm Sci. 2020; 9: 529-548.
8. Shalin C, Kumar KV, Vimal V. Formulation, evaluation and optimization of niosomal gel of loratadine. World J Pharm Pharm Sci. 2017; 6: 971-992.
9. Sawant D, Dandag PM, Gadad AP. Formulation and evaluation of sparfloxacin emulsomes-loaded thermosensitive in situ gel for ophthalmic delivery. J Sol Gel Sci Technol. 2015; 77: 654–665.
10. Sathyavathi V, Hasansathali AA, Ilavarasan R, Sangeetha T. Formulation and evaluation of niosomal in situ gel ocular delivery system of brimonidine tartrate. Int J Life Sci Pharma Res. 2012; 2: 82-95.
11. Gilhotra RM, Saroot R. Design and characterization of bioadhesive ophthalmic films of flurbiprofen. Thai J Pharm Sci. 2011; 35: 29-39.
12. Chaudhari PD, Desai US. Formulation and evaluation of niosomal in-situ gel of prednisolone sodium phosphate for ocular drug delivery. Int J App Pharm. 2019; 11: 97-116.