

“Formulation And Evaluation Of Netarsudil Insitu Gel”



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ABSTRACT

In present study, ophthalmic eye drops solution are available in the Indian market with strength of 0.02 w/v. As of now, the Indian market does not have any formulations of In- situ gel. Netarsudil ophthalmic solutions have an extensive drug loss during the time of administration due to that drug may not reach at the site of action. As a result, this medication was created as In-situ gel to improve patient compliance and increase the bioavailability of Netarsudil. In-situ gel was prepared by adopting Cold method, which is relatively easy and using pH triggered system. Preliminary studies were taken by using different excipients gelling agent and viscosity enhancers. Sodium alginate, Carbopol 940 and HPMC E4M, HPMC K4M, HPMC E50LV used as gelling agent and viscosity enhancers respectively. By this check best polymer for the optimization study. Three dependent responses, R1 (Gelling time), R2 (Viscosity), and R3 (% CDR), were explored in order to optimise the formulation using the two parameters F1 (Quantity of HPMC E50LV- viscosity enhancers) and F2 (Carbopol 940- gelling agent). Formulations with HPMC E50LV at 1.5mg and Carbopol 940 at 0.3mg (D2) were shown to have comparably better.

Key Words: ophthalmic solutions, viscosity enhancers, Cold method, Gelling time

1. INTRODUCTION:

1.1 Introduction to Disease:^{[1][2][5]}

Glaucoma is a group of eye diseases that can cause vision loss and blindness by damaging a nerve in the back of your eye called the optic nerve. The main reason of Glaucoma is that the drainage angle of an eye not working properly, fluids build up. Pressure inside the eye raises i.e. Intraocular Pressure and damaging the optic nerve and leads to blindness.

There are two main types of Glaucoma 1) Open angle Glaucoma and 2) Closed angle Glaucoma. In open angle glaucoma the eye does not drain fluid as well as it should (like a clogged drain). As a result, eye pressure builds and starts to damage the optic nerve. This type of glaucoma is painless and causes no vision changes at first. Closed angle Glaucoma or Narrow angle Glaucoma occurs when iris is very close to the drainage angle in their eye. The iris can end up blocking the drainage angle.

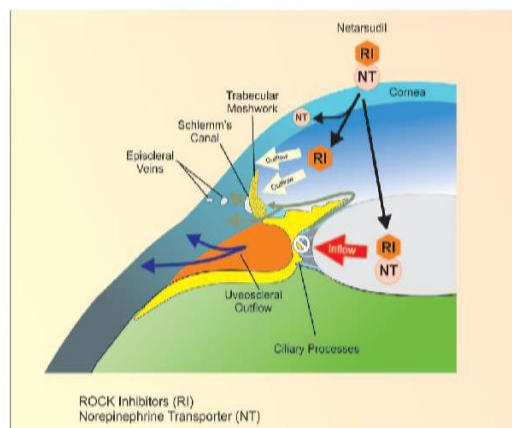
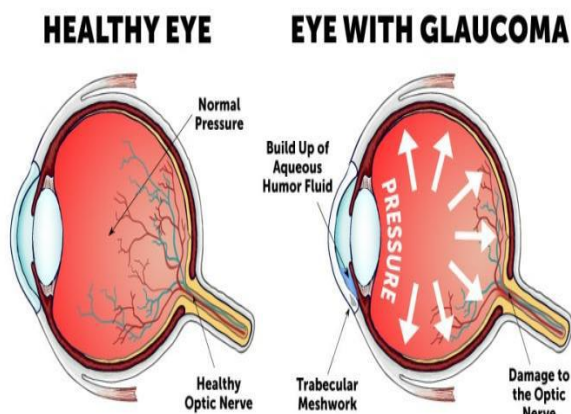


Figure 1.1 Glaucoma Condition Figure 1.2 Mechanism of Netarsudil

There are also various causes of glaucoma such as Impaired Aqueous Humor Drainage, Primary open-Angle Glaucoma in which the drainage angle remains open, but the trabecular meshwork's function is gradually impaired over time, causing a gradual increase in IOP another is Angle closure Glaucoma in which the drainage angle between the iris and cornea is blocked, leading to a sudden and significant increase in IOP.

There are certain risk factors of Glaucoma such as Age, Family history, Ethnicity, Various medical conditions such as Diabetes, heart disease, and high blood pressure may increase susceptibility.

1.1.1 Causes of Glaucoma^{[3][4]}

- High internal eye pressure, also known as intraocular pressure
- Age over 55
- Family history of glaucoma
- Corneas that are thin in the center
- Eye injury or certain types of eye surgery
- Certain medical conditions, such as diabetes, migraines, high blood pressure and sickle cell anemia

1.1.2 Treatment of open angle glaucoma^[6]

Beta adrenergic blockers: timolol, betaxolol, levobunolol,

Alpha adrenergic agonists: Dipivefrine, Apraclonidine, Brimonidine

Prostaglandin analogues: Latanoprost, Travoprost, Bimatoprost,

Carbonic anhydrase inhibitors: Acetazolamide, Dorzolamide

Miotics: Pilocarpine

1.2 Introduction to Drug

Netarsudil ophthalmic drug is used to treat glaucoma (a condition in which increased pressure in the eye can lead to gradual loss of vision) and ocular hypertension (a condition which causes increased pressure in the eye).

Netarsudil is a rho kinase inhibitor that reduces intraocular pressure (IOP) in patients with open angle glaucoma or ocular hypertension. It has a triple mechanism of action for IOP control 1) Increase aqueous outflow by inhibiting ROCK, 2) Decrease aqueous Production and 3) Decrease episcleral venous pressure.

1.3 Introduction to In-situ Gel^{[7][8]}

In situ gels are polymeric formulations that undergo a phase transition from a solution to a gel at the site of administration. This transition can be triggered by external factors such as temperature, pH, or ion concentration. The gelation process is typically reversible, allowing the gel to revert to a sol state upon changes in the triggering conditions.

In the pharmaceutical field, in situ gels are employed for controlled drug delivery. For example, a solution of a drug-loaded polymer can be administered, and as it comes into contact with the physiological environment (such as the body temperature or a specific pH level), it undergoes a gelation process, forming a gel that adheres to the site of application. This sustained-release mechanism helps in prolonging the therapeutic effect of the drug and minimizing side effects.

The choice of polymers is crucial in designing in situ gels, as they influence the gelation process and drug release characteristics. Polymers like thermo sensitive polymers, which respond to temperature changes, or pH-sensitive polymers, which respond to changes in acidity or alkalinity, are commonly used.

Advantages of Insitu gel

1. Prolonged drug release
2. Enhanced bioavailability
3. Patient Compliance
4. Site specific delivery
5. Reduced side effects
6. Improved therapy effectiveness
7. Reduced dosing frequency
8. Target specific sites

Disadvantages of Insitu gel

- One disadvantage of in situ gel formulation is the potential difficulty in achieving precise control over drug release rates, which may lead to variations in therapeutic efficacy.
- In situ gels can pose challenges in terms of formulation stability and shelf life, as some formulations may be prone to degradation or phase separation over time. This can impact the reliability of the drug delivery system.

2. MATERIALS AND METHOD:

Materials: Netarsudil, Sodium alginate, Citric acid, Carbopol 940, Disodium hydrogen phosphate, Benzalkonium chloride, HPMC E4M

Method:

This study has to be done at the initial stage of the formulation. In this study some physical and chemical property of drug and inactive ingredients would be checked individually and in combination of both. Pre-formulation studies provide insight into the drug's compatibility with excipients; therefore, formulation development will begin after the study. Study may give idea about the nature of the substances. Pre-formulation research's main objective is to give formulators knowledge that will help them create stable and bioavailable dosage forms.

Table 2.1 Selection of viscosity enhancers and Gelling agent

Ingredients(mg)	T1	T2	T3	T4	T5	T6
Netarsudil	0.02	0.02	0.02	0.02	0.02	0.02
HPMCK4M	1.5	-	-	1.5	-	-
HPMCE4M	-	1.5	-	-	1.5	-
HPMCE50LV	-	-	1.5	-	-	1.5
Sodiumalginate	0.3	0.3	0.3	-	-	-
Carbopol940	-	-	-	0.3	0.3	0.3
Citric acid	0.4	0.4	0.4	0.4	0.4	0.4
Disodiumhydrogen phosphate	1.125	1.125	1.125	1.125	1.125	1.125
Benzalkoniumchloride	0.02	0.02	0.02	0.02	0.02	0.02
Purifiedwater	100ml	100ml	100ml	100ml	100ml	100ml

Table 2.2 Layout of 3² full factorial design

IndependentVariables					
F1			F2		
Concentration of HPMC E50LV (Viscosity enhancers)(mg)			Concentration of Carbopol 940(Gelling agent)(mg)		
-1	0	+1	-1	0	+1
0.5	1.5	2.5	0.2	0.3	0.4
DependentVariables					
R1		R2		R3	
Viscosity(cps)		GellingTime (Seconds)		%DrugRelease	

Table1.3 Formulation Layout of 3² Full Factorial Design

Formulation Code	Coded Value		ActualValue	
	Concentration of viscosity enhancers (HPMCE50LV) (F1)	Concentration of Gellingagent (Carbopol 940) (F2)	F1(mg)	F2(mg)
1	0	1	1.5	0.4
2	0	0	1.5	0.3
3	0	-1	1.5	0.2
4	-1	-1	0.5	0.2
5	1	0	2.5	0.3
6	1	-1	2.5	0.2
7	-1	1	0.5	0.4
8	1	1	2.5	0.4
9	-1	0	0.5	0.3

3. EVALUATION PARAMETERS:

3.1Organoleptic characterization : Received gift sample of Netarsudil was evaluated for appearance, color and odour.

3.2Melting point determination:

The Veego Melting Apparatus was used to determine the melting point of Netarsudil. A little sample of the drug is placed in a capillary that is sealed at one end and submerged in silicon oil bath. Gradually raising the temperature was permitted, and it was documented at what temperature the

medicine began to melt and at what temperature it finished melting.

3.3Solubility study:

Drug is dissolved in 1ml water drug is added continuously in water and shake at one point of time drug will not dissolve and supersaturated solution would prepare, filtered and analysed in UV-vis spectrophotometer.

3.4FTIR Studies:

The functional group detection by FTIR investigations was used to confirm that the

Netarsudil was pure. Peaks discovered in spectra were used to assess Netarsudil. Small quantity of sample transferred into mortar with KBr and triturate with paste and sample were put into holder and scanning the scan and check observed frequency.

Evaluation Parameters of *In situ gel*

The evaluation parameters of *In situ gel* are given below.

3.5 Visual appearance, homogeneity and viscosity:

Under fluorescent light against a white and black background, visual appearance and clarity were assessed for the presence of any particle matter.

3.6 Determination of pH:

The pH of the in-situ gels was measured using a digital pH metre following the addition of all components

3.7 Drug content analysis:

The drug content of in situ gel ophthalmic formulation was carried out by spectroscopic analysis technique. 1ml of the developed formulation was dissolved in 100ml of stimulated tear fluid and absorbance was taken in UV visible spectrophotometer.

3.8 Gelling strength:

A drop of the created formulation was placed in a beaker containing 50 millilitres of freshly prepared, concentrated calcium chloride solution, and the gelling time was visually inspected to determine the formulation's gelling capacity. Coding of gelation described in below table.

Table 3.8 Coding for the gelling capacity

Sr.No	Observation	Coding
1	No gelation	-
2	Gelation occurred in few minutes and remained for few hour	+
3	Gelation immediate, remained for few hour	++
4	Gelation immediate, and for extended period	+++
5	Very stiff gel	++++

3.9 Rheological studies :

The rheological characteristics of in-situ gelling systems that were created were assessed under non-physiological conditions (pH-5 and 25°C) and physiological conditions (pH-7.4 and 34°C) using varying shear rates (2, 4, 6, 10, 20, and 30 rpm). The viscosity was calculated by averaging two values, with the hierarchy of the shear rate reversed.

3.10 *In vitro* Drug release study:

Dissolution study was carried by using Franz diffusion apparatus with the use of stimulated fluid as a solvent. A temperature was maintained 37 ± 0.5 °C with the 100rpm rotation. The samples withdrawn at various time interval and analyzed in UV visible spectrophotometer. The formulation was placed in donor compartment and stimulated tear fluid was placed in receptor compartment. Cellulose filter was placed between the donor and receptor compartment. 1ml solution withdrawn at predetermined time interval and same volume replaced with fresh medium. Withdrawn samples

are diluted in volumetric flask with specific solvent and analyzed.

3.11 Stability study:

The goal of stability study is to ascertain how various environmental factors, such as temperature, humidity, and light, affect a drug substance's or drug product's quality over time. To ascertain the product's shelf life and advised storage conditions, a stability study is done. An accelerated stability study was conducted on prepared strips in accordance with the ICH Q1A guideline at 40 °C, 2 °C, 75 %, and 5 % relative humidity. The ICH Q1A guideline regulates stability testing of novel pharmaceutical compounds and products. An expedited stability study should take at least six months, as required by the ICH Q1A standard, but due to scheduling constraints, the research only lasted one month. The *in-situ gel* were examined after a month for a number of aspects, including appearance and drug percentage content, gelling time, viscosity, and percent CDR.

4. RESULTS AND DISCUSSION:

4.1 Organoleptic characterization

Various organoleptic characterization of Netarsudil is given below.

Table 4.1 Organoleptic characterization of Netarsudil

Sr No.	Characterization	Observation
1	Appearance	Whitemorphouspowder

2	Odour	Odorless
3	Taste	-

4.2 Melting Point Determination

Table 4.2 Melting Point of Netarsudil

Reference Melting Point	Observed Melting Point
384.3 ± 32.9 °C	386 °C

The observed melting point of drug is found within limits of reference drug hence it confirms the purity of experimental drug.

4.3 Solubility Studies

Table 4.3 Solubility of Netarsudil

Sr.no	Drugname	Solvent	Result
1	Netarsudil	Water	Soluble
2		DMSO	Soluble
3		StimulatedTearFluid	Freely Soluble
4		Ethanol	Insoluble

4.4 FTIR Studies

Pure Netarsudil was the subject of FTIR analyses in the 4000 to 500 cm⁻¹ wave number range. Based on the functional group that the medication contains, all necessary peaks are determined.

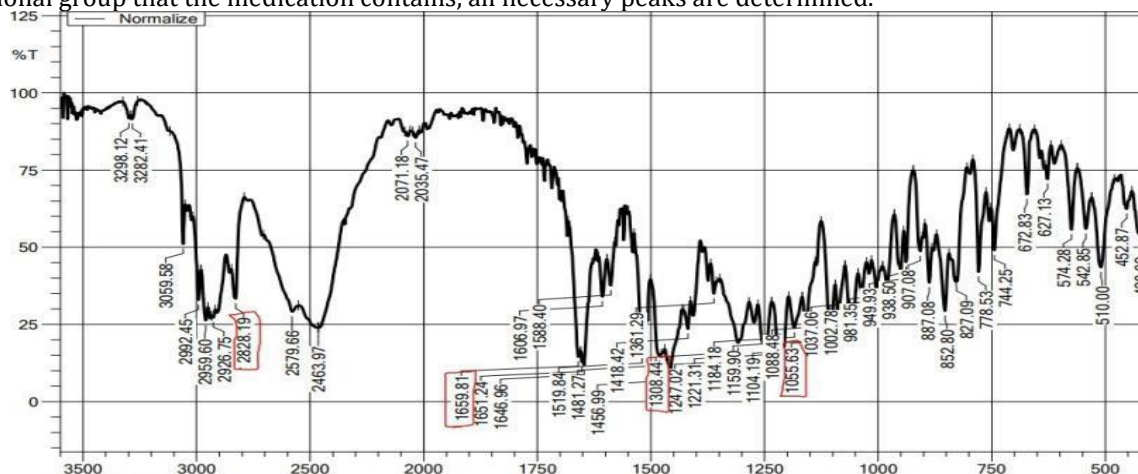


Figure 4.4 FTIR of Netarsudil

4.5 Visual appearance, homogeneity and viscosity

Sr.no	Evaluation Parameters	T1	T2	T3	T4	T5	T6
1	pH	6.6	7.1	7	6.5	6.9	7.2
2	Clarity	Non-Clear	Non-Clear	Non-Clear	Clear	Clear	Clear
3	Viscosity (cp)	1440	1725	1654	2541	2356	2450
4	Gellingtime (sec)	39	43	40	52	50	56

4.6 Determination of pH

Table 4.6 Evaluation of Factorial Batches (Average ± Standard Deviation (n=3))

Sr.no	Evaluation Parameters	D2
1	Transparency	Transparent
2	Clarity	Clear
3	pH	6.5 ± 0.05
4	Viscosity(cp)	2451 ± 0.019
5	Gellingtime	68 ± 0.04

4.7 Drug content analysis

Table 4.7 Drug content analysis (Average $\pm$ Standard Deviation (n=3))

Sr.no	Evaluation Parameters	D2
1	%DrugContent	99.25 $\pm$ 0.114
2	%CDR inHours	85.56 $\pm$ 0.253

4.8 Gelling strength

4.9 Rheological studies

In-vitro drug release of all factorial batches from D1-D9 are given below table.

Table 4.9 In-vitro Drug release of Factorial Batches

4.9 In vitro Drug release study

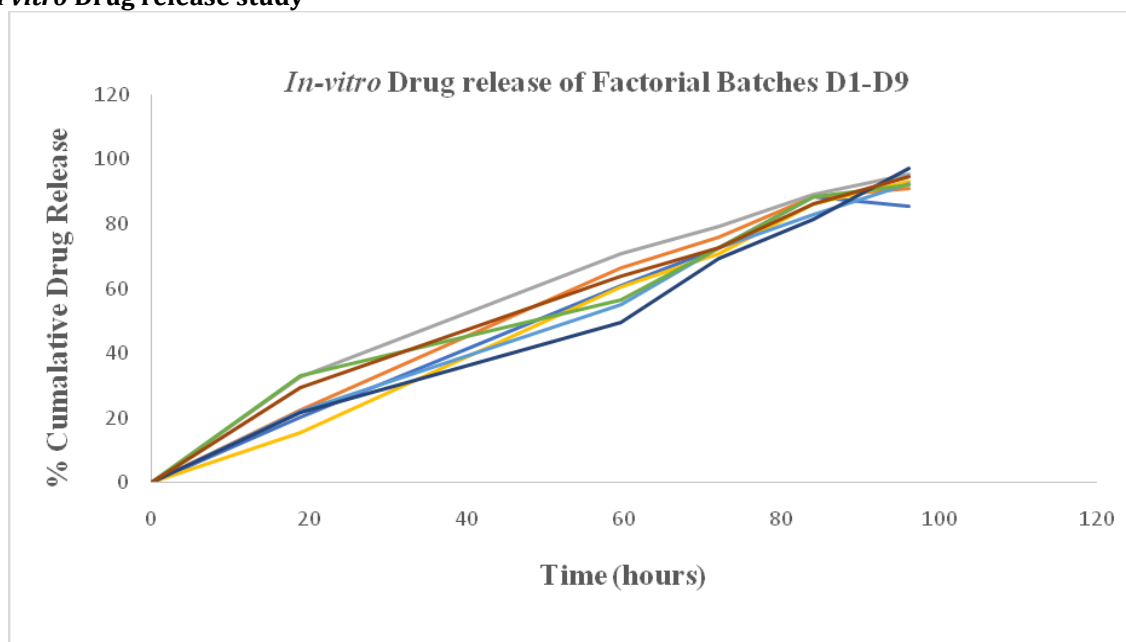


Figure 4.9 In-vitro Drug release of Factorial Batches D1-D9

From above evaluation of factorial batches, it is observed that all the formulation has uniform clarity, transparency and pH. So, it is concluded that basic parameter of all the batches shows good results.

Comparing all batches, formulations with lower viscosity enhancer concentrations exhibit low viscosity in cps and it may increase the % drug release. A gelling agent's concentration will rise together with gelling time increase and it may increase gelling strength. Gelling time and gelling strength are directly correlated with Gelling agent concentration. Additionally, the concentration of the gelling agent ingredient affects the drug release. the film may also increase. The gelling strength and gelling capacity is directly proportional to the concentration of Gelling agent, as is the thickness of

the gel, which may also depend on the concentration of Gelling agent. Stickiness may also increase the concentration of Gelling agent increases.

- pH was found to be within the permitted range when compared to all other batches. The entire gel's pH is approximately 7, which is likewise acceptable D2.
- Comparison of all dissolution data of factorial batches, batch D2 had a maximum drug release of 85.56 percent at 15hours, according to in-vitro drug release data. Batch D2 has the gelling time 68(seconds) and the viscosity of 2451. As a result, it is established that the D2 batch is the most suitable batch for further development.

Since the concentration of Gelling agent increases across all batches, the gelling strength.

4.11 Stability study

Table 4.11 Stability Study

Condition	40°C $\pm$ 2°C / 75% RH $\pm$ 5% RH	
Evaluation	Initial of observation	After 1 month

Appearance	Clearandtransparent	Clearandtransparent
Drugcontent	98.26 ± 0.294	97.95 ± 0.512
Gellingtime(sec)	62 ± 0.02	60± 0.221
Viscosity(cps)	2334 ± 0.62	2241± 0.51
%CDR in hours	90.12 ± 0.325	91.56± 0.285

5. SUMMARY & CONCLUSION:

It is noticeable effect of gelling agent on gelling time and effect on viscosity enhancers on viscosity of formulation and in-vitro dissolution. Overlay and contour plots were created by a design expert 12. Two batches, V1 and V2, are chosen from this overlay plot to serve as the validation model. The values derived from a batch evaluation show that the results are fairly close to the values predicted. This highlights the significance and consistency of the model. Collected data from the overlay plot which is near to final optimized batch. This batch has 62 seconds-Gelling time, a 2334 cps viscosity, and a 15hours CDR of 90.12%.

Comparison between *In-vitro* dissolution study of marketed ophthalmic solution and prepared *in-situ* gel gives idea that prepared *in-situ* gel gives better *in vitro* release in sustained manner and dosage of frequency also reduce by preparing *in-situ* gel in comparison to the ophthalmic eye drops solution. A one-month accelerated stability investigation was then conducted on the check point batch, and the findings were deemed satisfactory. As a result, it can be inferred from the research and results that the in-situ gel will result in a sustained drug release and improved bioavailability. This method reduces the frequency of dosage. Because of this, the In-situ gel has better and more promising results.

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