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Development and Evaluation of in Situ Gelling Ophthalmic Eye Drop of Netarsudil for Anti-Glaucoma Activity

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KEYWORDS

Netarsudil, In Situ Gel.

ABSTRACT:

Conventionally used eye medication like eye drops or other preparations may cause loss of drug due to lower viscosity and the bioavailability believes to be 35-40%. In order to reduce the loss of medications, in-situ preparation are formulated in liquid form, but when reaches to the body temperature of 370C the viscosity increases that causes conversion of liquid to the gel form. Due to this, there is very less loss of medication and the bioavailability reaches to the range of 80-85%. In the current study, evaluated the organoleptic properties of the drug along with physicochemical studies of the drug including melting point, solubility, uv spectroscopy, calibration of drug. The gelation temperature of all the formulations was found in the range of 36.90C to 37.80C. % drug content of all the formulations were calculated and it found to be between 83.56% to 95.25%. Highest % drug content is obtained in formulation F-5.

1. INTRODUCTION

The more desirable dosage forms must deliver drugs in a drop form, should not interfere with vision problems by creating little to no refractive index and infrequent installation. Such conditions could be achieved through in- situ gel forming systems(1). Polymers are widely used for the in-situ gel formulation and it follows pseudo plastic and reversible phase transition (sol-gel-sol) behaviour in order to reduce blinking, increase pre-corneal residence time and enhance ocular drug bioavailability(2).

The challenge in the formulation is to circumvent the protective barriers of the eye without causing permanent tissue damage. Ophthalmic ointments ensure superior drug bioavailability by increasing the contact time, minimizing the dilution by tears, and resisting nasolacrimal drainage. Major disadvantage of ointment, providing blurred vision, due to this it could be used either night time or for treatment on the outside and edges of the eyelids(3,4). Suspension as ophthalmic delivery systems rely on the assumption that particles may persist in conjunctival sac. Precorneal drug loss can be minimal, such as retarding drainage by using diffusion-controlled, nonerodible polymeric insert.(5) The major disadvantage of inserts is the lack of patient acceptance owing to difficult administration(6). The development of newer, more sensitive diagnostic techniques and therapeutic agents render urgency to the development of more successful ocular delivery systems(7). The primitive ophthalmic solution, suspension, and ointment dosage forms are clearly no longer sufficient to combat these diseases, and current research and development efforts to design better therapeutic systems are the primary focus of this research work. The aim of the present investigation is to formulate an in situ gel using novel gum system. In situ gel solution increases the residence time and also sustain the release mechanism of the drug(8,9).

2. MATERIAL AND METHODOLOGY

Netarsudil gift sample was from Insoco Remedies Ltd.

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PREFORMULATION STUDIES:

Preformulation studies

The pre-formulation studies like physical characteristics, melting point, solubility studies, calibration, uv spectroscopy was done.

Netarsudil Ultra Violet Spectroscopy –

100 mg of a drug-Netarsudil mixed with 100 ml of 7.2 pH phosphate buffer. This mixture had a concentration of 1000 µg/ml. The concentration was reduced to 50 µg/ml solution using 7.2 pH phosphate buffer. The sample was evaluated in UV spectrophotometer(10).

Calibration of Netarsudil

Standard calibration curve of Netarsudil in buffer solution of phosphate at 7.2 pH

A 100 mg Netarsudil drug was diluted in 100ml buffer solution of phosphate with 7.2 pH and volume was made-up up to using the same buffer solution. which is called as stock-I solution.

In this process, taken 10 ml of the first solution (Stock-I) and mixing it with phosphate buffer in a special container called a volumetric flask. We fill it up to 100 ml, creating what we call Stock-II solution. Next, we take different amounts of Stock-II (1 ml, 2 ml, 4 ml, 6 ml, 8 ml, and 10 ml) and add more buffer to make new solutions. These new solutions have different strengths of the drug, ranging from 10 to 100 µg/ml. We then use a machine called a UV-visible spectrophotometer to measure how much light each solution absorbs at 230 nm. Finally, we draw a graph showing how the amount of drug relates to how much light it absorbs(11).

Formulation of In-situ gel

Ophthalmic in-situ gel containing netarsudil was formulated by using cold method. Initially, a small amount of purified water was taken in a 100 ml of beaker along with necessary quantities of poloxamer 188 and poloxamer 407, dissolved through magnetic stirrer. Further required quantity of HPMC K4M was added to the polymer solution and stirred for 30 minutes. Subsequently, required quantity of Netarsudil, 0.9% w/v NaCl, 0.3% sodium citrate dihydrate, and 0.01% benzalkonium chloride was added followed by stirring the formulation for 30 minutes. Seven different formulations were prepared by adjusting the concentrations of HPMC K4M (0.10 gm to 0.25 gm), 14-17% concentration of poloxamer 407, with 5% concentration of poloxamer 188 in all the formulations. Formulated in-situ gels were refrigerated at 4^0C overnight. Further adjusted the volume upto 50 ml using purified water, stirred for 10 minutes. The pH of all the formultions were adjusted by using 0.1N NaOH drop by drop. At last, 10 ml of each formulation was filtered using sterile 0.22 micro-ml syringe filters and transferred to clean vials.

Table 1: Showing formulation of Netarsudil im-situ gel

Ingredients	**Formulation code (50 ml)**						
	NF-1	**NF-2**	**NF-3**	**NF-4**	**NF-5**	**NF-6**	**NF-7**
Netarsudil (g)	0.01	0.01	0.01	0.01	0.01	0.01	0.01
HPMC K4M (g)	0.10	0.15	0.20	0.25	0.10	0.15	0.20
Poloxamer 407 (%)	14	14	15	15	16	16	17
Poloxamer 188 (%)	5	5	5	5	5	5	5
Sodium citrate dihydrate (%)	0.3	0.3	0.3	0.3	0.3	0.3	0.3
0.1N NaOH (ml)	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Benzalkonium chloride (gm)	0.05	0.05	0.05	0.05	0.05	0.05	0.05

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Purified water q.s. (ml)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Evaluation of formulated in-situ gel

The formulations were evaluated for appearance, clarity and pH to evaluate the behavior of all the formulations.

Viscosity

The viscosity of the prepared in situ gels was assessed using a Brookfield viscometer DV-II + pro model with spindle no.61 at various shear rates ranging from 10 to 100 rpm. The measurements were conducted at temperatures of 25±1°C (12).

Gelling capacity

A small amount of the eye drop is placed in a special liquid that's similar to tears. This mixture is kept at body temperature, about 37°C, then watch how quickly the eye drop turns into a gel and how long it takes for the gel to dissolve.

In vitro drug release study

The release study conducted in vitro utilized a Franz diffusion cell method. Within this setup, the formulation was positioned in the donor section, while freshly prepared simulated tear fluid was placed in the receptor section. A dialysis membrane treated with simulated tear fluid after soaking overnight. This membrane has been attached to one end of the diffusion cell, with a rotational speed of 50 rpm and a temperature of 37 ± 0.5°C maintained in the medium. Samples of 1ml were taken at varied intervals of 0, 30, 60, 120, 180, 240, 300, 360 minutes with 10 ml of a fresh medium replacing each withdrawal. The samples taken were diluted to 10 ml in a volumetric flask with simulated tear fluid and analyzed using a UV spectrophotometer at the drug's particular λmax. The cumulative drug release percentage (% CDR) was then determined, and the data obtained underwent curve fitting analysis for the drug release investigation(13).

Sterility test

The sterility testing of formulation (NF-5) was done to detect the presence of microbial growth in the formulation. The media used for the sterility testing includes soya bean digest for fungi and fluid thioglycolate medium for detection of bacterial growth.

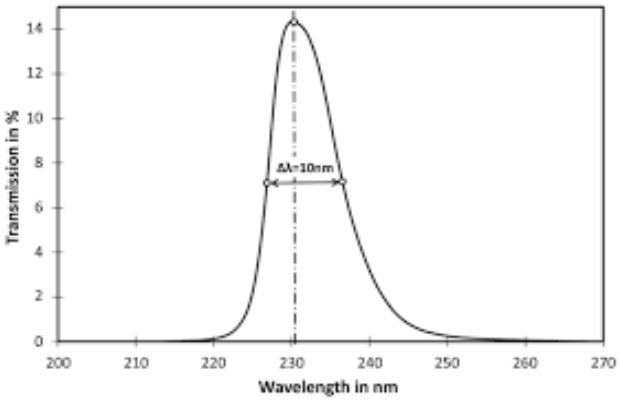
The test was performed by utilising direct inoculation method as mentioned in the I.P. using soyabean casein digest medium (SCDM) and fluid thioglycolate medium (FTG) medium.

3. RESULT DISCUSSION

The physical characterization of drug was done and it was found to be powdered form with light yellow colour. The melting point of drug was determined and was found to be 257^0C.

UV spectroscopy

Absorbance of Netarsudil is determined under UV spectrometer and it was found to be 230 nm.



Graph 1: Figure showing UV spectroscopy of Netarsudil

Netarsudil standard calibration curve of in 7.2 pH phosphate buffer at λmax 230 nm

Table 2- Netarsudil absorbance was recorded at different concentration using 7.2 pH phosphate buffer at λmax 230 nm

Concentration (µg per ml)	Absorbance (nm)
10	0.249
20	0.544
40	0.859
60	1.31
100	1.658

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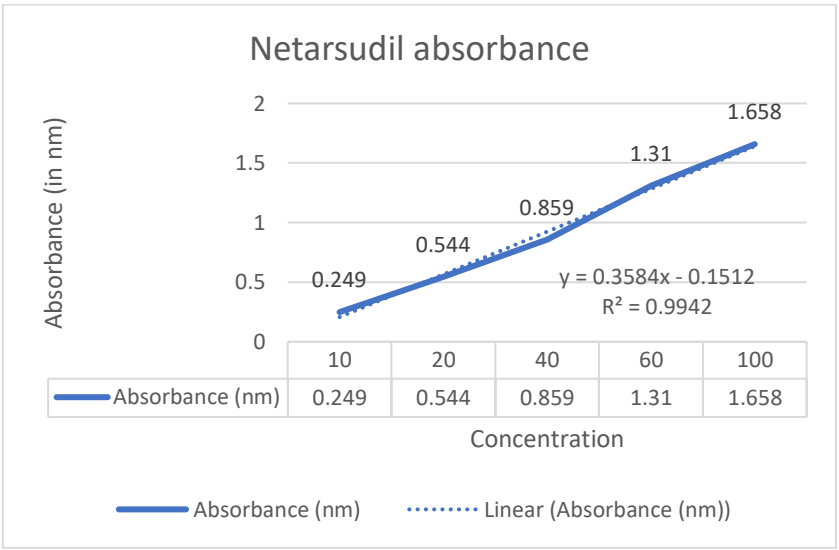

Graph 2: Figure showing calibration curve of Netarsudil

EVALUATION OF NETARSUDIL IN-SITU GEL

Appearance Clarity pH

The result of appearance, clarity and pH of all the formulations of netarsudil loaded in-situ gel in discussed in the table given below

Table 3: Results of appearance, clarity and pH of Netarsudil in situ gels

Formulation Code	Appearance	Clarity	pH
NF-1	Free flowing liquid	Clear	7.1 ± 0.1
NF-2	Free flowing liquid	Clear	7.2 ± 0.1
NF-3	Slightly viscous liquid	Clear	7.1 ± 0.1
NF-4	Slightly viscous liquid	Clear	7.0 ± 0.1
NF-5	Viscous liquid	Clear	7.3 ± 0.1
NF-6	Viscous liquid	Clear	7.4 ± 0.1
NF-7	Highly Viscous liquid	Clear	7.2 ± 0.1

Viscosity

The results of viscosity of Netarsudil in situ gels upon increasing angular velocity from 10-100 rpm is shown in the table 4.

Table 4: Viscosity of Netarsudil in situ gels (cps) at 25°C ± 1°C

Angular velocity (rpm)	Viscosity of Netarsudil in situ gels (cps) at 25°C ± 1°C						
	NF-1	NF-2	NF-3	NF-4	NF-5	NF-6	NF-7
10	65.28 ± 0.21	71.33 ± 0.13	73.25 ± 0.04	76.24 ± 0.14	81.24 ± 0.14	85.04 ± 0.12	89.45 ± 0.14

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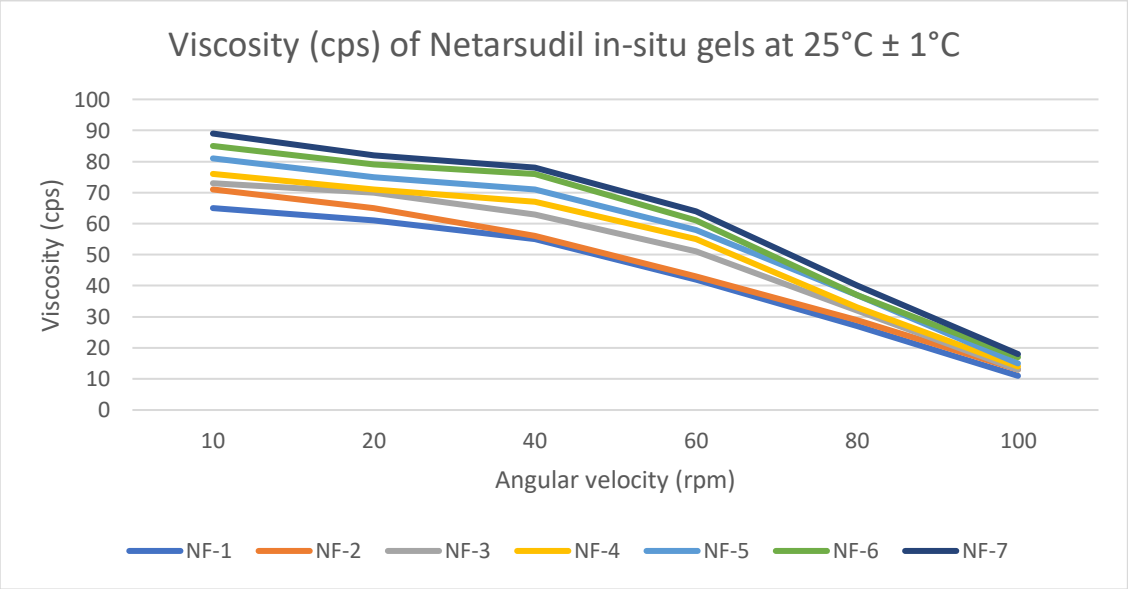
20	61.12 ± 0.22	65.14 ± 0.12	70.21 ± 0.10	71.89 ± 0.04	75.28 ± 0.14	79.04 ± 0.13	82.25 ± 0.17
40	55.12 ± 0.14	56.13 ± 0.07	63.05 ± 0.12	67.01 ± 0.4	71.12 ± 0.15	76.28 ± 0.14	78.15 ± 0.06
60	42.15 ± 0.16	43.78 ± 0.13	51.12 ± 0.12	55.05 ± 0.11	58.23 ± 0.05	61.12 ± 0.06	64.41 ± 0.09
80	27.05 ± 0.21	29.52 ± 0.45	32.41 ± 0.14	33.15 ± 0.18	37.11 ± 0.11	37.89 ± 0.05	40.46 ± 0.12
100	11.07 ± 0.04	13.12 ± 0.11	13.84 ± 0.06	14.12 ± 0.11	15.11 ± 0.09	17.15 ± 0.12	18.11 ± 0.14



Graph 3: Viscosity of Netarsudil in situ gels (cps) at 25°C ± 1°C

Gelling capacity Gelation temperature.

The results of gelling capacity, gelation temperature and drug content of Netarsudil in situ gels is represented in Table 5.

Table 5: Results of gelling capacity, gelation temperature and drug content for Netarsudil in situ gels

Formulation Code	Gelling capacity	Gelation temperature
NF-1	++	37.1^0C
NF-2	++	37.5^0C
NF-3	+	37.8^0C
NF-4	++	36.90C
NF-5	+++	37.1^0C

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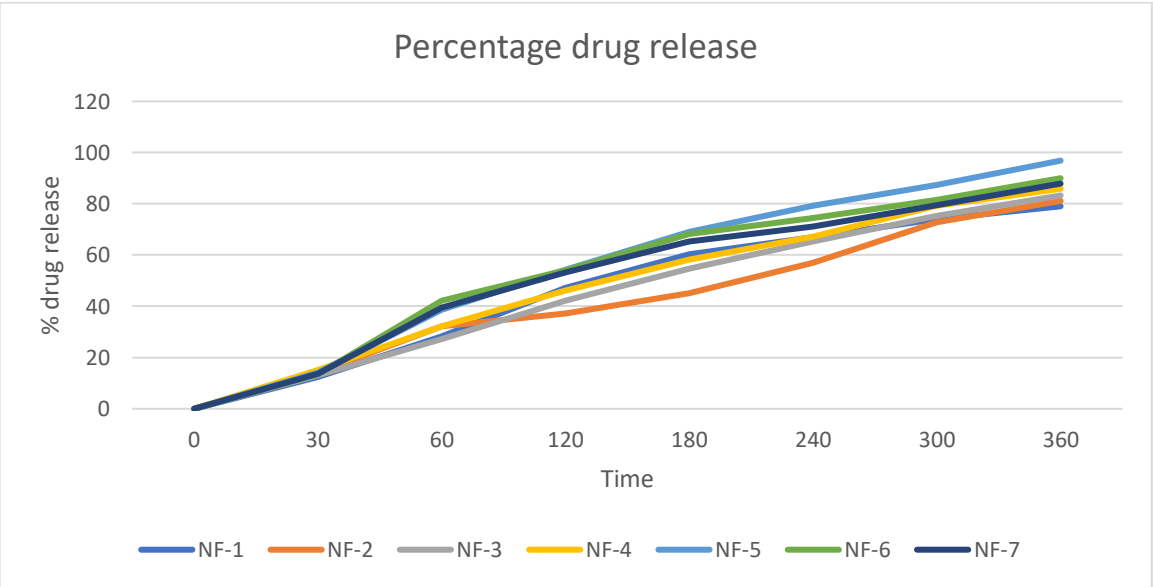
NF-6	+++	37.2^{0}C
NF-7	+++	37.3^{0}C

In vitro drug release study

Both the formulations of drugs, shown the drug release upto 10 h. In Netarsudil the formulations, NF-5 was found to be selected as optimized formulations. The in vitro drug release data for the formulations is presented in Table 6.

Table 6: Showing percentage drug release of all the formulation

Time (in minutes)	% Cumulative drug release of Netarsudil in situ gels						
	NF-1	NF-2	NF-3	NF-4	NF-5	NF-6	NF-7
0	0	0	0	0	0	0	0
30	12.35	14.05	13.11	15.15	14.08	13.56	13.74
60	28.14	32.12	27.25	32.15	38.55	42.14	39.58
120	47.12	37.11	42.16	46.11	54.27	54.12	53.15
180	60.22	45.05	54.78	58.14	69.12	68.25	65.28
240	67.06	57.05	65.25	67.10	79.15	74.52	71.15
300	74.11	72.89	75.21	79.25	87.28	81.55	79.52
360	79.01	81.12	83.25	85.86	96.84	89.99	87.88



Graph 4: Showing percentage drug release of all the formulation

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4. CONCLUSION

The Pre-formulation studies were carried to evaluate the properties of drug, the melting point of drug was found to be 257^{0}C, and it was found to be soluble in Phosphate buffer, Methanol and freely soluble in water. The UV spectral analysis was done which was found to be 230 nm at concentration of 50µg/ml, which confirms the spectral analysis of Netarsudil. The pH of all the formulation were found in the range of 7.0 to 7.4 that complies with the pH of the eyes. The gelation temperature of all the formulations were found in the range of 36.9^{0}C to 37.8^{0}C. % drug content of all the formulations were calculated and it found to be in the range of 83.56% to 95.25%. Highest % drug content is obtained in formulation F-5. % Cumulative drug release of all the formulations was calculated upto 6 hours, it was found to be in the range of 79.01% to 87.88%. Highest % drug release is shown in F-5 formulation as 96.84%. So the formulation F5 is considered as optimized formulation.

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