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Research Article

Formulation and Characterization of Fluorometholone Nanosuspension for Ophthalmic Drug Delivery by Precipitation Method

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Abstract: The objective of present study was to prepare a novel stable Fluorometholone ophthalmic Nanosuspension which has advantage over conventional ophthalmic suspension such as blurred vision, burning, stinging and irritation upon instillation. The viscosity was increased to provide additional advantage of long duration of action. Precipitation method was used to prepare Fluorometholone ophthalmic Nanosuspension. The type of polymer and stabilizer used showed effect on the particle size and zeta potential of Fluorometholone. Viscosity of prepared Nanosuspension was carried out which is sufficient to give better retention with cornea. The in-vitro drug release study showed that the optimized Nanosuspension released 98.87% of the drug within 8 hours. Stability study was carried out as per ICH guidelines.
Keywords: Nanosuspension, Ophthalmic delivery, Fluorometholone, Bioavailability.

INTRODUCTION

The challenges in ophthalmic drug delivery system are due to unique anatomy and physiology of eye. The conventional formulation like solutions, suspension and ointments shows disadvantages such as rapid precorneal elimination, high variability, and drainage by gravity and absence of controlled release. So overcome to these problems newer pharmaceutical ophthalmic formulation such as in-situ gel, Nanoparticle, liposome, Nanosuspension, Microemulsion, Iontophoresis and ocular inserts have been developed in last three decades increase the bioavailability of the drug as a sustained and controlled manner [1]. Fluorometholone is insoluble drug; hence preparation of Nanosuspension can lead to colloidal dispersion having solution like properties with increased retention [2]. Addition of viscosity imparter is an additional advantage. Mainly two types of techniques are available for preparation of Nanosuspension, (1) Bottom up technique (2) Top down technique i.e. High pressure homogenization, lipid emulsion, media milling and dry co-grinding. High pressure homogenization is a reported technique for preparation of Fluorometholone Nanosuspension. The present research work was aimed to develop an optimized formulation & process for Fluorometholone Nanosuspension by Precipitation method [3-5].

MATERIALS & METHODS

Fluorometholone was a gift-sample from Sentiss pharmaceuticals, Gurgaon, Haryana, India. Hydroxy propyl methyl cellulose E-5 (HPMC E-5), Polyvinyl alcohol was purchased from Research Lab Fine Chem, Mumbai. All other chemicals & reagents were of analytical grade.

Preparation of Nanosuspension [6-8]

Accurate quantity of hydroxyl propyl methyl cellulose and polyvinyl alcohol was weighed and dissolved in 10 ml of water. Accurate quantity of Fluorometholone was weighed and dissolved in 5ml methanol as a solvent. Solution prepared in step 1 can be mixed using high pressure homogenizer at about different rpm. Add solution prepared in step 2 slowly with the help of syringe in mixture prepared in step 3. Using high pressure homogenizer at speed different rpm for different hr the Nanosuspension was prepared. Addition of NaCl, polysorbate 80 and Benzalkonium chloride to step 5 under homogenization.

Table 1: Formulation variables (3^2 factorial design)

Formulation code		Coded Values		
	X_1	Gm	X_2	Gm
F19	+1	2.6	**-1**	1.4
F20	+1	2.6	0	2.0
F21	+1	2.6	+1	2.6
F22	0	2.0	-1	1.4
F23	0	2.0	0	2.0
F24	0	2.0	+1	2.6
F25	-1	1.4	-1	1.4
F26	-1	1.4	0	2.0
F27	-1	1.4	+1	2.6

Sterilization [9]

Nanosuspension was prepared in sterile room. The formulation was filled in final container that was washed and rinsed with distilled water. Container Sealed with regular screw caps and sterilized at 121 °C for 20 min.

Formulation optimization

The size of Nanosuspension depends on the viscosity of medium & interfacial tension. Therefore, the amount of viscosity imparter (HPMC E-5) & surfactant (Polyvinyl alcohol) were optimized using 3^2 factorial design. Nine batches were prepared using 3 different concentrations of HPMC E-5 & Polyvinyl alcohol (Table 1). Amount of all other ingredients were constant, i.e. 0.1% fluorometholone, Sodium chloride 1.6681gm, and 0.004% benzalkonium chloride.

Process optimization

Process variables for high-speed homogenization are homogenization speed and homogenization time. Three levels for homogenization speed were selected within the range of 15,000 to 25,000 rpm and for homogenization time 20 to 60 min. based on trial experiments done in our lab.

Table 2: Formulation prepared at variable speed and time

Sr. no.	Excipients	FF1 [15,000 RPM] [20min]	FF2 [20,000 RPM] [40min]	FF3 [25,000 RPM] [60min]
1	Drug	0.1	0.1	0.1
2	HPMC	1.4	1.4	1.4
3	PVA	2.6	2.6	2.6
4	NaCl	1.6681	1.6681	1.6681
4	BKC	0.004	0.004	0.004
5	EDTA	0.13	0.13	0.13
6	Polysorbate 80	0.05	0.05	0.05
7	Methanol	5	5	5
8	Distilled water	100	100	100

Evaluation

1. Physical appearance

The prepared Fluorometholone Nanosuspension was inspected visually for their color, homogeneity, consistency.

2. pH

pH of all formulations was determined by using pH meter (DIGITAL pH METER). The pH meter was calibrated before each use with standard pH 4 and pH 7 buffer solutions. 20ml of formulation was taken in suitable beaker and pH was measured.

3. Specific gravity

Specific gravity bottle is used to check the density of the formulation prepared and in turn compared with the density of water. Weight of empty gravity bottle is taken as (M1), weight of specific gravity bottle containing the preparation is considered as (M2), weight of gravity bottle containing water is taken as (M3). Considering this data we can find. Weight of preparation: (M2-M1), Weight of purified water: (M3-M1)

$$\text{Specific gravity} = \frac{Weight\ of\ preparation}{weight\ of\ an\ equal\ volume\ of\ water}$$

4. Particle size measurement [10]

Particle size distribution of nanosuspension can be determined by photon correlation spectroscopy that analyzes fluctuations in light scattering due to Brownian motion of the particles, using Zeta sizer 1000 HS [Malvern Instruments, UK].

5. Poly- dispersivity index

The average diameters and poly-dispersity index of samples were measured by Photon Correlation

Spectroscopy. The measurements should be performed at 25^0C.

6. Viscosity

The viscosity of different formulation was determined at room temperature using a Ostwald viscometer at laboratory scale.

7. Drug content determination [11]

Drug concentration in NSanosuspension of Fluorometholone was measured by spectrophotometer. Fluorometholone content in suspension was measure and added with known quantity of in solvent [Methanol] and it gets miscible. Absorbance was measured after suitable dilution at 238 nm in UV/VIS spectrophotometer [JASCO V-630, Japan] and % drug content was calculated.

8. In- vitro Drug Release Study

8.1 In *Vitro* Diffusion study [12]

In vitro study of the formulated ophthalmic Nanosuspension was carried out by using diffusion cell through egg membrane as a biological membrane. Diffusion cell with inner diameter 24mm was used for the study. The ophthalmic Nanosuspension was placed in donor compartment and freshly prepared 100 ml artificial tear fluid (sodium chloride 0.678g, sodium bicarbonate 0.218g, calcium chloride dehydrated 0.008g, potassium chloride 0.138g, purified water q.s 100 ml) in receptor compartment. Egg membranes were mounted in between donor and receptor compartment. The position of the donor compartment was adjusted so that egg membrane just touches the diffusion medium. The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at 37^0C ± 0.50^0C. 1ml of sample is withdrawn from receive compartment after 1, 2, 3, 4, 5, 6, 7 & 8 hrs and same volume of fresh medium is replaced. The withdrawn samples was diluted to 10 ml in a volumetric flask with simulated tear fluid and analyzed by UV spectrophotometer at 238 nm.

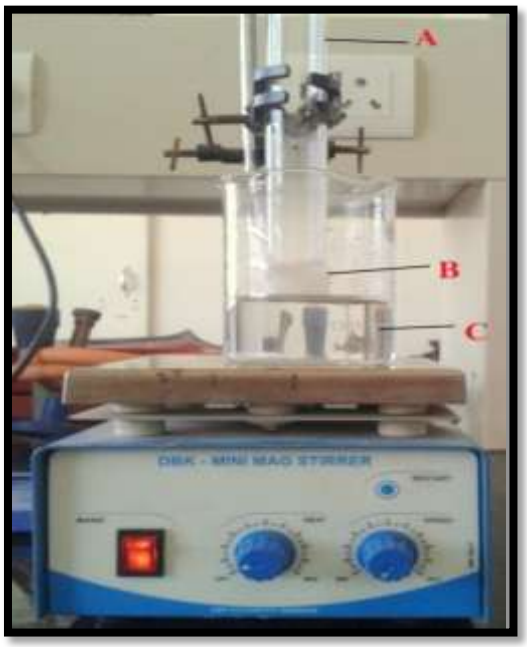

Fig-1: Laboratory designed diffusion cell (A- Tube containing formulation, B- Egg membrane, C- Beaker containing simulated tear fluid)

8.2 In *vitro* dissolution study

The rotating glass vial method: size of the vial was 1.5 cm diameter x 3.3 c height. It was attached to the end of basket drive in off centered manner in the dissolution jar of USP apparatus type 1. The jar having adequate quantity of water was used as secondary water bath for the bottle. The MT was accurately weighed, and transferred to glass vial containing 5 ml simulated tear fluid, stopper using a rubber stopper, sealed with Teflon. This was attached to the shaft of the USP apparatus type 1 and firmly held with thread. The shaft was positioned in the secondary water bath at 37^0C ± 0.50^0C and rotated separately at 25 rpm. Samples measuring 1 ml each were withdrawn at 1 hr, 2 hr, 4 hr, 6 hr and 8 hr. thus, the total duration was aimed to be 8 hrs. Each aliquot withdrawal was followed by immediate replenishment with 1 ml of the medium at the same temperature. The samples were diluted to 10 ml with simulated tear fluid. UV absorbance of these was measured at 238 nm on a Jasco V630 spectrophotometer against an appropriate blank. The concentration of drug in the samples was calculated from the standard pots of the drug in the simulated tear fluid.

Fig-2: Dissolution apparatus

8.3 In *vitro* corneal permeation study

Whole goat eyes were transported from the local butcher shop to the laboratory in cold (4ºC) normal saline within 1 h of slaughter. The corneas were carefully excised along with 2 to 4 mm of surrounding scleral tissue and washed with cold normal saline until the washing was free from protein. Diffusion cell with inner diameter 24mm was used for the study. The ophthalmic nanosuspension was placed in donor compartment and Freshly prepared 100 ml artificial tear fluid (sodium chloride 0.670g, sodium bicarbonate 0.200g, calcium chloride dehydrated 0.008g,purified water q.s 100ml.) in receptor compartment. Isolated cornea was mounted in between donor and receptor compartment. The position of the donor compartment was adjusted so that cornea just touches the diffusion medium. The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at 37°C ± 0.5°C. 1ml of sample is withdrawn from receiver compartment after30 min, 1, 2, 3, 4, 5, 6, 7 & 8 hrs and same volume of fresh medium is replaced. The withdrawn samples was diluted to 10ml in a volumetric flask with methanol and analyzed by UV spectrophotometer at 238 nm.

9. Eye irritation Studies [9]

Eye irritation study of nanosuspension was evaluated using isolated goat cornea. Whole eye balls of goat were obtained from local butcher. Eye balls were washed with cold saline to remove the proteins and then preserved in Krebs solution. In this study three eye balls were used. From three eye balls one was put in simple saline solution to get negative control, another eye ball was put in formulation for 8 hours, and last eye ball was put in NaOH solution as positive control. With the help of histopathology lab (Baroda clinical laboratory) obtained T.S of three eye ball. Prepared slides are examined under inverted microscope.

RESULTS AND DISCUSSION

Physical appearance

Physical characteristics of nanosuspension are given in Table-3.

Determination of pH

pH of nanosuspension formulation is given in Table-4.

Table 3: Physical characteristics of nanosuspension

Batch	Parameters		
	Appearance	Homogeneity	Consistency
FF2	Buff white color solution	Phase separation under centrifugation	Buff solution like consistency

Table 4: pH of nanosuspension formulation

Sr. No.	Formulation [Batch no.]	PH
1.	FF2	7.3± 0.1

The pH value was found to be satisfactory for the ophthalmic formulation and at this pH value the drug will be stable in the formulation as literature survey concludes that Fluorometholone is stable pH 6.2-7.4.

Specific gravity

Specific gravity bottle was used to check the density of the formulation prepared and in turn compared with the density of water. Weight of empty gravity bottle is taken as (M1), weight of specific gravity bottle containing the preparation is considered

as (M2), weight of gravity bottle containing water is taken as (M3). Considering this data we can find Specific gravity bottle is used to check the density of the formulation prepared and in turn compared with the density of water. The optimized batch had the following results.

Weight of preparation: (M2-M1) = (27.0-18.7) =8.3gm/ml
Weight of purified water: (M3-M1) = (26.7-18.7) =8gm/ml

Specific gravity = $= \frac{Weight\ of\ preparation}{weight\ of\ an\ equal\ volume\ of\ water} =$ 1.037gm

Particle size measurement

Particle size of nanosuspension is given in Table-5.

Poly- dispersivity index

Poly-dispersivity index of nanosuspension is given in Table-6.

Table 5: Particle size of nanosuspension

Formulation batch	Particle size
FF2	101.3

Table 6: Poly-dispersivity index of nanosuspension

Formulation batch	Poly – dispersivity index
FF2	0.301

Viscosity

Viscosity is the resistance to flow, which is an important physicochemical property for topical preparations because it influences spreadability and drug release as well as jellification. Viscosity of formulation was measured using Ostwald's viscometer (Table-7).

Table 7: Viscosity of Nanosuspension

Sr. No.	Viscosity [cp]
1.	32.97±0.055

Drug content determination

The drug content of the batch FF2 Was found to be 98.5%

In *Vitro* Drug release Study

Diffusion study of the different batches shows following results.

Table 8: Drug release by diffusion study

Time	F1	F2	F3	F4	F5	F6	F7	F8	F9
30	21.1	22.6	29.73	22.6	33.06	23.08	29.73	25.03	19.66
1	39.57	40.07	40.47	26.52	50.74	32.64	40.5	40.06	28.01
2	48.49	50.21	42.79	32.02	62.11	42.5	44.72	44.14	38.34
3	54.22	54.86	52.26	45.15	72.79	50.21	52.46	53.43	43.92
4	59.99	62.64	62.22	53.29	75.85	57.19	60.08	62.15	49.28
5	65.44	70.01	74.76	60.67	80.5	64.69	70.09	74.82	56.06
6	68.76	72.8	86.2	70.72	84.2	70.93	79.27	79.47	60.92
7	73.54	83.66	90.78	78.56	87.54	72.89	89.68	82.38	70.93
8	78.49	88.66	98.18	85.84	89.84	78.52	95.76	87.51	86.09

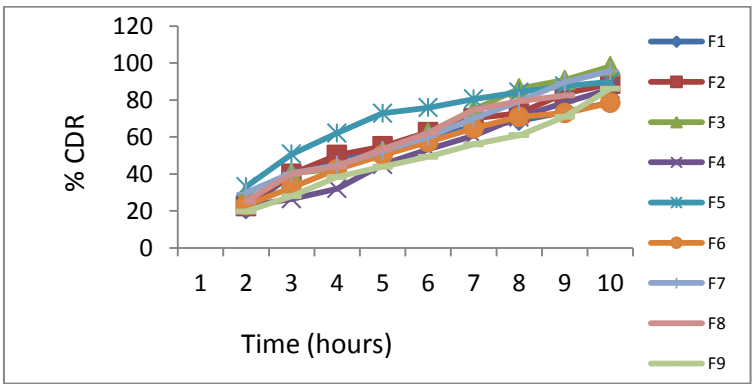

Fig-3: Drug release of different batches

Drug release study was also carried out by rotating glass vial method. The results of the dissolution study were given in table no.10

Table 9: Drug release by dissolution study

Time	% Release
1 hr	12.38
2 hr	30.11
3 hr	47
4 hr	55.29
5 hr	69.63
6 hr	87.75
7 hr	93
8 hr	98.87

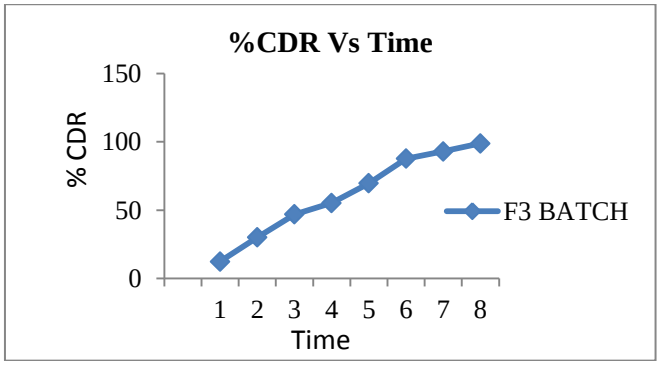

Fig-4: drug release of optimized batch

From the in vitro dissolution and diffusion data the release of batch F3 found best hence batch F3 was selected.

***In vitro* permeation studies**

The vitro permeation studies were carried out for the final selected batch F3. The result of permeation study was described in table no.11

Table 10: The results of permeation study

Time [hr]	% Permeation
30 min	29.73±0.00051
1 hr	40.47±0.00065
2 hr	42.79±0.00096
3 hr	52.26±0.00062
4 hr	62.22±0.00031
5 hr	74.76±0.00064
6 hr	86.2±0.00021
7 hr	90.78±0.0006
8 hr	98.18±0.0092

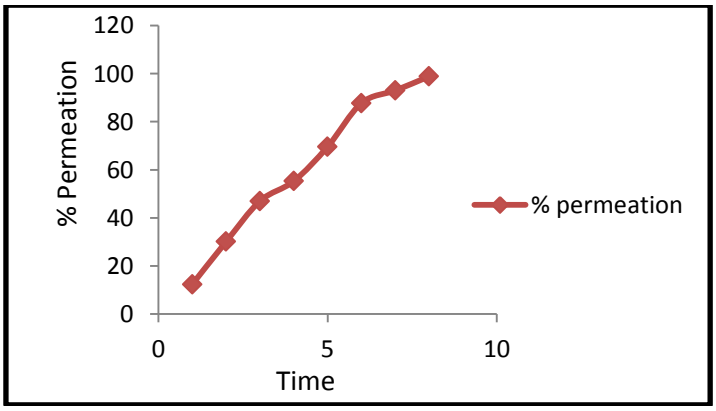

Fig-5: Release profile batch F3

Release Kinetics

The diffusion kinetics of optimized batch was applied to various diffusion models such as zero order, first order, Hixon crowell, Higuchi and Korsemayer-Peppas. The best fitted model gives the highest R^2 value and least slope value. Thus, zero order, first order, Higuchi model, korsemeyer-Peppas fits best for the diffusion data of the optimized batch as it showed the highest value R^2.

Results of Surface plot of HPMC and PVA depending on zeta potential and Particle size shown in Fig-6.

Table 11: Model fitting of batch F3

Zero order	First order	Hixon crowell	Higuchi	Korsemeyer Peppas
0.987	0.987	0.982	0.987	0.987

Table 12: Best model fitting of batch F3

Sr no	Model fitting	r^2 value
1	Zero order	0.987
2	First order	0.987
3	Hixon crowell	0.987
4	Korsemeyer Peppas	0.987

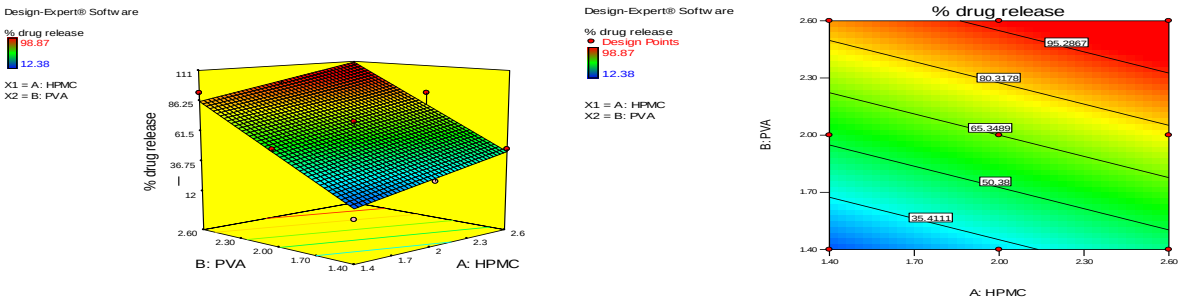

Fig-6: Surface plot of HPMC and PVA depending on zeta potential and Particle size

1. Eye irritation test

Irritation test was conducted on nanosuspension (F3) to check possible irritation effect to the ocular tissue on *in-vivo* application. The microscopic images of ocular tissue showed blue colour in negative control (fig.7) and pink colour in positive control (fig.8) which showed hemorrhage. Test sample also showed blue colour (fig.9) so the investigated Fluorometholone ophthalmic nanosuspension was classified as practically non-irritant.

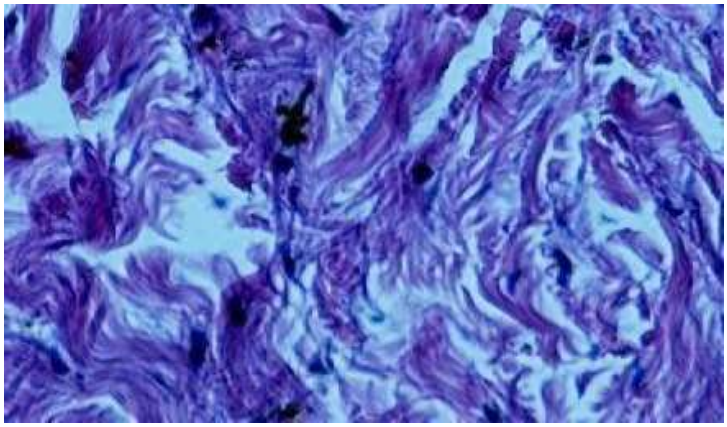

Fig-7: Negative Control

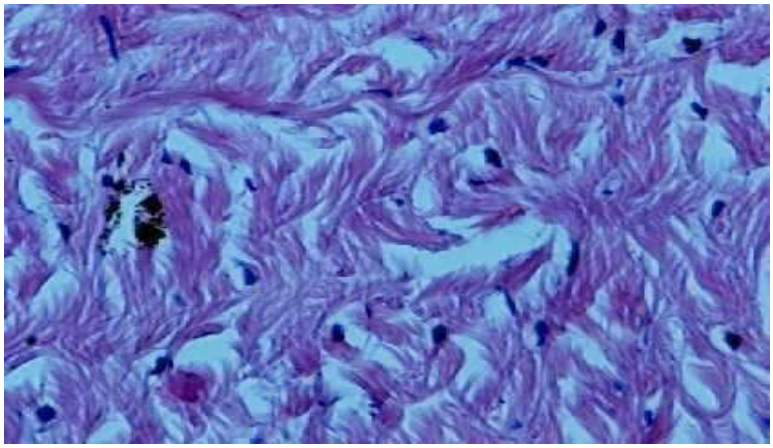

Fig-8: Positive Control

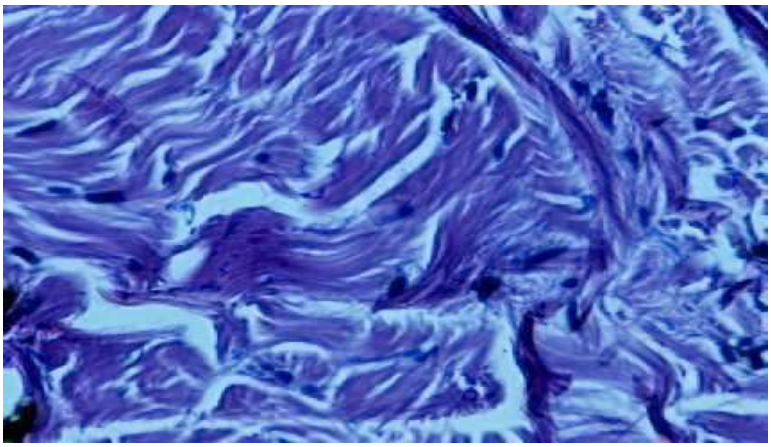

Fig-9: Test Sample

Stability studies

Optimized formulations were subjected to stability studies as per ICH guidelines. Various parameters such as Physical appearance, drug content, were measured before and after 30 and 60 days of stability. Results of stability studies are shown in table no…. Physical appearances of all formulations were unaffected or did not show any significant changes.it states that, optimized batch was stable.

Table 13: Stability study of optimized batch no. F3

Sr. no.	Observations	Before accelerated	After accelerated	
			30 days	60 days
1	% Drug content	98.5± 0.0002	97.78±0.0007	96.41± 0.0006
2	Physical	White color suspension	White color suspension	White color Suspension

CONCLUSION

The preparation of Fluorometholone ophthalmic nanosuspension was attempted using high speed homogenization techniques to improve solubility of drug. The type of polymer and stabilizer used showed effect on the particle size of Fluorometholone. No major drug polymer interaction was detected using FTIR. Ophthalmic nanosuspension may give better acceptance due to its small size, which may cause less irritation & blurring potential as compared to normal suspension. The prepared nanosuspension showed sustained action. The viscosity studies revealed that upon simultaneous dilution with tear fluid viscosity drastically increased which may enhance ocular residence time drastically.

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