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RESEARCH ARTICLE

Formulation and evaluation of Fluconazole ophthalmic gel by ion gelation method

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ABSTRACT

Developed the novel antibacterial drugs with the improved efficacy by using different polymers. the formulation and evaluation of an ophthalmic delivery system of an antibacterial agent Fluconazole, based on the concept of ion gelation. In present study Carbopol 934 and Chitosan were used as polymers. Carbopol 934 was used as a pH sensitive polymer and chitosan as a drug carrier. The prepared formulations were evaluated for pH, clarity, viscosity, drug content, gel strength, *in vitro* drug release, antibacterial activity, isotonicity test and stability. The formulations were therapeutically efficacious, sterile, stable and provided sustained release of the drug over a period of time.

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CONTENTS

1. Introduction

Drug delivery to the eye can be broadly classified into anterior and posterior segments. Any drug delivery systems tend to alter the release of drug rate or site of absorption of the drug or delivers the drug at its site of action is broadly categorized under novel drug delivery system .This drug delivery method shows significant effect on its efficacy. Novel drug delivery includes many advantages like duration of action of drug will be increased, newer side effects will be reduced. This method helps to achieve the targeted or specific sites by the drug and shows the maximum therapeutic efficacy by reducing the unwanted side effects, the dose of the drug will be adjusted precisely.

Nanoparticles

Recently the interest is evolving to develop a drug delivery system with the use of biodegradable polymers. Nanoparticles have huge awareness as potential drug delivery devices appliance of targeted and controlled release for various absorptive tissues by improving the intracellular penetration. Nanoparticles are submicron [<1micron] in size or the colloidal systems made of polymer. The advantages of nanoparticles include, the drug

will be administered through injection by intravenously due to their smaller size. Nanoparticles have higher loading capacity due to their larger surface area. They can pass through the sinusoidal spaces in the bone marrow and spleen due to their small size and shows longer circulation time period in the biood**.** Monoclonal antibodies can be attached to nanoparticles to enhance their specificity.

The prepared nanoparticles can be formulated into gels. Effective topical treatment of a drug is that which sufficiently permeate into the skin and reach the desired infection site. Nanoparticles do improve permeability of drug and further decrease irritation potential due to entrapment.

Ophthalmic Gels

Among various conventional dosage forms the extensive research has carried in designing of polymeric drug delivery systems. Opthalmic gels provides a promising site for local effect as well as systemic drug delivery because of its smooth large surface area. Occular bioavailability of drug is depending upon some physiological properties of drug and physiological factors. There are physiologic factors, which can affect a drug's ocular bioavailability including protein binding, drug metabolism and lacrimal drainage. In addition to physiologic factors affecting ocular bioavailability, other factors as the physicochemical characteristics of the drug substance, and product formulation are important. Because the cornea is a membrane-barrier containing both hydrophilic and lipophilic layers, drug substances having both hydrophilic and lipophilic characteristics permeate it most effectively. It is advantageous for corneal penetration to adjust the pH of solution to increase the fraction of unionized drug in the instilled dose. Drugs, which are highly water-soluble, do not readily permeate the cornea. Some of antifungal agents are griseofulvin, Fluconazole, itraconazole, Fluconazole etc.

Ideal ophthalmic drug delivery must be able to sustain the drug release and to remain in the vicinity of front of the eye for prolong period of time. Consequently it is imperative to optimize ophthalmic drug delivery, one of the way to do so is by addition of polymers of various grades, development of viscous gel, development of colloidal suspension or using erodible or non-erodible insert to prolong the precorneal drug retention. Bioadhesive systems utilized microparticle suspension or polymeric solution.

2. Materials and methods

Fluconazole, β-Cyclodextrin, Chitosan, are collected from Yarrow chem products, Mumbai, Carbopol-940 was collected from rolex chemical industries, Sodium tripoly phosphate, Triethanolamine, Poly ethylene glycol, are collected from Finar chemicals limited, Ahmedabad, Propylene glycol collected from Thomas baker, Mumbai. Glycerol from merck specialities. Pvt. Ltd.

Preparation of standard calibration curve of Fluconazole:

From the standard curve of Fluconazole, It was observed that the drug obeys Beer's law in the range 2-10µg/ml and the equation was generated, absorbance and concentration was used to calculate the drug content and & CDR of the dosage form.

Determination of λmax:

The standard solution of Fluconazole (10µg/ml) was scanned in the wavelength region of 200-400nm and the λmax found to be as 284nm.

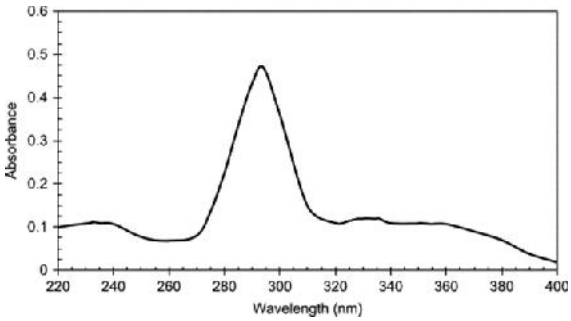

Fig 1: λmax of Fluconazole at 284nm

Preparation of calibration curve at 284nm: The working standard solutions of Fluconazole were scanned in the UV region and absorbance were observed against distilled water as a blank at 284nm. Finally the calibration curve was plotted between concentration (x-axis) and absorbance (y-axis).

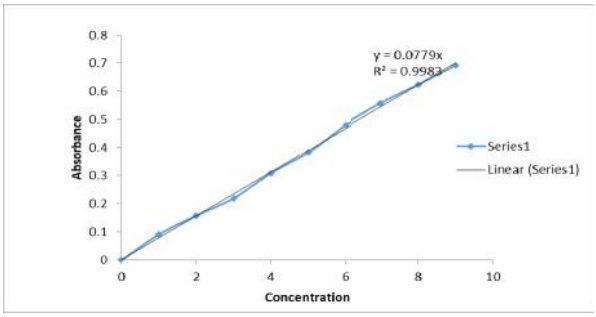

Fig 2: Calibration curve of Fluconazole

Table 1: Calibration curve data of Fluconazole

S.No	Concentration (µg/ml)	Absorbance
1.	0	0
2.	1	0.091
3.	2	0.158
4.	3	0.219
5.	4	0.308
6.	5	0.382
7.	6	0.479
8.	7	0.558
9.	8	0.623
10.	9	0.691

Preformulation Parameters

Compatibility studies:

The drug-excipient interaction study was carried out using FT-IR technique i.e, by potassium bromide pellet method. In FT-IR drug –excipient interaction study, it was found that Fluconazole was compatible with all excipients used in formulation. There were no extra peaks were observed. Thus excipients chosen for the formulation were found to be compatible with active ingredient and have no physical interaction with the active pharmaceutical ingredient.

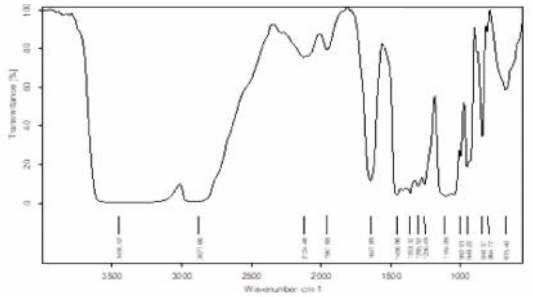

Fig 3: FTIR spectra of pure drug fluconazole and with all excipients

Formulation of Fluconazole Nanoparticles by ionic gelation method

Preparation of drug solution: weigh accurately 1 gm of Fluconazole and dissolve it in a 10ml of 0.1% glacial acetic acid. To these add 0.1gm of β-cyclodextrin and chitosan of different concentrations. The prepared drug solution is sonicated for 15 minutes using Ultrasonicator to form a nanoparticulate drug solution.

Formulation of Fluconazole GEL:

Fluconazole nanoparticles were prepared by using ultrasonication method; the nanoparticles were incorporated in carbopal gel. The % entrapment efficiency, drug content, spreadability, invitro drug release, test for anti fungal activity, gel strength were conducted.

a) Preparation of carbopol gel: Weigh accurately about 0.1gm of carbopol and dissolved it in a 4.5ml of water. Add 2ml of propylene glycol and 0.5ml poly ethylene glycol. Mix the contents and add 1ml of glycerine. To these add a drop 0.03ml methyl paraben and 0.06ml of propyl paraben. Mix all the ingredients and add 0.15ml of triethanolamine drop by drop.

b) Preparation of ophthalmic gel: Equal quantity of nanoparticulate drug solution and sodium tri poly phosphate added to the previously prepared carbopol gel.

3. Results and discussion

Characterization of ophthalmic gel:

The formulated ophthalmic gel was subjected to evaluation parameters such as entrapment efficiency invitro diffusion studies, spreadability, anti-microbial activty, gel strength.

Clarity:

The formulations were visually checked for the clarity.

pH: pH of each formulation was determined by using Digital pH meter (Digital pH meter 335). This was previously calibrated by pH 4 and pH 7. The pH values were recorded immediately after preparation.

Rheological study-Viscosity:

The rheological properties of solution & gels were determined by the Brookfield viscometer; type DV-II + PRO using spindle no.61 and 63. Viscosity of the formulations was taken at two different pH i.e. at pH 6 and at pH 7.4 with varying shear rate.

Drug Content:

The drug content was determined by taking 1 ml of the formulation and diluting it to 100 ml with distilled water. Aliquot of 5 ml was withdrawn and further diluted to 25 ml with distilled water. Fluconazole concentration was determined at 287 nm by using UV-Visible spectrophotometer.

Measurement of the gel strength:

A sample of 50 g of the gel was put in a 50 ml graduated cylinder. A weight was placed on the gel surface. The gel strength, which is an indication for the ophthalmic gel at physiological temperature, was determined by the time in seconds required by the weight to penetrate 5 cm into the gel. All measurements were performed in triplicate (n=3). The apparatus used for measuring gel strength is shown in Fig. 1

Antibacterial activity:

An agar diffusion method was used for the determination of antibacterial activity of formulations. Standard Petri dishes (9 cm diameter) containing medium to a depth of 0.5 cm were used. The sterility of the lots was controlled before use. Suspension was prepared by suspending 1-2 colonies of *Staphylococcus aureus* from 24hr cultures in Nutrient agar medium into tubes containing 10 mL of sterile saline. The tubes were diluted with saline inoculum (0.5mL) was spread over the surface of agar and the plates were dried at 35°C for 15 min prior to placing the formulation. The bores of 0.5 cm diameter were prepared and 2 drops of formulation (0.3 % w/v) were added in the bores. After incubation at 35°C for 24 hrs, the zone of inhibition around the bores was measured.

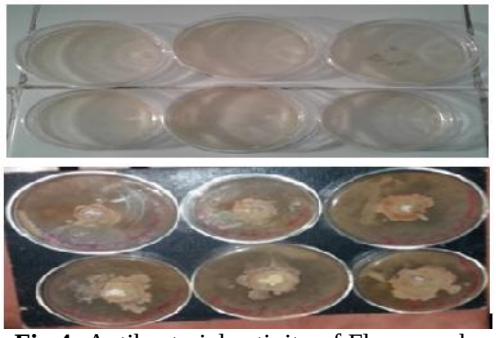

Fig 4: Antibacterial activity of Fluconazole

Isotonicity Evaluation: The F6 formulations were mixed with few drops of diluted blood on a slide blood sample treated with fluconazole (10mg/mL) during 60 minutes. The diluted blood was prepared by using Grower‟s solution and Slide was observed under microscope at X1000 magnification. The shape of blood cells were compared with standard marketed ophthalmic formulation.

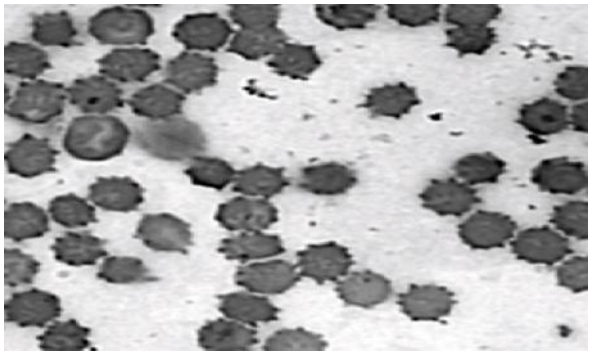

Fig 5: Isotonicity of F6 Fluconazole (10mg/mL) during 60 minutes

Test for Sterility:

Method: The sterility test was carried out as per IP (2014) method. The three medium were taken for this test i.e. fluid thioglycolate medium, artificial fluid thioglycolate medium and soyabean casein medium. The three set were prepared each set containing three tubes of each medium. The first set was a negative control for this sterile media is used, second set was a positive control for this sterilized media inoculated with *Staphylococcus aureus* (MH1714) was used and third set was a test. The 1mL sterile optimized formulation was taken and this formulation was diluted with 100mL sterile water for injection, from this formulation was diluted with 100mL sterile water for injection, from this 5mL test solution was added in each medium. The formulation was incubated for not less than 14 days at 20-250C in the fluid thioglycolate medium and at 20-25^{0}c in soyabean casein digest medium to find out growth of bacteria in formulation.

Spreadability:

Spreadability of the formulation was determined by placing a gel on surface of a glass slide which is placed on a scale. the scale is attached to flat surface connected to a trolley. when the weight is placed on the end of the plate the glass slide it detaches from the scale. the spreadability was calculated by

Spreadability=weights taken × length of glass slide /time taken to seperate the plates.

Entrapment efficiency:

The prepared formulation of ophthalmic gel was dissolved in solvent tear fluid and centrifuged at 100rpm for one and half hour. The supernatant liquid was collected and followed by serial dilutions.

Entrapment efficiency=

Total drug - Diffused drug / total drug ×100

In-vitro Drug Release Study:

In vitro release study of the formulated ophthalmic gel 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. 1 mL formulation 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, potassium chloride 0.248g, distilled water q.s 100mL) was placed in receptor compartment. Egg membrane was 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°C ± 0.5°C. 1mL of sample was withdrawn from receiver compartment after 30 min, 1, 2, 3, 4, 5, 6, 7,8 & 9 hrs and same volume of fresh medium was replaced. The withdrawn samples were diluted to 10mL in a volumetric flask with distilled water and analyzed by UV spectrophotometer at 284 nm.

Curve Fitting Analysis:

In order to describe the kinetic of the release process f drug in all formulations various equations were used, such a zero-order rate equation, which describes the system where release rate is independent of the concentration of the dissolved species. The first order equation describes the release from the systems were dissolution rate is dependent on the concentration of the dissolving species.

Higuchi square root equation describes the release from system where solid drug is dispersed in insoluble matrix, and the rate of drug release is related to the rate of diffusion. The korsemeyer-peppas equation is used to analyze the release of pharmaceutical polymeric dosage form, when the release mechanism is not well known or when more than one type of release phenomenon could be involved. The data obtained from in-vitro dissolution studies were fitted to zero ordered, first order, Higuchi, Korsemeyer-Peppas equation.

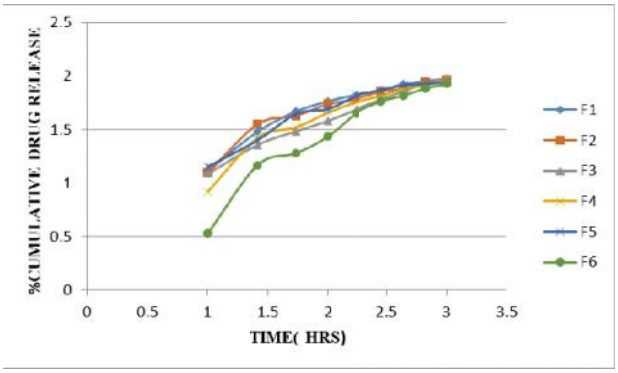

Fig 6: %Cumulative drug release

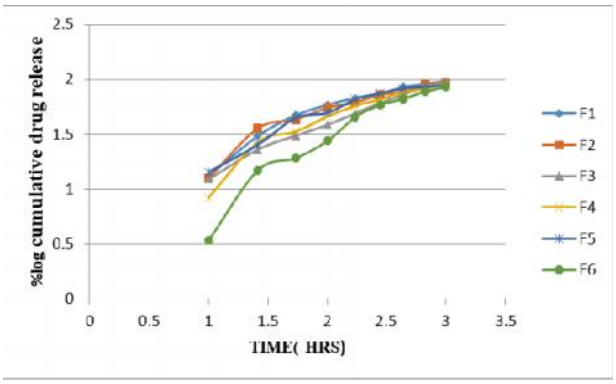

Fig 7: Time vs %log cumulative drug release (first order kinetics)of formulations F1 to F6

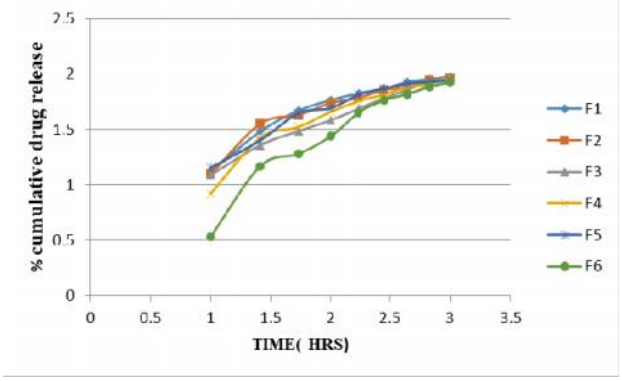

Fig 8: Square root of time vs cumulative %drug release (Higuchi's release mechanism) of formulations F1toF6

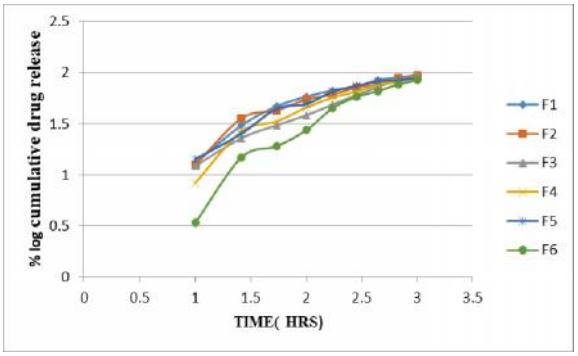

Fig 9: Square root time vs %log cumulative drug release (korsmeyer Peppas model) of formulation F1toF6

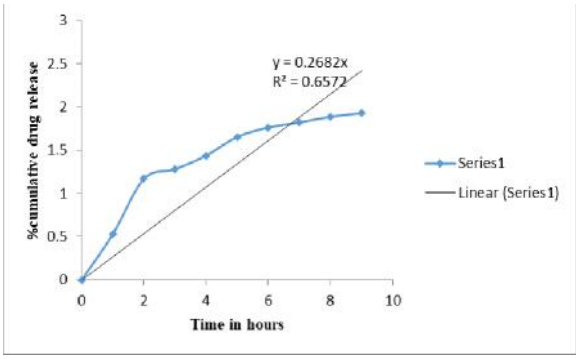

Fig 10: Time vs % cumulative drug release of formulation F6

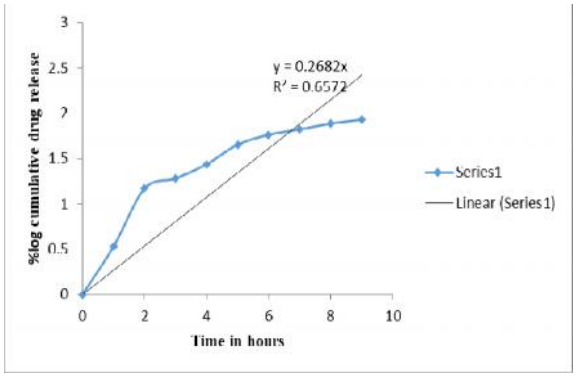

Fig 11: Time vs %log cumulative drug release (first order kinetics) of formulation F6

Evaluation Parameters

Characterization of physical parameters:

The results obtained after assessment of biopharmaceutical parameters were tabulated as fallows. From the table it was clear that as the chitosan concentration increases viscosity increases, drug release, spread ability was decreased after physical examination of all formulation they were found to be homogenous.

Entrapment efficiency:

Fluconazole was loaded in the gel and its entrapment efficiency is of 91.25 -94.02% was observed it was found that enhanced concentration of chitosan increases entrapment efficiency of formulation F6 was considered as optimised, because of its high entrapment efficiency

Drug Content:

The maximum drug content was found to be as 91.24% for formulation F7,and lowest as 82.6% for formulation F1 among all prepared formulation highest drug content was found in F6 formulation.

In-vitro diffusion studies:

In the first hour of diffusion studies drug release was observed ranging from 3.38-14.2,for all formulation. In-vitro drug release studies were performed over a period of 24hrs maintaining sink conditions .Percentage cumulative drug release were obtained as 89.25, 89.12, 91.48, 92.20, 93.64, 94.02 respectively .The drug release was retained with increase in concentration of chitosan, better sustained release of drug was attained with F6 formulation. In-vitro release data was subjected to zero order, first order and higuchi and korsmeyer peppas models in order to establish drug release mechanism and kinetics of drug release from gels.

When the data was subjected to zero and first order kinetics model a linear relationship was observed with high R^2 values for zero order models compared to first order model and it suggested that formulation followed zero order release. Higuchi model was applied to invitro release data linearity was obtained with high R^2 values suggested that drug release from gel followed diffusion mechanism. In order to define perfect model which will represent a better fit for invitro release data, korsemeyers peppas model was applied which will define the exact mechanism. Good linearity with high R^2 values was observed with this model.

Discussion:

Fluconazole is an ophthalmic and topical anti-bacterial agent used in the management of Allergic conjunctivitis, Trachoma, Blepharitis. The basic idea behind the development of such a system is to maintain a sustained drug release from the dosage form. Fluconazole is suitable candidate for formulation into sustained dosage form in order to prolong the release of drug. The drug-excipient compatibility studies were carried out by using FTIR technique. Based on the results, excipients were found to be compatible with Fluconazole. In preformulating, estimation of Fluconazole was carried out by systronics UV spectrophotometer at λmax 284nm using distilled water, which had a good reproducibility and this method was used in entire study. Formulation was prepared by using ionic gelation method. The response drug content, entrapment efficiency, diffusion, spread ability, in vitro drug release was evaluated Drug content ranging from to82.6 % to 91.24% entrapment efficiency values are ranged from 91.25% to 94.02% and in -vitro drug release studies are also studied. The In-vitro drug release study of Fluconazole was carried out by using In-vitro diffusion apparatus.100ml of using tear fluid was taken in a beaker. The solution was stirred with 100rpm by maintaining the temperature of 37˚c ± 5˚c. The drug release data were explored for this type of release mechanism followed. The best fit with the higest determination R^2 coefficients was shown by both the models (zero and peppas) followed by Higuchi model which indicate the drug release via diffusion mechanism. However as indicated by the values of R both of the models (zero and peppas) followed by Higuchi model were found to be efficient in describing the release of Fluconazole.

Table 2: Formulation table for the preparation of Fluconazole ophthalmic gel

S.No	Ingredients	F_1	F_2	F_3	F_4	F_5	F_6
1	Chitosan (gm)	0.1	0.2	0.3	0.4	0.5	0.6
2	Glacialacetic acid (ml)	1%	1%	1%	1%	1%	1%
3	β-cyclodextrin (gm)	0.1	0.1	0.1	0.1	0.1	0.1
4	Fluconazole (gm)	0.3	0.3	0.3	0.3	0.3	0.3
5	Carbapol (gm)	0.1	0.1	0.1	0.1	0.1	0.1
6	Propyleneglycol (ml)	2	2	2	2	2	2
7	Polyethylene glycol (ml)	0.5	0.5	0.5	0.5	0.5	0.5
8	Glycerin (ml)	1	1	1	1	1	1
9	Sodiumtripolyphoshate(gm)	0.01	0.01	0.01	0.01	0.01	0.01
10	Triethanalamine (ml)	0.15	0.15	0.15	0.15	0.15	0.15
11	Distilled water (ml)	4.5	4.5	4.5	4.5	4.5	4.5

Table 3: Antibacterial activity of Fluconazole

S.no	Formulation	Staphylococcus aureus
		Minimum zone of inhibition
1.	F_1	27.82
2.	F_2	27.69
3.	F_3	26.38
4.	F_4	25.98
5.	F_5	25.14
6.	F_6	24.6

Table 4: Results for Spreadability

S.No	Formulations	Length of glass slide (cm)	Weights taken (gm)	Time (sec)	Spreadability
1	F_1	7.5	25	30	6.25
2	F_2	7.5	31	42	5.35
3	F_3	7.5	35	50	5.25
4	F_4	7.5	38	57	5.0
5	F_5	7.5	40	62	4.83
6	F_6	7.5	42	67	4.70

Table 5: Drug content, Entrapment efficiency, viscosity and spreadability

Formulation code	Drug content (%)	Entrapment efficiency(%)	spreadability	Viscosity (dynes/cm)
F_1	82.6	89.25	5.75	1.32×10^{-3}
F_2	83.4	89.12	6.65	1.72×10^{-3}
F_3	84.32	91.48	6.25	1.78×10^{-3}
F_4	86.89	92.20	6.0	1.8×10^{-3}
F_5	89.8	93.64	5.83	1.9×10^{-3}
F_6	91.24	94.02	5.70	1.92×10^{-3}

Table 6: In-vitro release profile of first order formulation F_1 to F_6

S.No	Time (hrs)	% Cumulative drug release					
		F1	**F2**	**F3**	**F4**	**F5**	**F6**
1	1	13.04	12.47	12.24	8.3	14.2	3.38
2	2	30.02	35.53	22.78	26.5	25.19	14.7
3	3	46.67	42.68	30.49	33.19	44.26	19.03
4	4	58.09	54.67	38.27	45.62	49.38	27.36
5	5	67.21	61.01	48.77	57.09	65.21	44.95
6	6	73.46	71.62	60.07	65.13	74.13	57.73
7	7	84.59	76.67	72.50	76.43	82.29	66.17
8	8	90.23	87.54	85.18	81.80	84.64	76.86
9	9	94.39	93.62	92.80	91.60	88.57	84.87

Table 7: In-vitro release profile of first order formulation F1toF6

S.No	Time	%Log cumulative drug release					
		F1	**F2**	**F3**	**F4**	**F5**	**F6**
1	1	1.115	1.095	1.087	0.919	1.152	0.528
2	2	1.480	1.550	1.357	1.423	1.401	1.167
3	3	1.669	1.630	1.484	1.521	1.646	1.279
4	4	1.764	1.737	1.582	1.659	1.693	1.437
5	5	1.827	1.785	1.688	1.756	1.814	1.652
6	6	1.866	1.855	1.778	1.813	1.869	1.761
7	7	1.927	1.884	1.860	1.883	1.915	1.820
8	8	1.955	1.942	1.930	1.912	1.927	1.885
9	9	1.974	1.971	1.967	1.961	1.947	1.928

Table 8: In-vitro release profile of Higuchi model for formulations F1 to F6

S.No	Square root of time	%Cumulative drug release					
		F1	**F2**	**F3**	**F4**	**F5**	**F6**
1	1	13.04	12.47	12.24	8.3	14.2	3.38
2	1.414	30.02	35.53	22.78	26.5	25.19	14.7
3	1.732	46.67	42.68	30.49	33.19	44.26	19.03
4	2	58.09	54.67	38.27	45.62	49.38	27.36
5	2.236	67.21	61.01	48.77	57.09	65.21	44.95
6	2.449	73.46	71.62	60.07	65.13	74.13	57.73
7	2.645	84.59	76.67	72.50	76.43	82.29	66.17
8	2.828	90.23	87.54	85.18	81.80	84.64	76.86
9	3	94.39	93.62	92.80	91.60	88.57	84.87

Table 9: In-vitro release profile of korsmeyer-Peppas model for formulations F1toF6

S.No	**Squre root of time**	**% log cumulative drug release**					
		F1	**F2**	**F3**	**F4**	**F5**	**F6**
1	1	1.115	1.095	1.087	0.919	1.152	0.528
2	1.414	1.480	1.550	1.357	1.423	1.401	1.167
3	1.732	1.669	1.630	1.484	1.521	1.646	1.279
4	2	1.764	1.737	1.582	1.659	1.693	1.437
5	2.236	1.827	1.785	1.688	1.756	1.814	1.652
6	2.449	1.866	1.855	1.778	1.813	1.869	1.761
7	2.645	1.927	1.884	1.860	1.883	1.915	1.820
8	2.828	1.955	1.942	1.930	1.912	1.927	1.885
9	3	1.974	1.971	1.967	1.961	1.947	1.928

4. Conclusion

In the present study, we made an attempt to formulate Fluconazole loaded nano particles with variable concentrations of chitosan and incorporated them in a ophthalmic gel prepared by using carbopol940 as a gelling agent. Synthesised formulations were characterised for parameters such as p^H, spreadability, viscosity, Entrapment efficiency, drug content, in vitro diffusion studies, invitro permeation studies, antifungal activity. Among the developed formulations,F6 was considered as an optimized one as it showed better entrapment efficiency, drug content, antibacterial activity when compared with the other formulations. Sustained drug release was observed with F6 because of presence of high concentrations of chitosan. Thus it was concluded that ophthalmic gel serve as a promising carrier for the delivery of antibacterial drug, Fluconazole for the treatment of bacterial infections of an eye like Allergic conjunctivitis, Ttrachoma, Blepharitis.

5. References

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