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Formulation and Evaluation of Ocular *In-Situ* Gels of Besifloxacin

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ABSTRACT

Besifloxacin, a fourth-generation broad-spectrum antibiotic indicated for infective ophthalmitis, is intended to be released over a sustained period using ion-sensitive ocular *in situ* gels, which were the focus of the current work. The present analysis concludes that designing and creating oral *in-situ* gels of Besifloxacin requires a careful selection of polymers and medication. According to I.R. and U.V. investigations, the polymer is chosen, sodium alginate and HPMC were compatible with pefloxacin. It was found that the two polymers' different concentrations had an impact on the gel's viscosity, spreadability, and drug release. Gel formulations demonstrated good stability and uniformity. However, the gel formula, which showed the most significant percentage of drug release and favorable rheological characteristics, ended up being the formula of choice. Based on *in vitro* release investigations. Formulation F3 gives better and quicker patient improvement. When compared to other formulations, the F3 formulation is the most optimal. There is room for additional pharmacokinetic research because the outcomes of the studies that have already been done are encouraging. With this prolonged drug delivery system, the medicine's bioavailability can also be increased, benefiting patient efficacy, compliance, and therapeutic usefulness.

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INTRODUCTION

Ophthalmic drug distribution is complex because the eye's specific architecture limits drug absorption into the deeper tissues. The disadvantages of the available ophthalmic medication delivery methods, such as inserts, ointments, and suspensions, were patient compliance issues, impaired vision, and heterogeneity [1].

Due to slower drainage from the cornea, increasing a drug formulation's viscosity in the precorneal region can boost bioavailability. Several approaches for in-situ gelling devices have been investigated to address this issue. Ion activation, pH, and temperature can all activate these systems.

In-situ gels are created from polymers that experience phase transition due to environmental physicochemical change. They are quickly injected into the conjunctival sac of the eye as a solution [2].

The polymer modifies its structure to form a gel when it comes into contact with the lachrymal fluid. Due to the gel formation, this delivery system has a long retention time and is as simple to use as an ophthalmic solution.

Ion-sensitive in-situ gels can produce a gel on the ocular surface by cross-linking with the cations found in tear fluid. Buffers can be manufactured at the ideal pH for ocular delivery and can be precisely and quickly injected at room temperature [3].

MATERIALS AND METHODS

Besifloxacin was obtained from Aurobindo Pharma Ltd, Hyderabad, India, as a gift sample, HPMC E50, sodium alginate, K4M. Other chemicals came from Mumbai's S.D. Fine Chemicals. Analytical-grade materials and solvents were also used.

Preparation of *In situ* gel

The necessary amounts of HPMC- E 50 LV/HPMC-K4M and sodium alginate were dissolved in water while continuously stirring. The dispersion process was used to create the polymeric solution. Besifloxacin solution was continuously stirred into the polymeric solution [Table 1]. Agents for preservation and isotonicity were introduced [4]. The solution's pH was determined to be 6.3–6.5.

Evaluation Parameters

Evaluation of Gels

Gels were evaluated for their clarity, pH, viscosity, spreadability, skin irritation test, drug content, in vitro diffusion, and in vivo studies by using standard procedure. All analyses were carried out in triplicate, and average values were reported [5].

Clarity

Visual inspection was used to assess the clarity of different formulations under the black and white background [6], and It was given the following grades: turbid (+), transparent (+), and apparent (+++).

P.H.

In 25 ml of purified water, 2.5 grams of gel were precisely weighed and mixed 82. Digital pH meters were used to measure the pH of the dispersion [7]. (Systronics μ pH system 362).

Homogeneity

All developed. After being placed in the container, the appearance of the gels and the presence of any aggregate were checked for homogeneity [8].

Spreadability

Glass slide and wooden block measuring tools were used to determine it. The excess sample was put for the spreadability test between two glass slides and squeezed to a uniform thickness for 5 minutes with a 1000 g weight. 50 g of weight was put into the pan [9]. The spreadability was measured by the Time it took to separate the two slides or when the upper glass slide moved over the bottom plates (S). Spreadability was determined using the following formula:

$$S = ML/T$$

Where,

S = Spreadability

M = Weight tide to upper slide

L = Length moved on the glass slide

T = Time taken to separate the slide from each other

Viscosity Measurement

The viscosity of the gels was measured using a Brookfield DV-II + Pro viscometer and a tiny sample adaptor with the spindle number SC4-18/13R. The torque applied to the gel ranged from 10% to 100%. The "Local" software was used to determine the viscosity [10].

Drug Content

The Besifloxacin 50 cc of phosphate buffer 6.8 was used to dissolve 100 mg of gel. The volumetric flask holding the gel solution was agitated on a mechanical shaker for two hours to obtain complete drug solubility. This solution underwent filtering and spectrophotometer estimation [11].

Extrudability

The Pfizer hardness tester was used to conduct the extrudability test. The aluminum tube was filled with 15gm of gel. To adequately secure the line, the plunger was adjusted. For 30 seconds, 1 kg/cm2 of pressure was applied. The weighing was done on the gel that was extruded. At three equally spaced locations along the tube, the process was repeated. A test was conducted in triplicates [12].

Through Dialysis Sac

The device is a cylindrical glass tube with an internal diameter of 22 mm and a height of 76 mm that was opened on both ends. One end of the line was fixed to the dialysis sac, which had previously been soaked in water for 15 minutes, and 100 mg of the gel formulation equivalent to 1 mg of besifloxacin was evenly disseminated on the surface. The preparation now fills the inner circumference of the tube. The assembly was adjusted so that the lower end of the line carrying the gel barely touched (1-2 mm depth) the surface of the diffusion medium, which was a 250 ml beaker containing 200 ml of phosphate buffer with a pH of 6.8 that was kept at 37.2 C in a water bath. The contents were swirled using a magnetic stirrer at a speed of 100 10 rpm. The dialysis sac is a barrier between the gel phase and water (the sink phase). At intervals of 1, 2, 3, 4, 6, 8, 10, and 12 hours, 5 ml of the receptor fluid was taken. A spectrophotometer set at 280 nm was used to estimate the release of the medication, and 5 ml of pH 6.8 phosphate buffer was changed immediately after each estimate [13].

Table 1: Formulation Design of In situ Gelling System

Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8
Drug	2	2	2	2	2	2	2	2
Sodium Alginate	500	1000	1000	500	1500	500	-	-
HPMC K15M	500	1000	500	1000	500	1500	500	1000
Ethylcellulose	-	-	-	-	-	-	1000	500
Calcium carbonate	2000	2000	2000	2000	2000	2000	2000	2000
Methylparaben	198	198	198	198	198	198	198	198
Distilled water (up to 100ml)	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Drug Release Kinetics for Prepared Besifloxacin *In-situ* Gel

Data from the in vitro release was plotted in several kinetics models to evaluate the kinetics of release [14, 15].

Zero Order Equation

The graph was drawn as percent medication release vs. days.

$$C = K_0 t$$

First Order Equation

Log cumulative% medication remaining was used to depict the graph against Time in days.

$$Log\ C\ =\ \log\ C_0\ -\ Kt\ /2.3$$

Higuchi Kinetics

The cumulative % drug release vs. square root of Time was used to produce the graph.

$$Q = Kt^{1/2}$$

Korsmeyer–Peppas Equation

To evaluate the drug release mechanism, which was then plotted as log cumulative% drug release vs. Time in Peppa's equation.

$$Mt/\ Ma\ =\ Kt$$
$$Log\ Mt/\ Ma =\ Log\ K\ +\ n\ \log t$$

Stability Study

Besifloxacin *In-situ* Gel Stability tests were run on the formulas developed for this investigation [Table 2]. Stability research on the best formulation of F3 was carried out following ICH recommendations under various humidity and temperature conditions for 3 or 6 months.

The samples were withdrawn after 3 and 6 months and were analyzed for their Clarity; Spreadability; Viscosity; Drug content, and *In-vitro* drug release. The results revealed no significant changes in Clarity, Spreadability, Drug content, and In-vitro drug release for F3 formulation.

Table 2: Stability Study Storage Condition

Study	Storage Condition
Long term	25°C + 2°C/60%RH+ 5% RH
Intermediate	30°C+ 2°C/65%RH+ 5% RH
Accelerated	40°C+ 2°C/75%RH+ 5% RH

RESULTS AND DISCUSSION

Pre-Formulation Studies

The first stage in creating any formulation is to conduct pre-formulation investigations.

The main objective of this investigation was to determine whether the medicine was compatible with the polymers that were being employed.

Drug-Polymer Compatibility

To study the possible interactions between the Drug and excipient formulation. The prominent peaks obtained for the combinations were nearly identical to the medications.

Pure materials' I.R. spectra did not significantly differ from one another. Besifloxacin as well a mixture of polymer and drug [Figure 1, Figure 2 and Figure 3] [Table 3, Table 4 and Table 5].

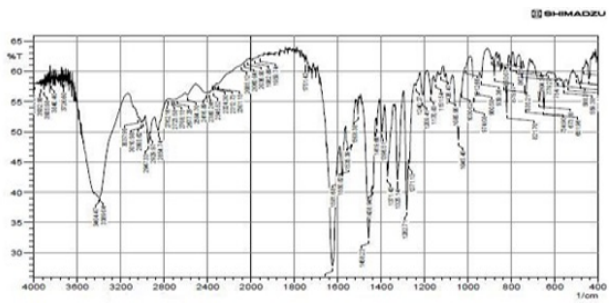

Figure 1: FT-IR Spectra of Pure Besifloxacin

Evaluation of Gels

Clarity

All gels were found to be translucent viscous. All gels

Table 3: FTIR Spectrum Data for Pure Besifloxacin

IR absorption bands (cm^{-1})		Bond	Functional Group
Observed Peak	Characteristic Peak		
3009.0	3000-3100	O-H strech	Monometric alcohol
1338.0	1330-1540	C-H Bend in plane	Alkanes
1225.4	800-1300	-C-O strech	Alcohol, ethers
1005.4	800-1300	-C-O strech	Alcohol, ethers
999.1	800-1300	-C-O strech	Alcohol, ethers
877.5	800-1300	-C-O strech	Alcohol, ethers
805.3	800-1300	-C-O strech	Alcohol, ethers

Table 4: I.R. Spectrum Data for Pure Sodium Alginate, HPMC K15M and Ethyl Cellulose

I.R. absorption bands (cm^{-1})		Bond	Functional Group
Observed peak	Characteristic peak		
3009.0	3000-3100	O-Hstrech	Monometric alcohol
3005.0	3000-3100	O-Hstrech	Monometric alcohol
1500.0	1330-1540	C-H Bend in plane	Alkanes
1153.3	800-1300	-C-Ostrech	Alcohol,ethers
1111.3	800-1300	-C-Ostrech	Alcohol,ethers
1005.4	800-1300	-C-Ostrech	Alcohol,ethers
999.1	800-1300	-C-Ostrech	Alcohol,ethers
805.3	800-1300	-C-Ostrech	Alcohol,ethers
754.5	600-1500	C-Clstrech	Alkanes
657.0	600-1500	C-Clstrech	Alkanes

Table 5: I.R. Spectrum Data for Mixture of all Ingredients

I.R. absorption bands (cm^{-1})		Bond	Functional group
Observed peak	Characteristic peak		
3135.6	3010-3330	N-Hstrech	Aromatic ring
2981.3	2500-3000	C-Hstrech	Alkens aromatic ring
2861.2	2650-2880	C-Hstrech	Alkens aromatic ring
1547.8	1660-1580	C=Ostrech	Alkenes
976.6	800-1300	C-Cstrech	Alcohols, ethers
845.3	800-1300	C-Cstrech	Alcohols, ethers
687.4	600-1500	C-Clstrech	Alkanes
612.4	600-1500	C-Clstrech	Alkanes

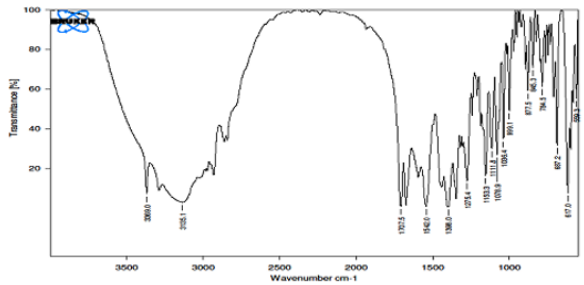

Figure 2: FT-IR Spectra of Pure Sodium Alginate, HPMC K15M and Ethyl Cellulose

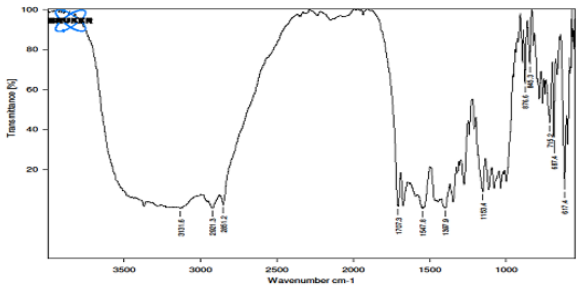

Figure 3: FT-IR Physical Mixture of all Ingredients (Formulation F3)

Table 6: Values of Created Gel's Evaluation Metrics

Formulations	Clarity	Ph	Homogeneity	Spreadability (G.Cm/S)	Viscosity (Cps)	%Drug	Skin irritation	Extrudability
F1	+	6.3	Good	18.07	9122	101.46	-	+
F2	+	6.3	Good	25.45	8776	98.74	-	+
F3	++	6.9	Good	18.70	9223	99.13	-	++
F4	+	6.4	Good	27.27	8824	97.29	-	+
F5	+	6.7	Good	18.07	8954	98.65	-	+
F6	+	6.6	Good	22.72	8874	99.48	-	+
F7	+	6.4	Good	19.12	9257	99.67	-	+
F8	+	6.4	Good	18.94	9783	99.64	-	+

Note: + Satisfactory, + + Good, + + + Excellent

Table 7: *In-vitro* Drug Release of Besifloxacin

Time (hrs)	Cumulative % Drug Released							
	F1	F2	F3	F4	F5	F6	F7	F8
1	21.47	15.84	20.16	19.81	18.99	19.64	22.83	15.94
2	28.49	22.42	30.56	26.77	25.55	26.04	36.81	28.48
3	35.86	33.51	37.37	31.47	31.65	33.90	51.56	43.22
4	43.66	43.91	49.12	37.60	42.84	45.85	64.78	57.73
6	51.66	52.60	58.66	44.51	50.62	54.33	79.27	72.51
8	58.22	59.77	64.45	51.92	58.93	61.93	80.12	81.34
10	72.49	71.45	77.06	63.28	71.04	74.75	89.13	90.34
12	80.82	81.33	96.08	72.77	78.38	83.77	90.57	95.56

Table 8: Kinetic Values Obtained from In-Vitro Released Data of Formulation F1-F8

Code	Zero-order R^2	First order R^2	Higuchi R^2	Peppas R^2	N
F1	0.972	0.981	0.995	0.990	0.518
F2	0.979	0.991	0.991	0.985	0.668
F3	0.977	0.987	0.988	0.992	0.576
F4	0.973	0.975	0.993	0.990	0.490
F5	0.977	0.990	0.994	0.987	0.580
F6	0.977	0.990	0.993	0.986	0.592
F7	0.965	0.969	0.992	0.988	0.659
F8	0.983	0.990	0.984	0.989	0.803

were free from the presence of particles [Table 6].

P.H.

The pH value of all developed formulations gels (F1-F8) was 6.2 – 6.9.

Homogeneity

All fully formed (F1-F8) gels displayed excellent uniformity and were lump-free. The prepared materials were considerably more transparent and clear [Table 6].

Spreadability

The spreadability rating shows that a tiny amount of shear can quickly spread the gel [Table 6]. Indicating Spreadability of HPMC K4Mcontaining Besifloxacin gel was good as compared to other gel Spreadability of gels in the range of 18.07-27.27g.cm/sec.

Viscosity Measurement

Using a Brookfield viscometer, the viscosity of variously prepared Besifloxacin gels was evaluated. Every system of prepared gels' rheological behavior

Table 9: The *In-Vitro* Drug Release Profile of F3 During Stability Tests

Time (hours)	Cumulative % Drug Release (X±S.D)*			
	Initial	1 month (25^0C- 60%RH)	Two month (40^0C-70%RH)	Three month (60^0C-80%RH)
0	0	0	0	0
1	20.16	19.53	20.64	19.94
2	30.56	28.34	31.42	29.46
3	37.37	36.46	37.96	35.46
4	49.12	48.92	51.24	47.92
6	58.66	58.65	60.67	58.64
8	64.45	65.12	64.94	63.79
10	77.06	78.64	77.64	75.94
12	96.08	97.34	96.92	95.38

*Mean ± S.D, n=3

was investigated [Table 6]. The proportion of the solid fraction, which creates the structure, to the liquid fraction determines the consistency of a gel system. The range of viscosity for variously designed gels was 8776 to 9826 centipoises.

Drug Content

The percentage drug content of 97.24 to 101.46% of all created gel formulations was discovered to fall within this range [Table 6]. It was determined that the formulations' drug content percentages were satisfactory. Consequently, the techniques used to make gels were deemed appropriate.

Extrudability

When applying the gel and ensuring that the patient accepts it, the extrusion of the gel from the tube is crucial. A sufficient consistency is necessary to extrude the gel from the line since high-consistency gels may not do so, while low-viscosity gels may flow easily [Table 6]. It was discovered that HPMC gel compositions have good extrudability. Although the extrudability of other gels was satisfactory.

Skin Irritation

Patient accepts lack of skin irritation in gel formulation. A test for skin irritation was run, but no skin reddening occurred. It was discovered that none of the gel formulations caused outrage [Table 6]. Thus, observations suggest that these gels are suitable for topical use.

In-Vitro Drug Diffusion Studies

Purified water was used as the dissolution medium for the *in-vitro* drug release investigations. The findings were tabulated and graphically depicted by placing Time (hrs) on the X-axis and Cumulative percentage drug release on the Y-axis [Table 7 & Figure 4].

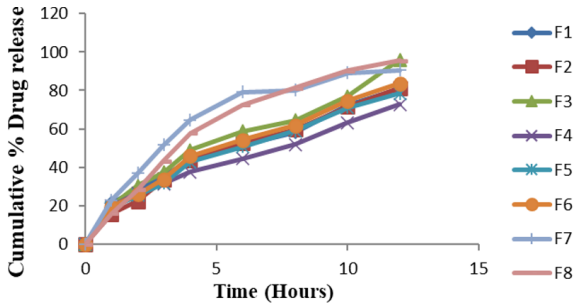

Figure 4: Comparative Diffusion Profile of F1 to F8 Formulations

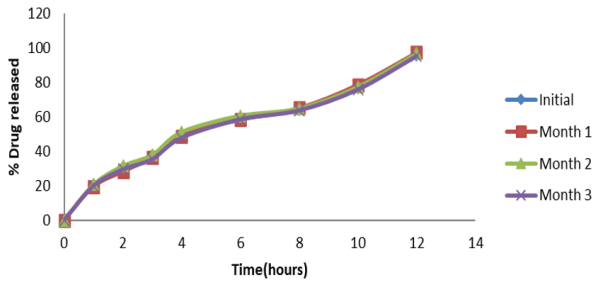

Figure 5: The *In-Vitro* Drug Release Profile of F3 During Stability Tests

In Vitro Drug Release Kinetics for Besifloxacin *In-Situ* Gel

To compare the dissolving profiles of the best formulation F3, various model-dependent techniques (Zero order, First order, Higuchi, and Korsemayer-Peppas plots) were used. According to the output of these models, the formulation F3 follow Peppas is the model that fits data the best. This is a result of a previously established fact based on the fitted R2 value. Formulation F3 has a Korsemayer-Peppas release exponent (n) value of 0.576, which is more than 0.45 and indicates non-fickian diffusion [Table 8].

Stability Data

The chosen formulation The F3 formulation was kept in storage for three months at 40°C + 2°C / 75% 5% R.H. Following storage, samples were examined for 1, 2 and three months [Figure 5 and Table 9].

CONCLUSION

The current analysis concludes that designing and creating oral in-situ gels of Besifloxacin requires a careful selection of polymers and medication. According to I.R. and U.V. investigations, the polymer chosen, sodium alginate, and HPMC were discovered to be compatible with the medication besifloxacin. It was found that the two polymers' different concentrations had an impact on the gel's viscosity, flowability, or drug release. Gel formulations demonstrated good durability or uniformity. But, a gel formula that exhibited the highest percentage of drug release and favorable rheological characteristics ended up being the formula of choice. Formulation F3 gives better and quicker patient improvement. There is room for additional pharmacokinetic research because the outcomes of the studies that have already been done are encouraging. When compared to other formulations, the F3 formulation is the most optimal. Based on in vitro release investigations, the formulations of the Besifloxacin gels used in this inquiry were found to be satisfactory, according to the thesis's results. With this prolonged drug delivery system, the medicine's bioavailability can also be increased, benefiting patient efficacy, compliance, and therapeutic usefulness.

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Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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