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Formulation and Evaluation of a Novel *In Situ* Gum Based Ophthalmic Drug Delivery System of Linezolid

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Abstract

A major problem in ocular therapeutics is the attainment of optimal drug concentration at the site of action, which is compromised mainly due to precorneal loss resulting in only a small fraction of the drug being ocularly absorbed [1]. The effective dose administered may be altered by increasing the retention time of medication into the eye by using *in situ* gel forming systems. The aim of the present investigation is to prepare and evaluate novel *in situ* gum based ophthalmic drug delivery system of linezolid. Hydroxypropyl guar (HPG) and xanthum (XG) were used as gum with the combination of hydroxyethyl cellulose (HEC), carbopol (CP), and sodium alginate as viscosity enhancing agents. Suitable concentrations of buffering agents were used to adjust the pH to 7.4. All the formulations were sterilized in an autoclave at 121°C for 15mins. The formulations were evaluated for clarity, pH measurement, gelling capacity, drug content estimation, rheological study, *in vitro* diffusion study, antibacterial activity, isotonicity testing, eye irritation testing. The developed formulations exhibited sustained release of drug from formulation over a period of 6hr thus increasing residence time of the drug. The optimized formulations were tested for eye irritation on albino rabbit (male) using

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the Draize test protocol with crossover studies. The formulations were found to be non-irritating with no ocular damage or abnormal clinical signs to the cornea, iris or conjunctiva observed. Thus these *in situ* gelling systems containing gums may be a valuable alternative to the conventional systems.

Key words

Linezolid • *In situ* gel • Draize test • Gelling capacity • Rheological evaluation • *In vitro* diffusion study.

Introduction

Ophthalmic drug delivery is one of the most interesting and challenging factors facing the pharmaceutical research scientist [1, 2]. The anatomy, physiology, and biochemistry of the eye render this organ exquisitely impervious to foreign substances. The challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage [3]. The development of newer, more sensitive diagnostic techniques and therapeutic agents render urgency to the development of more successful ocular delivery systems. 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.

Results

In situ gel formulations were prepared using various polymers such as hydroxy ethyl cellulose, carbopol, sodium alginate, and gums such as hydroxypropyl guar, xanthum gum. Linezolid was used as model drug and cyclodextrin was used as

solubilising agent. All the formulations were prepared in the concentration range of 0.5gm/100ml of linezolid.

The prepared *in situ* gel formulations were evaluated for clarity, pH measurement, gelling capacity, drug content estimation, rheological study, *in vitro* diffusion study. The pH of *in situ* gel solution was found to be 7.4 for all the formulations. The formulation should have an optimum viscosity that will allow for easy instillation into the eye as a liquid (drops), which would undergo a rapid sol-to-gel transition (triggered by ion exchange) as shown in table II.

Tab. II. Evaluation parameters

Formulation code	**pH measurement**	**Gelling capacity**	**Drug content in (%)**
L_1	7.4	++	93.8
L_2	7.4	++	89.2
L_3	7.4	++	89.9
L_4	7.4	++	89.85
L_5	7.4	++	94.8
L_6	7.4	+++	84.80
L_7	7.4	+++	80.34
L_8	7.4	++	91.36
L_9	7.4	+++	91.18
L_{10}	7.4	+++	76.78
+: Gels after few minutes, dissolves rapidly. ++: Gelation immediate remains for few hours. +++: Gelation immediate, remains for extended period.			

Rheological evaluation of all the formulation exhibited Newtonian flow before gelling (as shown in figure I) and exhibited pseudoplastic flow after gelling (as shown in figure II and table III) in the eye. There was 5-fold increase in the viscosity after gelling. Additionally, the gel formed *in situ* should maintain its integrity without dissolving or eroding for a prolonged period.

Tab. III. Rheological studies of formulation L_1 & L_8

RPM	Viscosity in mPas. (Before Gelling) (spindle no L2)		Viscosity in mPas. (After gelling) (spindle no L3)	
	L_1	L_8	L_1	L_8
20	1272.5	1428	5630.1	4923
30	937.1	1142	4014	3721
50	669.1	725.9	2664	2872.1
60	604	641.7	2293.0	1925.7
100	380.4	354.9	1491	823.1
200	166.7	198.1	794	521.5
200	166.7	197.9	794.3	521.5
100	380.4	354.9	1491	823.1
60	604.2	641.7	2293.0	1925.7
50	669.1	725.9	2664	2872.1
30	937.1	1142	4014.2	3721
20	1272.5	1428	5630.1	4923
L_1: 0.25% HPG, 0.3% HEC, 0.5% SA, L_8: 0.2%XG, 0.5% SA, 0.4% CP				

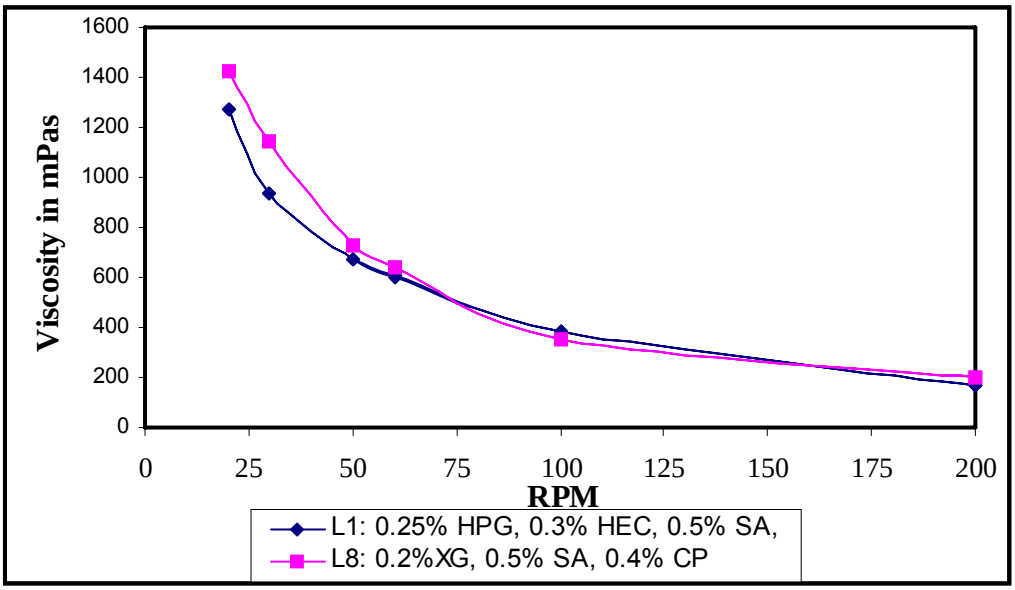


Fig. I. L_1 & L_8 formulation (Before gelling)

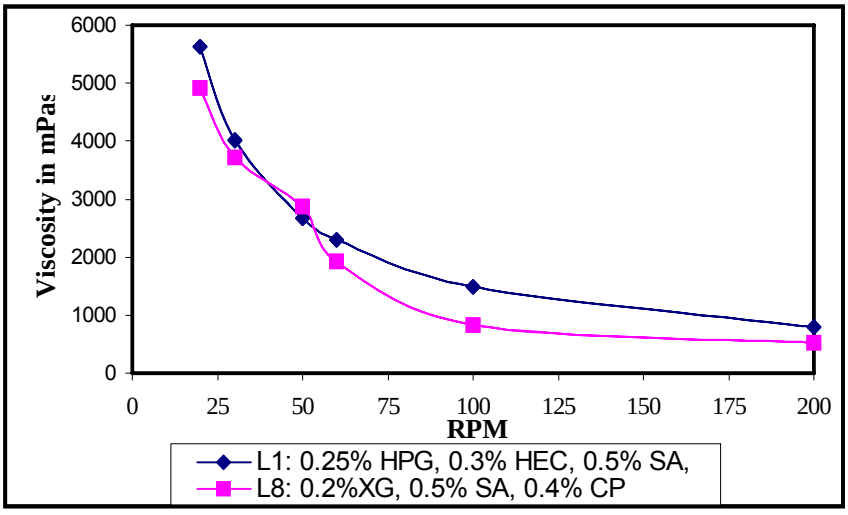


Fig. II. L_1 & L_8 formulation (After gelling)

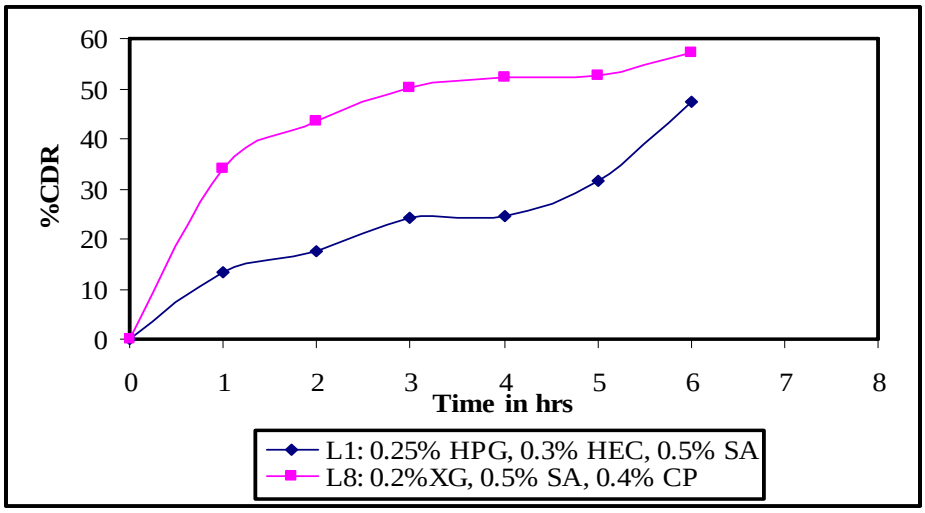
Formulation L_1 & L_8 exhibited good gelling capacity and good *in vitro* release with fickinain type of diffusion mechanism when subjected to PCP DISSO software analysis. For all the formulation, the best fit model was Krosmeyers peppas and followed fickian diffusion mechanism for drug release (table IV depicts the curve fitting data of L1 and L8 formulations).

Tab. IV. Curve fitting data for the release rate profile of formulation L_1 toL_{10}

Model		**L_1**	**L_8**
Krosmeyers – peppas	k	0.2861	0.3411
	n	0.1600	0.1274
	R	0.9855	0.9959
Zero order	k	0.0831	0.0941
	R	0.4751	0.2652
First order	k	-0.008	-0.0009
	R	0.4761	0.2666
Higuchi matrix	k	0.1817	0.2071
	R	0.9035	0.8708
Hixson Crowel	k	-0.0003	-0.0003
	R	0.4758	0.2662
L_1: 0.25% HPG, 0.3% HEC, 0.5% SA, L_8: 0.2%XG, 0.5% SA, 0.4% CP			

From the i*n vitro* results it was observed that the L_8 formulation contains xanthum gum, sodium alginate and carbopol (57.08%) has shown highest % cumulative drug release (% CDR) at 6^{th} hrs where as the L_1 formulation contains HPG with the varying concentration of Hydroxyethyl cellulose and Sodium alginate has shown 47.20 % CDR (as shown in figure III). Hence L_1 and L_8 formulation were taken for further study.

Fig. III. Comparative *in vitro* diffusion profile of L_1 & L_8 formulations



%CDR … % Cumulative drug release

The optimized formulations were subjected to isotonicity, antibacterial activity, *in vivo* ocular testing in rabbits and accelerated stability studies. One month accelerated stability study was carried out as per ICH guidelines, it was evident from the data that there was no change in clarity, pH, gelling capacity, rheological evaluation, and drug content and *in vitro* diffusion of the drug from the formulation.

Isotonicity testing of L_1 and L_8 formulation exhibited no change in the shape of blood cells (bulging or shrinkage), which reveals the isotonic nature of the formulation and compared with that of standard marketed ophthalmic of ciprofloxacin.

Isotonicity testing

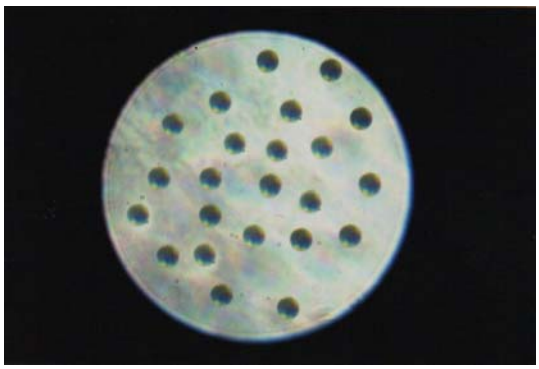


Fig. IV. Blood cells with ciprofloxacin as standard

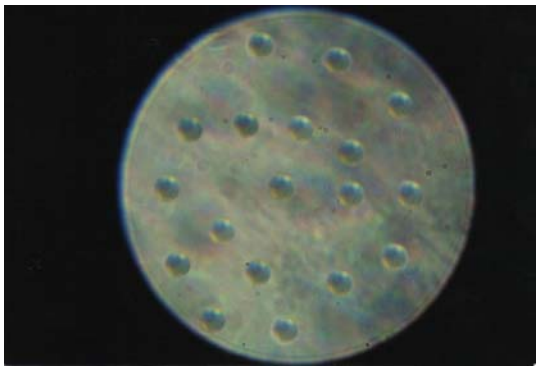


Fig. V. Blood cells with linezolid formulation (L_1)

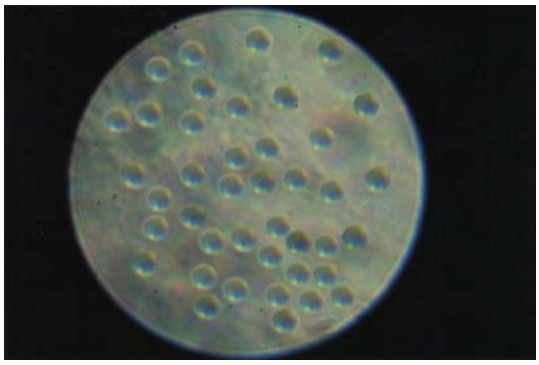


Fig. VI. Blood cells with linezolid formulation (L_8)

Antibacterial sensitivity test-MIC was carried out using serial dilution method. In L_1 formulation, pure drug sample & L_8 formulation the MIC conc. was found to be 4mcg/ml. Hence it was concordant with that of the standard linezolid and no reduction in the efficacy of the formulation was observed.

Tab. V. Antibacterial activity test-MIC for 3 samples

Conc. in mcg/ml	**Turbidity in L_1 formulation**	**Turbidity in pure drug sample (standard)**	**Turbidity in L_8 formulation**
128	–	–	–
64	–	–	–
32	–	–	–
16	–	–	–
8	–	–	–
4	–	–	–
2	+	+	+
1	+	+	+
0.5	+	+	+
0.25	+	+	+
NC	–	–	–
MC	–	–	–
DC	–	–	–
PC	+	+	+

NC = Negative control, MC = Media control, DC = Drug control,
PC = Positive control, – = Presence of clear solution (Inhibition),
+ = presence of turbidity (No inhibition)

In vivo eye irritation testing was carried out using rabbits and as per Draize test protocol. Optimized formulations L_1 & L_8 were used for this test. The formulations were found to be non-irritating with no ocular damage or abnormal clinical signs to the cornea, iris or conjunctivae observed. Hence the formulation was suitable for the eye instillation.

Tab. VI. Eye irritation testing: Rabbit corneal observations for opacity and area of cornea involved

Opacity	**Normal Rating for opacity**	**Rating for formulation**		**Area of cornea involved**	**Normal Rating for corneal area involved**	**Rating for formulation**	
		L_1	**L_8**			**L_1**	**L_8**
No opacity	0 none	0	0	25% or less (not 0)	1	0	0
Diffuse area, details of iris clearly visible	1 slight	0	0	25% to 50%	2	0	0
Easily visible transulescent areas, details of iris slightly obscure	2 mild	0	0	50% to 75%	3	0	0
Opalescnent areas, no details of iris	3 moderate	0	0	Greater than 75%	4	0	0
Opaque, iris invisible	4 Severe	0	0	–	-	0	0

Tab. VII. Rabbit conjunctiva observation

Redness	**Normal Rating**	**Rating for formulations**	
		L_1	**L_8**
Vessels normal	0 none	0	0
Vessels definitely injected above normal	1 slight	0	0
More diffuse, deeper crimson red with individual vessels not easily discemible	2 moderate	0	0
Diffuse beefy red	3 Severe	0	0

Tab.VIII. Rabbit iris observations

Values	Normal Rating	Rating for formulations	
		L_1	L_8
Normal	0 none	0	0
Folds above normal, congestion, swelling, iris reacts to light	1 slight	0	0
No reaction to light, haemorrhage, gross destruction	2 Severe	0	0

Discussion

By varying the concentration of polymers with two different gums ratio, it is to obtain the increased residence time and sustained drug release. Both the gums, HPG and xanthan are suitable candidates for ophthalmic *in situ* gel system. The combination of and appropriate ratio of hydroxypropyl guar, xanthan:polymers is an important factor in achieving increased duration of action and also release from the dosage form to achieve sustained effect. L_1 formulation containing 0.25% HPG, 0.3% HEC and 0.5% SA has shown release of 47.20%. L_8 formulation containing 0.2% XG, 0.5% SA, 0.4% CP has shown release of 57.08%. The formulation was liquid at the formulated pH (7.4) and underwent rapid gelation upon coming in contact with ions present in the tear fluid. The gel formed *in situ* afforded sustained drug release over 6 hr periods. The formulations exhibited therapeutic efficacy. Stability data recorded over a 1-month period under accelerated temperature conditions indicated the stability of the formulation.

The developed formulation is a viable alternative conventional eye drops by virtue of its ability to enhance bioavailability through its longer precorneal residence time and ability to sustain drug release.

Conclusion

The present work was carried out to develop a novel *in situ* gum based ophthalmic drug delivery system of linezolid. The methodology adopted for preparation of *in-situ* gel solution was very simple and cost effective. It is newer approach to improve easy eye instillation, residence time and bioavailability and prolong drug release. From the study conducted, the following conclusions were drawn, by varying the concentration of polymers with two different gums ratio, it is to obtain the increased residence time and sustained drug release. Among the novel gum systems used such as hydroxypropyl guar and xanthan gum. Xanthan was found to be best gum and viscosity enhancer in combination with polymers with respect to increased duration of action and drug release. The study revealed that an appropriate ratio of hydroxypropyl guar, xanthan to polymers is an important factor in achieving increased duration of action and also release from the dosage form to achieve sustained effect. The gel formed *in situ* afforded sustained drug release over 6 hrs periods. The formulations exhibited therapeutic efficacy. The developed formulation is a viable alternative conventional eye solution by virtue of its ability to enhance bioavailability through its longer precorneal residence time and ability to sustain drug release.

Experimental

Materials

Hydroxypropyl guar (Emcure), Xanthan gum (Lucid colloids Ltd), Hydroxyethyl cellulose (Microlabs), Carbopol 934P (Noveon, Mumbai), Sodium alginate (Microlabs), (Laboratory grade), Cyclodextrin (Zydus Biogen, Ahmedabad), Benzylkonium chloride(Astra Zeneca Bangalore), and Linezolid (Cipla Vikroli. Mumbai)

Preparation of in situ gel

The polymeric dispersion was prepared by dispersing required quantity of gums and polymers in water using a magnetic stirrer and allowing it to swell overnight. In the aqueous solution of cyclodextrin linezolid was added with agitation until it is fully dissolved [4]. Buffering and osmolality adjusting agents were added. The above solution was added to the polymer dispersion slowly [3]. The pH of the solution was adjusted to 7.4 using 0.1 N NaoH/ 0.1N HCL. The ingredients of the formulations are depicted in table I.

Tab. I. Formulation design of *in situ* gelling system

Form-ulation Code	Ingriedients						
	Linezolid	**Cyclo-dextrin**	**HPG**	**Xanthan gum**	**HEC**	**Sodium alginate**	**Carbo-pol 934**
L_1	0.5gm	2.5gm	0.25gm	-	0.3gm	0.5gm	-
L_2	0.5gm	2.5gm	0.25gm	-	0.25gm	-	0.3gm
L_3	0.5gm	2.5gm	0.25gm	-	-	0.5gm	0.4gm
L_4	0.5gm	2.5gm	0.25gm	-	0.5gm	-	0.3gm
L_5	0.5gm	2.5gm	0.25gm	-	-	0.3gm	0.4gm
L_6	0.5gm	2.5gm	-	0.2gm	0.3gm	0.5gm	-
L_7	0.5gm	2.5gm	-	0.2gm	0.25gm	-	0.3gm
L_8	0.5gm	2.5gm	-	0.2gm	-	0.2gm	0.2gm
L_9	0.5gm	2.5gm	-	0.2gm	0.5gm	-	0.3gm
L_{10}	0.5gm	2.5gm	-	0.2gm	-	0.3gm	0.4gm
All the formulations contain Benzylkonium chloride—0.01% W/V, Citric acid—0.2% W/V, Boric acid—0.3% W/V, Sodium chloride —0.9 % W/V, Disodium EDTA 0.0625% W/V, Sodium Metabisulfite---0.02% W/V.							

Physical parameters

The formulated *in situ* gel solution was tested for clarity, pH, gelling capacity, and drug content estimation. The results are as shown in table II.

Gelling capacity

The gelling capacity of the prepared formulation was determined by placing a drop of the formulation in a vial containing 2ml of freshly prepared simulated tear

fluid and visually observed. The time taken for its gelling was noted [5, 6]. The results are as shown in table II.

Rheological studies

The viscosity measurements were done using Brookfield viscometer DV-2 model. The *in situ* gel formulations were placed in the sampler tube. From the literature it was evident that, the formulation before gelling should have a viscosity of 5 to 1000 m Pa s. And after ion gel-activation by the eye, will have a viscosity of from about 50-50,000 m Pa s. The samples were analyzed both at room temperature at 25°C and thermostated at 37°C ± 0.5°C by a circulating bath connected to the viscometer adaptor prior to each measurement. [7–10]

The angular velocity of the spindle was increased 20, 30, 50, 60, 100, 200 and the viscosity of the formulation was measured. All the formulations exhibited Newtonian and pseudoplastic flow characteristics before and after gelling in the simulated tear fluid respectively. The formulations L_1 and L_8 exhibited the required viscosity range, hence this formulation were taken for further study. Results are as shown in table III & figure I & II.

In vitro drug release studies

In vitro release study of *in situ* gel solution was carried out by using Franz diffusion cell. The formulation containing 5 mg/ml concentration of linezolid was placed in donor compartment and freshly prepared simulated tear fluid in receptor compartment. Between donor and receptor compartment dialysis membrane is placed (0.22µm pore size). 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 at predetermined time interval of 1hr for 6 hrs and same volume of fresh medium was replaced [3–5]. The withdrawn samples were diluted to 10ml in a volumetric flask with acetonitrile:methanol:water (4:4:2) and analyzed by UV spectrophotometer at 254nm using reagent blank. The drug content was calculated using the equation generated from standard calibration

curve (y= 0.0505 x+0.0291). The % cumulative drug release (%CDR) was calculated**.** The data obtained was further subjected to PCP DISSO software for curve fitting for drug release data [11]. The best fit model was found to be Krosmeyers peppas with the regression in the range of 0.9204-0.9959 and the formulation exhibited fickinian diffusion mechanism with a value of 0.1095-0.1609. Formulation L_1 and L_8 exhibited required release characteristics with the regression value of 0.9855, 0.9959 respectively as show in table IV & figure III.

The L_1 and L_8 formulation were further subjected isotonicity evaluation, antibacterial activity, eye irritation testing and accelerated stability studies.

Accelerated stability studies

Formulation L_1 & L_8 were placed in ambient colored vials and sealed with aluminium foil for a short term accelerated stability study at 40±2 °C and 75±5% RH as per International Conference on Harmonization states Guidelines [12, 13]. Samples were analyzed every month for clarity, pH, gelling capacity, drug content, rheological evaluation, and *in vitro* dissolution.

The L_1 and L_8 formulation were subjected to accelerated studies and were analyzed for isotonicity evaluation, antibacterial activity and eye irritation testing.

Isotonicity evaluation

Isotonicity is important characteristic of the ophthalmic [13]. Isotonicity has to be maintained to prevent tissue damage or irritation of eye. L_1 and L_8 were subjected to isotonicity testing, since they exhibited good release characteristics and gelling capacity and the required viscosity. Formulations were mixed with few drops of blood and observed under microscope at 45X magnification and compared with standard marketed ophthalmic formulation containing ciprofloxacin. Figure IV, V & VI, depicts the isotonic nature of Standard ciprofloxacin, L_1 and L_8 respectively. The shape of blood cell was compared with standard marketed ophthalmic formulation containing ciprofloxacin.

Antibacterial activity

The microbiological growth of bacteria is measured by concentration of antibiotics and this has to be compared with that produced by known concentration of standard preparation of antibiotic. To carryout microbiological assay serial dilution method was employed [14, 15]. Test organism recommended for linezolid oxazolidinone antibiotic is *Staphylococcus aureus* [16, 17].

Three samples were tested for MIC, and they are coded as A, B, and C. A = formulation L_1, B = pure sample, C= formulation L_8. It was communicated that the activity of the compound against *Staphylococcus aureus* is to be tested by MIC. The concentration of linezolid in both standard and test taken was 5 mg/ml. 51 µl of the above solution contains 255µg of the drug. 14 sterile test tubes were arranged in the rack and numbered as 1 to 14.To the 1st test tube 2000µl of BHI broth was added. To the remaining test tube 1000µl of BHI was added. 51µl of BHI broth was pipetted out using sterile micropipette and was discarded. To this 51µl of drug solution was added (2000µl contains 256µg of drug). The concentration in the 1st test tube was 128µg/ml of linezolid, and then 1000µl of the solution was transferred from tube no1 to tube no 2. and mixed well. This procedure was repeated till the second last tube to obtain the concentration of 128µg/ml, 64µg/ml, 32µg/ml, 16µg/ml, 8µg/ml, 4µg/ml, 2µg/ml, 1µg/ml, 0.5µg/ml, 0.25µg/ml respectively. The last two tubes contain 1000 µl of media. One tube is considered as media control and another tube as drug control. 10µl broth of the *Staphylococcus aureus* was inoculated in all the test tubes except in negative control and incubated at 37 °C for 24hrs to observe the growth. After the incubation period the tubes were observed for showing inhibition of growth and calculation of MIC was done and results were tabulated. The results are as shown in table V.

Ocular irritancy

The Draize technique was designed for the ocular irritation potential of the ophthalmic product prior to marketing [18]. According to the Draize test, the amount of substance applied to the eye is normally 100µl placed into the lower cul-de-sac

with observation of the various criteria made at a designed required time interval of 1hr, 24hrs, 48 hrs, 72hrs, and 1week after administration [18, 19]. Three rabbits (male) weighing 1.5 to 2kg were used for the present study. The sterile formulation was instilled twice a day for a period of 7 days, and a cross-over study was carried out (a 3 day washing period with saline was carried out before the cross-over study). Rabbits were observed periodically for redness, swelling, watering of the eye. The results are as shown in table VI, VII, & VIII.

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