DESIGN, DEVELOPMENT AND EVALUATION OF CATIONIC GUAR AND HYDROXYPROPYLGUAR BASED *IN SITU* GELS FOR OPHTHALMIC DRUG DELIVERY

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ABSTRACT

The poor bioavailability and therapeutic response exhibited by the conventional eye drops due to rapid corneal loss is overcome by the use of ion-activated gel forming systems that are instilled as drops; these undergo gelation in cul-de-sac mode. The present study describes the design, development and evaluation of *in situ* ophthalmic drug delivery of antibacterial agent, linezolid, based on ion-activated guar gum derivatives. Novel polymers such as Cationic guar with hydroxypropyl guar are being used as gelling as well as viscosity enhancing agents. Differential scanning calorimetric studies have revealed that linezolid is compatible with all the excipients in the formulation. The study also aims at rheological characterization, effect of sterilization (moist heat) and effect of aging on the viscosity of *in situ* gels by calculating consistency index (K), flow behaviour index (n value) using power law model. The *in vitro* drug diffusion study for the developed formulations has also been carried out. The formulation CG-HPG2, exhibiting good physical stability subsequent to sterilization and storage and further retaining the consistency index (K) and flow behavior index (n value), was chosen as the optimized formulation. The gel formed *in situ* revealed the sustained release of the drug for up to 12 hrs. Stability data recorded over a period of 6 months at elevated temperature conditions revealed the formulation to be stable. *In vivo* ocular toxicity studies revealed non irritant and non toxic nature of the formulation. Therefore, the developed guar gum derivative based ophthalmic *in situ* gel by virtue of its prolonged corneal residence time and sustained drug release could be considered a viable alternative to the conventional eye drop formulation in achieving enhanced bioavailability.

INTRODUCTION

Guar gum is known for its viscosity contribution in formulation and also for its ion-induced gelation effect[1]. Guar gum has certain drawbacks such as uncontrolled rate of hydration, fall in viscosity upon storage, susceptibility to microbial degradation and turbidly in aqueous dispersions. These drawbacks are overcome by derivatization to hydroxy alkyl derivatives[2]. *In situ* ophthalmic gels are expected to provide prolonged corneal contact time, reduced pre-corneal drug loss and ease of administration in comparison to the conventional eye drops, suspensions or ointments[3].

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To exhibit the above criteria, viscoelastic property or rheology plays an important role in the formulation of *in situ* gels[4]. Cationic guar and hydroxypropyl guar (HPG) have been used in the present investigation for the ion-induced gelation effect. It is evident from the literature that a cationic polymer in conjunction with a non ionic polymer leads to enhanced gelling ability and sustained release of drugs [5,6]. Linezolid is a synthetic antibacterial agent of a new class of antibiotics, the oxazolidinones, which has clinical utility in treatment of infectious disease caused mainly by aerobic gram positive bacteria and certain gram negative bacteria and anaerobic bacteria. Linezolid binds to a site on the bacterial 23S ribosomal RNA of the 50S subunit and prevents the formation of a functional 70S initiation complex, which is an essential component of the bacterial translational process[7]. Assessment of the potential compatibility

between the API and the different excipients is an essential part of the preformulation study prior to the final formulation of a dosage form especially when novel excipients such as Cationic guar and HPG are intended to be used in the formulations. To predict the shelf life of the dosage form, one should know the stability aspects of the API in the presence of other components of the formulations[3,4]. The study investigates the compatibility of antibiotic linezolid with Cationic guar, HPG, benzalkonium chloride and boric acid in the preformulation stage by subjecting to differential thermal analysis (DSC) for formulations stored at elevated storage conditions as per ICH guidelines[8]. The study also aims rheological characterization, study of effect of sterilization (moist heat) and effect of aging on the viscosity of *in situ* gels by calculating consistency index (K) and flow behaviour index (n values) using the power law model[9,10]. The developed formulations were subjected to *in vitro* diffusion studies. The optimized formulation was subjected to ocular toxicity study and accelerated stability studies for a period of 6 months according to ICH Guidelines.

MATERIALS AND METHODS

Materials

Linezolid (99.94% drug purity, Cipla Ltd, Mumbai), HPG and Cationic guar (Encore polymers, Ahmedabad) and benzalkonium chloride (Microlabs, Bangalore) were obtained as gift samples. The solvents used for HPLC (S. D. Fine Chemicals) were of AR grade.

Methods

Drug Polymer Compatibility studies[11]

Various blends of linezolid with HPG, Cationic guar, boric acid, benzalkonium chloride, were prepared in the ratio of 1:1 packed in amber colored screw capped glass bottles. The bottles were packed in black canvas and stored in stability chamber (Model TH 90 S, Thermo Lab Scientific Equipments Pvt. Ltd., India) set at 40±2^{0}C /75±5% RH over a period of six months. Sample no.1 (Cationic guar: HPG 1:1) and 2 (Linezolid: Cationic guar + HPG+ β-Cyclodextrin + Boric acid +Benzalkonium chloride blend) were subjected to stability testing according to ICH guidelines[8]. At the end of 6 months, the samples were subjected to differential scanning calorimetry (Model DSC Q20V24.4, Universal Instrument (Germany). DSC of the pure drug was also recorded. The thermograms of the blends were compared with that of the pure drug. The Thermograms for Linezolid, Cationic guar and HPG blend, Linezolid+ HPG+ other adjuvants are shown in Fig. 1.

Optimization of Cationic guar and HPG for *in situ* gelling ability

Various concentrations of Cationic guar alone and Cationic guar (0.125 to 0.75 % w/V) in combination with HPG (0.25-0.5% w/V) were prepared in phosphate buffer pH 7.4. The gum derivative(s) were allowed to hydrate overnight. The dispersions were tested for their *in situ* gelling ability.

The gelling ability was assessed by placing a drop of dispersion in a vial containing 2 ml of artificial tear fluid (ATF), freshly prepared, and maintained at 37^{0}C using a thermostat regulated water bath. The gelling ability was scored in terms of consistency, clarity and ability to flow freely. The composition of ATF employed was: 0.67g of sodium chloride, 0.20g of sodium bicarbonate, 0.008 g of calcium chloride and purified water to make 100 mL.

Preparation of *in situ* gelling ophthalmic formulations

The two main requisites of an *in situ* gelling formulations are, gelling capacity and viscosity build up[11]. The formulation needs to have an optimum viscosity that allows easy instillation of the formulation in the form of drops, which would undergo rapid sol to gel transition in the eye and the gel needs to preserve integrity without eroding for prolonged periods of time[12].

The polymer dispersions were prepared in phosphate buffer pH 7.4. The gum derivative(s) were allowed to swell overnight. Linezolid was added to

an aqueous solution of the cyclodextrin compound with agitation until complete dissolution. Agents for adjustment of osmolality and preservative were added. The solution was added to the polymer dispersion and volume was made up to 100 mL with phosphate buffer pH 7.4.The formulation details of *in situ* gelling systems of linezolid using Cationic guar and HPG is displayed in Table I.

Evaluation of *in situ* gelling ophthalmic formulations:

- **Gelling Capacity:** The procedure as mentioned earlier was followed for assessing gelling capacity of the formulations.
- **pH measurement:** pH of the formulations was recorded using digital pH meter (Model 510, Bench pH Meter, Eutech, India Std.) with special electrode for viscous gels and semisolids. Readings are compiled in Table II.
- **Rheological studies:** The viscosity determinations were carried out using Brookfield viscometer DV-2 model. From the literature, it is evident that in the sol form, the formulation is needed to have a viscosity of 5 to 1500cps. Further, after ion-gel activation in the eye, it is needed to have a viscosity of about 50-50,000cps[12,13]. Based on this viscosity range correct spindle/speed combination was selected from the Brookfield Inc.handbook.The samples were analyzed both at 25^{0}C (room temp) in the sol form and at thermostated temperature of 37^{0}C ± 0.5^{0}C (body temperature) in the gel form (after addition of ATF) by circulating water from a thermostatic water bath connected to the viscometer adapter prior to each measurement. To 10 ml of each of the formulation, approximately 2.8 ml of the ATF was added[14]. The angular velocity of the spindle was increased to 20, 30, 50, 60, 100, 200 and the viscosity of the formulation was recorded. The viscosity values for the sol and the gel form are contained in Table II. The rheograms of CG, CG-HPG1, CG-HPG2, CG-HPG3 in sol and gel form are compiled in Fig. 2 and 3 respectively.
- **Effect of sterilization on viscosity of *in situ* gelling formulations [4,9]**

  The formulations were subjected to sterilization by means of an autoclave at 121^{0}C with a sterilization cycle of 15 min to check the rigours of sterilization on formulations. After sterilization and addition of ATF, the samples were subjected to viscosity measurement at a spindle speed of 30 rpm. Spindle L2 was used for recording the sol reading and L3 was used for recording the viscosity of the gel. The viscosity values before sterilization and after sterilization are contained in Table III.
- **Variation of viscosity upon storage[4]**

  The sterile formulations were stored at 40^{o}C and at 25^{o}C for 1 month and three months respectively. The viscosity was recorded at the end of one month for samples stored at 40^{o}C and at 25^{o}C. Further the viscosity was recorded at the end of three months for samples stored at 25^{o}C.

  Viscosity of non-Newtonian fluids is characterized by more than one parameter and is better expressed in the form of a power law model $\mu a = K (1/n)^n x (4\pi N)^{n-1}$ [9,10]. where μa is the apparent viscosity (Poise), N, the spindle speed (RPS), K is the consistency index and the dimensionless index of flow behaviour. The values of ln (μa) and ln(4ϖN) were fitted into the equation for a straight line. From the slope and intercept values of the linear relationship, the flow behavior index "n" and consistency coefficient "K" were arrived at. The values for flow behavior index "n" and consistency coefficient "K" of freshly formed *in situ* gels, after sterilization, and after storage for 1 month and 3 months at 25^{o}C, after 1 month storage at 40^{o}C is contained in Table IV.
- **Determination of drug content[11]**

  1mL of the formulation was diluted to 25ml with acetonitrile: methanol: water (4:4:2 mixture). From this, 1mL was drawn and diluted to 25mL with acetonitrile: methanol: water (4:4:2) mixture. The absorbance was measured at 254 nm against the reagent blank. Linezolid obeys Lambert Beer's law

in the concentration range of 2-18 mcg/mL. The linear relationship revealed slope value of 0.0505 and intercept value of 0.0291 with a regression value of 0.9997. The drug content was arrived at by making use of the equation for straight line. The results are shown in Table II.

- ***In vitro* drug release Study[14, 15]**

  The *in vitro* release study from *in situ* gels was carried out by using 7 station diffusion cell apparatus (Model EDC 07 Electrolab, India).The dialysis membrane -50 (Himedia) was placed in-between donor and receptor compartment. The formulation(s) was placed in donor compartment and freshly prepared simulated artificial tear fluid of pH 7.4 was placed in the receptor compartment (43 mL capacity).The magnetic bead was set to rotate at 50rpm, the temperature of the medium was maintained at 37 ± 0.5^{0}C. Sample measuring 1 ml was withdrawn at predetermined time intervals of 1 hour for upto 6 hours and each time it was replaced with the same volume of fresh medium (at 37 ± 0.5^{0}C). The withdrawn samples were diluted to 10 ml in a volumetric flask with acetonitrile:methanol:water (4:4:2 V/V) mixture and the absorbance was recorded at 254nm againstthe reagent blank to obtain the linezolid drug content.

  The drug content was obtained by making use of linear equation generated from the calibration curve. The percentage CDR was plotted against time; release profile details are compiled in Fig.4.

- **Kinetic fitment[16]**

  The data obtained from the *in-vitro* diffusion studies was fitted to the kinetic models in order to obtain the order of release *i.*e. Zero order kinetics, First order kinetics, Higuchi model, Krosmeyer and Peppas release model by using PCP-DISSO-V2 software; the best-fit model for linezolid release was arrived at. The results are compiled in Table V.

- **Short term stability studies[8]**

  Stability of a pharmaceutical preparation can be defined as "the capability of a particular formulation in a specific container/closure system to remain within its physical, chemical, therapeutic and toxicological specifications throughout its shelf life."

  Procedure: In the present study, stability studies were carried out at 40^{0}C± 2^{0}C/75% RH± 5% for a period of 6 months for CG-HPG2 formulation. For stability study, the *in situ* gelling solutions were placed in amber colored ampoules and bottles (Type 1 Glass) and sealed with aluminum foil. These sample containers were placed in the stability chamber (Model TH 90S Make: Thermolab Scientific Equipments Pvt. LTD, India) consisting of two chambers. The samples were analyzed at the end of 1, 3, 6 months for the following parameters.

  Physical Appearance[3]: The samples were visually observed for any change in color or appearance.

  pH measurement[3]: pH was recorded using digital pH meter.

  Gelling capacity[13]: Was determined by adding a drop of *in situ* gelling formulation to a vial containing 2ml of freshly prepared simulated tear fluid and subjected to visual observation. The observations are compiled in Table VI.

Rheological evaluation:

The study was carried by using Brookfield viscometer model DV-2. The rheological data was subjected to the power law model to obtain the value of flow behavior index "n" and consistency coefficient "K" of the sample subjected to stability studies.

***In vitro* release studies:**

The study was carried out by the use of 7 station diffusion cell apparatus as explained earlier. The release profile of linezolid from CG-HPG2 formulation subjected to stability study is displayed in Fig. 5.

- ***In vivo* ocular toxicity studies of CG-HPG2 formulation**

  The animal experiment was performed using two Albino rabbits. The animals were quarantined

Table I: Formulation design of *in situ* gelling systems using Cationic guar and HPG

Ingredients (% w/V)	Formulation Code			
	Cg	CG-HPG1	CG-HPG2	CG-HPG3
Linezolid	0.5	0.5	0.5	0.5
β-Cyclodextrin	2.5	2.5	2.5	2.5
Cationic Guar	0.5	0.5	0.5	0.25
HPG	-	0.125	0.25	0.5
Benzalkonium Chloride	0.01	0.01	0.01	0.01
Boric Acid	0.3	0.3	0.3	0.3
Purified Water To Make	100	100	100	100

β- cyclodextrin is added to enhance the solubility of linezolid by forming a complex. The complex formed has greater solubility compared to the pure drug. Benzalkonium chloride is used as a preservative and boric acid is included to adjust the tonicity of the formulation.

Table II: Evaluation of formulations for gelling capacity, pH, viscosity and drug content

Formulation code	Gelling capacity	pH measurement	Viscosity Values at 30 rpm(n=3)(cps units)		Drug Content (%)±SD, n=3
			Sol form	Gel form	
CG	+++	7.39	231.6±2.02	1443.2±3.23	95.251±0.01
CG-HPG1	+++	7.40	1445.7±5.96	2336.53±2.49	96.896±0.001
CG-HPG2	++++	7.39	1312.3±4.87	4038.33±2.15	97.639±0.03
CG-HPG3	+++	7.44	940.3±5.63	2535.16±3.75	90.975±0.01

Table III: Viscosity values (cps) for *in situ* formed gels before sterilization, after sterilization and % viscosity variation values after 1 month, 3 months of storage at 25^{0}C and after 1 month storage at a 40^{0}C recorded at a spindle speed of 30 RPM

Formulation Code	Viscosity values for *in situ* formed gels(cps)	% Viscosity Variation (mean values ±sd, n=3)			
	Before sterilization[a] / After Sterilization[b]	After sterilization t=0	1month T=25^{0}C	3 months T=25^{0}C	1 month T=40^{0}C
CG	1443.2±3.23[a] / 1065.33±2.85[b]	-26.38±0.08	-9.87±1.2	-14.17±2.031	-20.13±0.65
CG-HPG1	2336.53±2.49[a] / 2218±3.65[b]	-5.18±0.05	-2.46±0.15	-4.85±2.128	-12.31±2.63
CG-HPG2	4038.33±2.15[a] / 4404.3±1.95[b]	9.11±0.12	6.56±1.56	8.69±3.189	-4.56±1.02
CG-HPG3	2535.16±3.75[a] / 2154.5±2.31[b]	-14.71±0.13	-8.53±1.02	-10.65±1.325	-14.32±2.38

Table IV: Flow behavior index (n) and consistency coefficient (K) values for freshly formed *in situ* gels, after sterilization, and after storage for 1 month and 3 months at 25^{0}C, after 1 month storage at 40^{0}C

	Formulation Code							
	CG		CG-HPG1		CG-HPG2		CG-HPG3	
	n	K	n	K	n	K	n	K
Freshly formed *in situ* gels	0.7789	8.7158	0.8008	9.3162	0.849	9.8161	0.5973	8.7741
After sterilization	0.6911	8.2311	0.7524	9.6141	0.8575	9.9826	0.6019	8.1397
t=25^{0}C/ 1 month	0.7228	6.5123	0.7924	7.799	0.7679	9.9787	0.7065	7.2709
t=25^{0}C/ 3 months	0.7802	6.4128	0.8198	7.703	0.5729	9.9718	0.7186	7.2084
t=40^{0}C / 1 month	0.8038	6.3695	0.8483	7.003	0.5669	9.8709	0.8017	7.0091

t =time period

Table V: *In vitro* linezolid release data fitting into various mathematical models

Model		Formulation Code			
		CG	CG-HPG1	CG-HPG2	CG-HPG3
Korsmeyer – Peppas	k	0.3411	0.2623	0.2458	0.3092
	n	0.1274	0.1366	0.1087	0.1156
	R	0.9059	0.9855	0.9990	0.9520
Zero order	k	0.0941	0.0767	0.0667	0.0832
	R	0.2652	0.3221	0.1555	0.2351
First order	k	-0.0009	-0.0008	-0.0007	-0.0007
	R	0.2789	0.3895	0.1572	0.2589
Higuchi matrix	k	0.2071	0.1813	0.1537	0.1821
	R	0.8609	0.8995	0.8558	0.8481
Hixson- Crowell	k	-0.0005	-0.0009	-0.0002	0.8666
	R	0.2569	0.3347	0.1566	-0.0013

Table VI: Clarity, pH, gelling capacity, viscosity values after gelation,% viscosity variation and drug content of CG-HPG2 formulation earlier to and after being subjected to stability testing at 40^{0}C(±2^{0}C)/75%(±5%)RH for a period of 1, 3 and 6 months

Time in month(s)	Clarity	pH	Gelling Capacity	Viscosity values after gelation (cps)	% Viscosity Variation (mean values ±sd, n=3)	% Drug content (n=3)
0	Clear	7.3	+++	4038.33±2.15	----	97.639±0.03
1 month	Clear	7.2	+++	3858.31±1.12	-4.56±5.02	95.258±0.03
3 months	Clear	7.4	+++	3749.5±3.15	-6.23±1.87	93.859±0.02
6 months	Clear	7.2	+++	3750.4±2.34	-6.32±0.95	90.231±0.01

Table VII: Ocular irritation, Intra ocular pressure and corneal thickness measurement after 7 day ocular toxicity test / study on rabbits

Sl.no	Formulation Code: CG-HPG2				
1.	**Ocular toxicity by Draize scoring method and examination by Slit lamp technique on 7th day**				
	Animal(s)	LE_C	RE_T	LE_C	RE_T
	Cornea (A)	0	0	0	0
	Iris (B)	0	0	0	0
	Conjunctiva (C)	0	0	0	0
	Chemosis(D)	-	-	-	-
2.	**Intraocular pressure using tonometer* (mm of Hg)**				
	0th Day	8.12	8.19	8.63	9.52
	7th Day	8.95	8.55	8.25	9.02
3	**Corneal thickness (mcm)#**				
	0th Day	380±2.303	375±1.23	380±2.030	379±3.006
	7th Day	380±3.09	379±2.08	376±2.007	374±2.05

LE_c- Left eye control, RE_T- Right eye test.

**Normal values of IOP of rabbits: 7-13 mm of Hg*

#*Normal co*neal thickness: 375-400 mcm

for 1 week prior to dosing. Rabbits were approximately 11 weeks old and weighed 1.9 to 2.5 kg and selected at random. Animals were housed individually in rabbit bracket cages equipped with water and food in an environment maintained at a temperature of 23 ± 1° C, 45–65% relative humidity; exposed to 12-hrs light/dark cycle.

Study Design: 7 day ocular toxicity study: The study was carried out in depth at Dr.M.M.Joshi Eye Institute, Hubli.The following parameters were recorded; intraocular pressure (IOP)[17] (by tonometer, icare), Corneal thickness (Pachymeter SP-3000); conjunctiva, cornea, aqueous humor and iris were visualized for any abnormalities (Slit lamp technique)[18,19]. Ocular irritancy measurement was carried out and scoring done according to Draize test protocol [17,18,19].

Procedure

Baseline study was carried out in the animals prior to administration of the formulations and the above mentioned parameters were recorded. The sterile formulations (40 µL) were instilled twice daily to the left eye of rabbit for a period of seven days (3rd day, washing was carried out with sterile saline before crossover study); the right eye was treated as control and left eye was treated as test site from the same animal. The IOP, conjunctiva, cornea, aqueous humor, iris, anterior chamber flare and corneal thickness were assessed at the end of 0th, 1st, 3rd, 7th day after instillation of the formulations. The observations were scored according to Draize method. Results are compiled in Table VII.

RESULTS AND DISCUSSION

Preformulation Studies

Compatibility studies were carried out by the technique of DSC

The thermograms of cationic guar and HPG, and that of the physical mixture after 6 months of accelerated stability studies, revealed the endothermic peak of linezolid at 175-176°C (according to literature-Melting point of linezolid is 173-181°C). The other peaks obtained were concordant with that of the

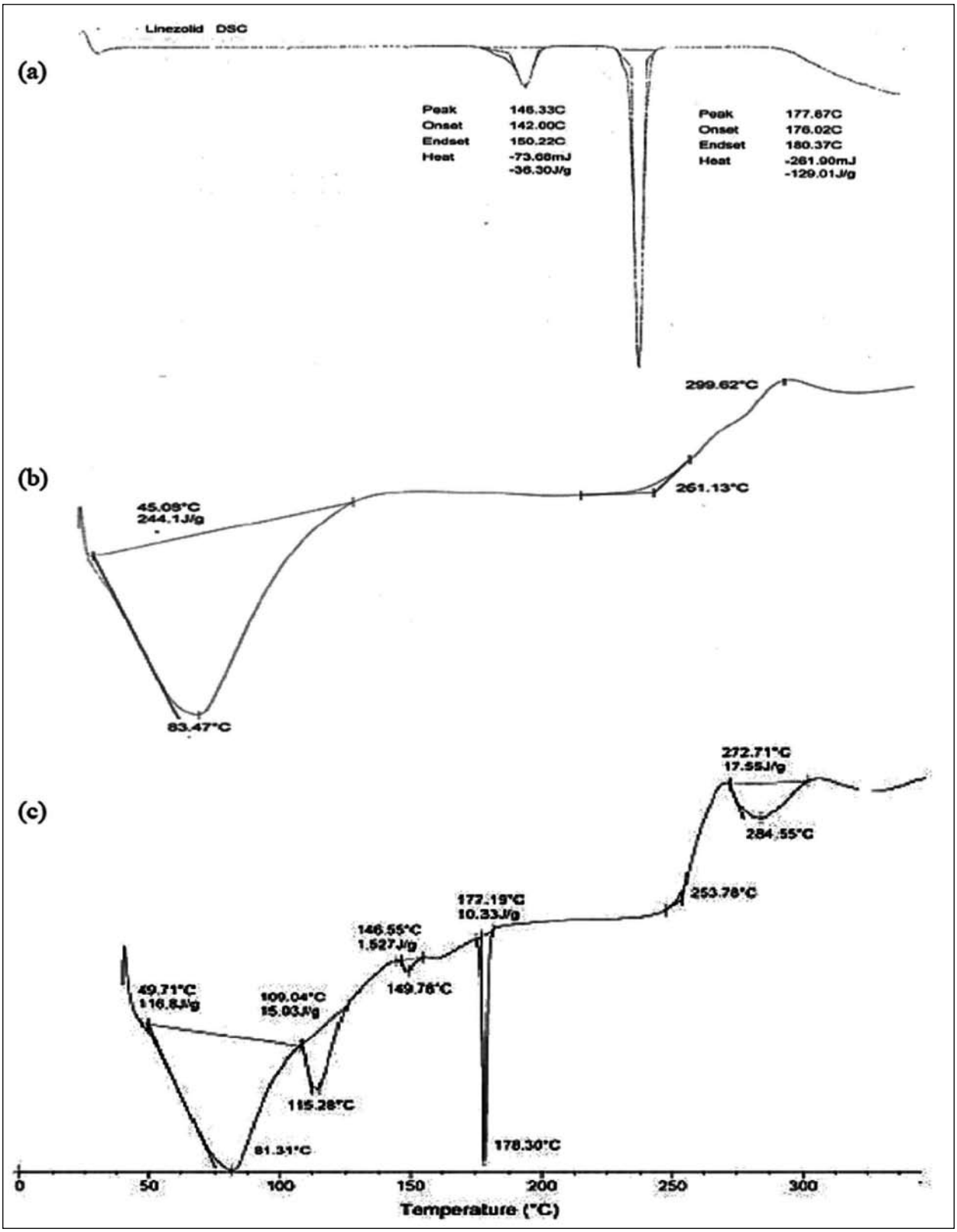

Fig. 1: Thermogram of Linezolid (a), Cationic guar and HPG (b), and that of physical mixture after 6 months of accelerated stability studies (c)

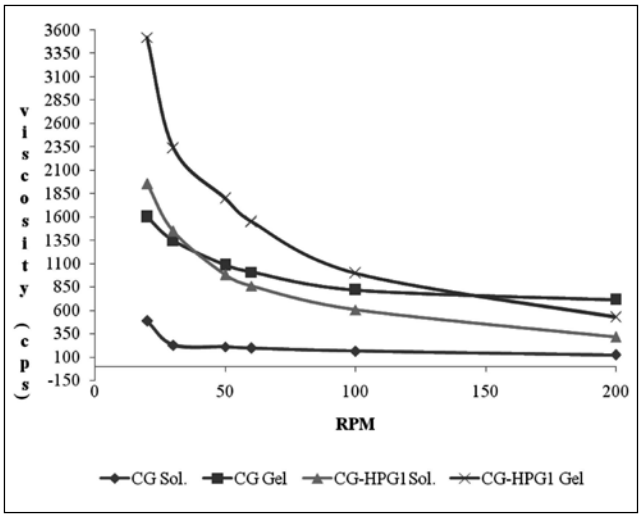

Fig. 2: Rheogram of CG and CG-HPG1 formulations in sol and gel form

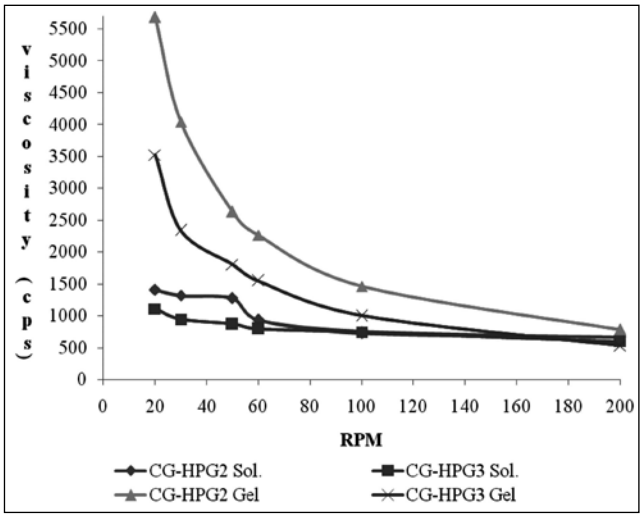

Fig. 3: Rheogram of CG-HPG2 and CG-HPG3 formulations in sol and gel form

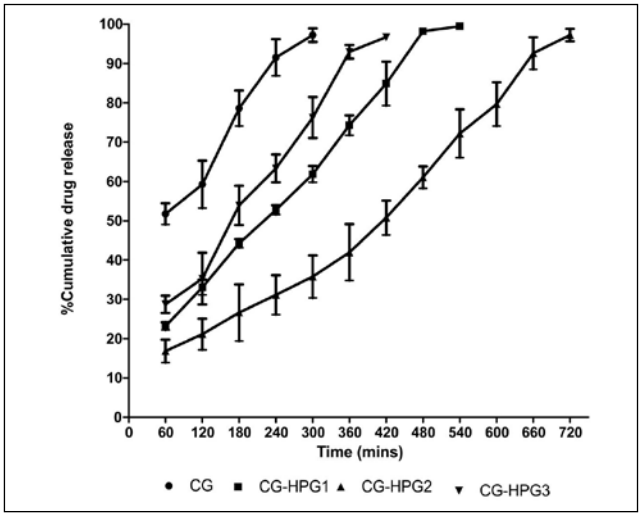

Fig. 4: Comparative *in vitro* release profiles from linezolid in situ gelling formulations as a function of time

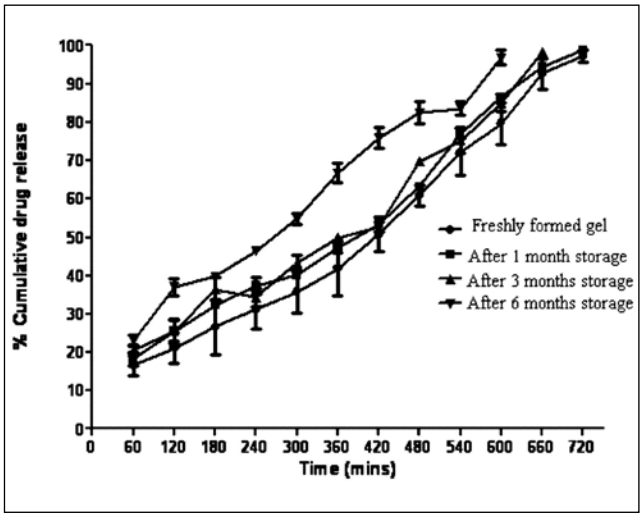

Fig. 5: Comparative *in vitro* release profiles from CG-HPG2 optimized formulations as a function of time after accelerated stability testing

endothermic peaks of individual gum, polymers, and excipients. Hence it was concluded that linezolid is compatible with Cationic guar and all otheradjuvants used in the formulations.

Cationic guar exhibited *in situ* gelling ability in artificial tear fluid. Increasing the concentration of Cationic guar from 0.5 to 0.75%w/V increased the gelling ability; 0.75%w/V dispersion was viscous, resisted flow and as suchomitted from the study. Cationic guar along with HPG exhibited enhanced gelling ability. Cationic guar (0.5%w/V) with increase in strengths of HPG (0.125%, 0.25%, 0.5% w/V) exhibited increased *in situ* gelling ability. Decrease in Cationic guar and increase in HPG concentration decreased the gelling ability and imparted yellow colour to the dispersion. Hence the dispersions which were clear and exhibiting free flowing characteristics were selected for designing the *in situ* gelling formulations.

Formulation design and preliminary evaluation of *in situ* gelling systems

The novel *in situ* gel system was formulated using a system of guar derivatives namely, Cationic guar and HPG.The formulations made were clear with a pH value ranging from 7.39 to 7.44. The gelling capacity remained unaltered after inclusion of the drug and excipients. The formulations exhibited free flowing characteristics making them ideal candidates for instillation to the eyes.

Rheological investigations

The formulations exhibited increased pseudoplastic flow pattern after gelling in ATF. There was 3 to 4 fold increase in the viscosity after gelling. The pseudoplastic nature is very much essential for the *in situ* gelling system to enhance the corneal residence time of the drug. The presence of Cationic guar tends to increase the viscosity of *in situ* gels as a result of increased gum-gum interaction. An optimum ratio of Cationic guar to HPG is needed to maintain the physical stability. As the amount of Cationic guar decreases, the physical stability (viscosity) decreases.

Effect of sterilization on viscosity

CG-HPG2 formulation exhibited slight increase in viscosity after sterilization and storage at 25^{0}C for 1 month and 3 months (8%) and slight decrease in viscosity (3 to 4%). Hence, formulation CG-HPG2 can be construed to have retained the viscosity on sterilization followed by storage at elevated temperature. In case of CG, CG-HPG1 and CG-HPG3, there is slight decrease in viscosity upon storage subsequent to sterilization.

The power law model was used to calculate the rheological parameters *viz:* consistency index (K) and flow behavior index (n). It is evident from the values obtained that CG, CG-HPG1 and CG-HPG3 exhibited increase in flow behavior index and decrease in consistency index subsequent to sterilization and storage at 25^{o}C for 1month and 3 months and at 40^{0}C for 1 month. CG-HPG2 exhibited decrease in flow behavior index and increase in consistency index, revealing an increase in the viscosity of the formulation on storage even at elevated temperature. It is evident from the literature that cationic polymer along with nonionic gum increases the hydration rate of the gum and also increases the gum-gum interaction, as a result increasing the physical stability upon sterilization. Further, the values of flow behavior index (n) were found to be less than unity after sterilization and storage at 25^{o}C for 1 month and 3 months and at 40^{0}C for 1 month indicating shear-thinning behavior (pseudoplasticity) of the formulations[13,14].

Drug release Studies

Fig. 4 shows percentage cumulative amount of drug release *vs* time profile. In case of formulation CG and CG-HPG3, a burst effect was seen and at the end of 6thhr, 92.95% of drug release was obtained (it may be due to less gum-gum interaction). In case of CG-HPG2, the formulation exhibited 50.76% drug release at 7th hr, revealing delayed drug release. Thus an optimum concentration of Cationic guar and HPG is required to retain the drug releasein contrast to the individual gum. Therefore, the results suggest that CG-HPG2 can be utilized as an effective *in situ* gel forming ophthalmic drug delivery system.

Furthermore, the release data was fitted to various mathematical model to assess the drug release pattern; the R^2 value revealed that Korsmeyer-Peppas model was the best-fit model. The diffusion coefficient (n) obtained was in the range of 0.1087-0.1366, release rate constant (k) was in the range of 0.2458-0.3411 and R^2 revealed a value of 0.9059-0.9990.The n value is indicative of Fickian diffusion mechanism.

Selection of optimized formulation

The criterion for selection of optimized formulation was based on *in vitro* release study and physical stability of *in situ* gelling formulation to withstand rigours of sterilization and storage for varied periods of time at lab temperature and at elevated temperature.

CG-HPG2 formulations exhibited a controlled *in vitro* drug release and did maintain physical stability subsequent to sterilization and storage at varied conditions and was therefore selected as the optimized formulation.

CG-HPG2 optimized formulation revealed good clarity, consistent pH and good gelling ability. The formulation revealed good physical stability; fall in viscosity was in the range of 4.56 to 6.32 % subsequent to 6 months of accelerated stability testing. CG-HPG2 formulation exhibited a flow behavior index of 0.8490 in freshly formed gel and a value of 0.7201 subsequent to 6 months accelerated stability testing.The consistency index was found to be 9.7760 prior to stability studies and 9.8663 after 6 months stability testing.From the above data, it can be concluded that the formulation is able to retain its viscosity subsequent to stability testing. The values of n less than 1 revealed the pseudoplastic behavior of the formulation.

As per ICH guidelines, the drug content in CG-HPG2 was determined at the end of I, 3and 6 months in samples stored at 40 ± 2°C/ 75%RH ±5%RH. The decline in drug content was in the range of 7-8%. CG-HPG2 formulation remained stable even after exposure to stressed conditions. The *in vitro* drug release study revealed similarity in the release pattern prior to and after being exposed to stability studies. CG-HPG2 formulation exhibited drug release profile without any burst effect and the drug release was extended upto 12 hrs in samples stored for 1 month. The drug release was extended upto 10 hrs in samples stored for 6 months; this may be due slight decrease in viscosity of the formulation but the formulation retained the sustained release without burst effect. The *in vitro* release profile for CG-HPG2 formulation as a function of time after accelerated stability testing is compiled in Fig. 5.

Ocular toxicity studies

Seven day ocular irritation study was carried out on rabbits. The baseline study was conducted out and both the eyes of the rabbits were tested for any abnormalities. Corneal thickness and intraocular pressure (IOP) of both the eyes were measured. Rabbits were assigned to receive the formulations. A crossover study was designed; while the right eye was treated, the left eye served as the control; both the eyes responded to light and no ocular lesions were observed. Conjunctiva was normal without any redness; iris was normal without any congestion or swelling. Cornea appeared without any ulceration, oedema or opacity. The total Draize score was found to be zero which revealed non irritant property of the formulation.The IOP values recorded for the left eye control as well as for the right test eye at the end of 0^{th} day and 7^{th} were within the normal range. Similarly the corneal thickness measured on the 0^{th} day and the 7^{th} day for both the control eye and the test eye were within the normal limits.

CONCLUSION

Linezolid, a broad spectrum antibacterial agent used in the treatment of ocular infections, was successfully formulated as an ion-triggered *in situ* gel forming eye drops comprising of Cationic guar and HPG. Good physical stability following sterilization and storage maintaining the flow behavior index and consistency index values makes CG-HPG2 formulation a promising novel guar gum derivative based *in situ* gelling formulation. The gel formed *in situ* yielded sustained release, wherein, 50% of release was seen at the end of 8^{th} hr. Stability data recorded over a 6 months period under accelerated temperature conditions indicated the formulation to be stable. Therefore, it could be concluded that by virtue of its prolonged corneal residence time and sustained drug release, the developed *in situ* gelling formulation is a viable alternative to the conventional eye drop formulation with assured enhancement in bioavailability.

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