SHORT COMMUNICATION

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Design, fabrication and evaluation of clotrimazole loaded polymeric microsphere based in situ ophthalmic gel

Manish Kumar Gupta[1], Vipul Sansare[*1], Birendra Shrivastava[1], Santosh Jadhav[2]

[1]School of Pharmaceutical Sciences, Jaipur National University, Jaipur- 302017, India
[2]SVPM'S College of Pharmacy, Malegaon, Maharashtra- 413115, India

Article History:

Received on: 05 Jan 2021
Revised on: 10 Feb 2021
Accepted on: 22 Feb 2021

Keywords:

Clotrimazole,
Corneal fungal infection,
In situ gel,
PLGA,
Polymeric microsphere

ABSTRACT

The present study was started with aim to design clotrimazole loaded polymeric microsphere based *in situ* ophthalmic gel for management of corneal fungal infections. The clotrimazole loaded microsphere was fabricated using various percentage of polylactide-co-glycolide polymer by solvent evaporation technique and evaluated for particle size, zeta potential and encapsulation efficiency. The polyvinyl alcohol was used as stabilizer. The formulation batch which showed good particle size and entrapment efficiency was selected for further study. The *in situ* ophthalmic gel of optimized drug loaded microsphere was formulated using various ratios of sodium alginate and hydroxy propyl methyl cellulose. The formulated gel was evaluated with respect to pH, *in vitro* gelling capacity, clarity, viscosity, *in vitro* antifungal activity and *in vitro* transcorneal permeation behavior. The fabricated drug loaded microspheres revealed acceptable particle size, zeta potential and encapsulation efficiency. The prepared *in situ* gels were clear and exhibited acceptable pH, rheological properties and *in vitro* gelation. The drug loaded microsphere based gel revealed superior *in vitro* antifungal activity against *Aspergillus niger* than conventional formulation and sufficient drug permeation across goat cornea. Thus, formulated clotrimazole loaded microsphere based *in situ* gel based systems can be a promising approach for sustained ophthalmic delivery of antifungal agents.

*Corresponding Author

Name: Vipul Sansare
Phone: +91-8692930764
Email: avipulsansare@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v12i2.4617

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INTRODUCTION

Fungal eye infection is extremely rare but major cause of ocular morbidity. Mycotic eye infections are commonly caused by yeast Candida albicans which is the most common cause of endogenous Endophthalmitis (Klotz *et al.*, 2000). The fungi such as *Fusarium solani* and *Aspergillus flavus* are also responsible for one-third of all traumatic infectious keratitis. Endophthalmitis is an infection of the inside of the eye i.e. the vitreous and/or aqueous humor whereas keratitis is an infection of the cornea of eye (Müller *et al.*, 2013). Clotrimazole (CLZ), 1-[(2-chlorophenyl)diphenylmethyl]-1H-imidazole is well known antifungal drug containing imidazole ring. Conventional ophthalmic drug administration involves local administration of antifungal drug by topical route (Mohanty *et al.*, 2015). However conventional ophthalmic drug delivery is challenging due to barrier properties of cornea and conjunctiva. The unique anatomy and physiology cornea presents constant challenge for formulators to circumvent the protective barriers of eyes. In addition

to this, poor ocular residence time of drug due to its loss by lacrimal fluid and nasolacrimal drainage are another hurdles for ocular drug delivery (Kaur *et al.*, 2008).

Various strategies like use of nanocarriers, *in-situ* ophthalmic gelling systems, improving rheological properties of systems were investigated and reported in literatures to increase the ophthalmic bioavailability of drugs by prolonging precorneal residence time of drugs (Das and Suresh, 2011). The water soluble polymers were reported to increase precorneal residence time and improve permeation of drugs across the cornea which eventually improves ocular bioavailability of drugs (Kakkar *et al.*, 2015).

Ophthalmic *in-situ* gelling systems contain environmentally sensitive polymers that will be structurally altered with the small alteration in parameters like pH, ionic strength and temperature in the environment (Wu *et al.*, 2019). *In-situ* ophthalmic gel are homogeneous polymeric solution before instillation into the eye; after instillation into eyes the system undergoes sol to gel transition in the ocular cavity to form viscous gels in response to change in environmental condition and gradually releases the drug in ocular cavity.

The present short communication focused on formulation and evaluation of CLZ encapsulated poly(lactic-co-glycolic acid) (PLGA) polymeric microsphere based *in situ* ophthalmic gel to prolong precorneal residence time of drug. The drug loaded microsphere were fabricated and loaded in *in situ* ophthalmic gel. The microsphere based gel showed acceptable gelling capacity, clarity, pH and rheological characteristics. The *in vitro* antifungal activity and *in vitro* permeation study revealed promising results.

MATERIALS AND METHODS

Materials

Clotrimazole (CLZ) was kindly gifted by Glenmark Pharmaceutical, Mumbai, India. Soya lecithin S-90 and S-100 were kindly gifted by Lipoid, Germany. Hydroxy propyl methyl cellulose, sodium alginate, Tween 20, Tween 80 and polyvinyl alcohol were purchased from SDFCL, Mumbai, India. PLGA was purchased from Sigma Aldrich, India. All other reagents and solvents were analytical grade and purchased locally.

Methods

Preparation and evaluation of CLZ loaded PLGA microspheres

The Clotrimazole encapsulated PLGA microspheres were fabricated by solvent evaporation technique using various ratios of drug and polymer (Garud and Garud, 2012). Briefly weighed quantity of drug and polymer were dissolve in dichloromethane (Table 1). The prepare organic phase then slowly added in aqueous dispersion medium containing 0.5% w/v polyvinyl alcohol with continuous stirring at 1000 RPM for 1 hr. The prepared microsphere based dispersion then centrifuged at 5000 RPM to separate unentrapped drug and redisperse in aqueous medium containing stabilizer. The prepared microspheres based dispersion evaluated with respect to particle size, zeta potential and encapsulation efficiency.

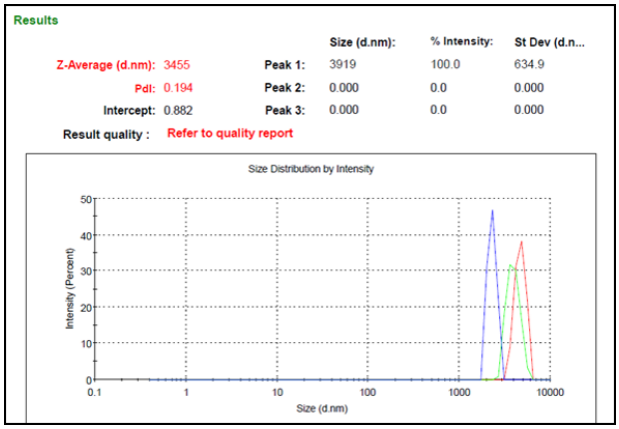

Figure 1: Particle size distribution of CLZ loaded microsphere (F3)

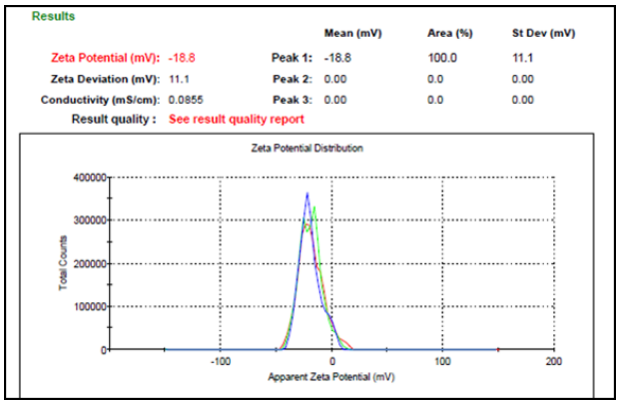

Figure 2: Zeta potential of CLZ loaded microsphere (F3)

The particle size and zeta potential of drug loaded microsphere were measured using Zetasizer Nano ZS (Malvern, Worcestershire, UK).

The encapsulation efficiency was measured as the percentage of CLZ entrapped in the microsphere. The percentage entrapment efficiency of CLZ in the CLZ loaded microsphere was determined using the indirect method. The CLZ loaded microsphere dispersions were subjected to ultra-centrifugation at

Table 1: Formulation of CLZ loaded microspheres

Formulation code	Drug: polymer ratio	Drug (mg)	Polymer (mg)	Dispersion medium volume (ml)	Stabilizer PVA (%w/v)
F1	1:1	250	250	20	0.5
F2	1:2	250	500	20	0.5
F3	1:3	250	750	20	0.5
F4	1:4	250	1000	20	0.5
F5	1:5	250	1250	20	0.5

Table 2: Formulation and characterization of microspherebased *in situ* gel

Formulation code	Polymer: copolymer ratio	HPMC (mg)	Sodium alginate (mg)	Dispersion medium volume (ml)
IOG 1	1:1	250	250	50
IOG 2	1:2	250	500	50
IOG 3	1:3	250	750	50
IOG 4	–	250	—	50
IOG 5	–	—	250	50

Table 3: Coding system used to study *in vitro* gelling capacity of prepared *in-situ* ophthalmic gel

Observation	Coding
No gelation	-
Gelation occur in few minute and retain for few hours	+
Gelation occur immediately and retain for few hours	++
Gelation occur immediately and retain for extended hours	+++
Very stiff gel	++++

Table 4: The results of CLZ loaded microsphere evaluation

Formulation code	Drug: polymer ratio	Particle size (nm)	Entrapment efficiency (%)	Zeta potential (mv)
F1	1:1	2870 ± 11.4	52.71 ± 1.4	-19.7 ± 3.14
F2	1:2	2789 ± 14.17	58.25 ± 2.41	-22.6 ± 2.83
F3	1:3	3455 ± 15.82	65.90 ± 2.17	-18.8 ± 2.74
F4	1:4	5467 ± 14.73	67.82 ± 3.46	-38.8 ± 1.64
F5	1:5	8915 ± 10.51	70.17 ± 2.43	-45.6 ± 1.47

Table 5: The gelling capacity, viscosity, clarity and pH of microsphere based *in-situ* ophthalmic gel

Formulation code	Polymer: copolymer ratio	Gelling capacity	Viscosity before gelation	Viscosity after gelation	Clarity	pH
IOG 1	1:1	+ +	535.40±2.14	847.83±2.69	Transparent	6.55±1.47
IOG 2	1:2	+ +	642.17±1.76	1130.81±3.75	Transparent	6.58±2.51
IOG 3	1:3	+ + +	837±2.78	1521.97±3.61	Transparent	6.53±2.16
IOG 4	–	-	224.5±2.67	376.54±4.51	Transparent	6.49±2.73
IOG 5	–	+	137.41±3.71	179.51±4.61	Transparent	6.60±2.59

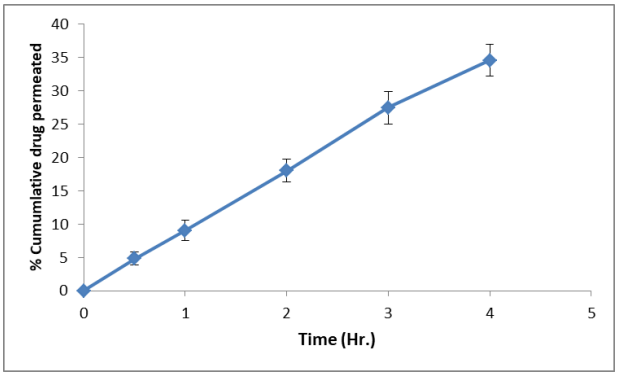

Figure 3: The *in vitro* permeation behavior of CLZ across goat cornea

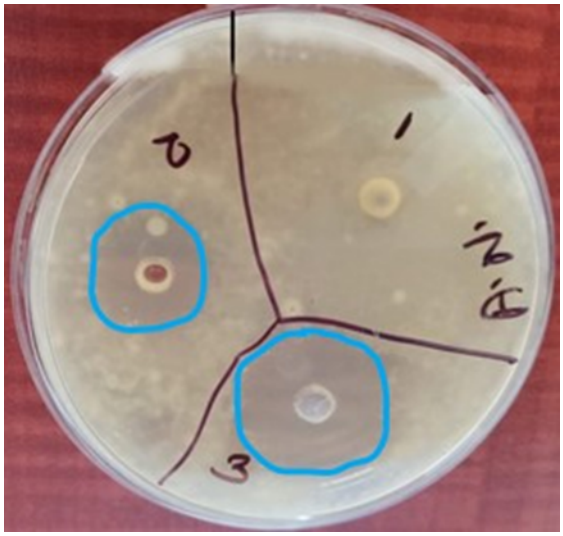

Figure 4: *In vitro* antifungal activity of 1. Vehicle control 2.Marketed formulation 3. *In-situ* gel against *Aspergillus niger* showing zone of microbial growth inhibition

80,000rpm for 1 hour at 4°C using Optima Max XP ultracentrifuge (Beckman Coulter, U.S.A.) to separate the unentrapped drug. The pellet of microsphere was formed at the bottom. The aqueous phase above the pellet (i.e., the supernatant) was carefully separated and measured. This required amount of aqueous phase (supernatant) was appropriately diluted and analysed by UV spectrophotometry (V-530, Jasco, Japan) at λmax of 243 nm.

Preparation of drug loaded microsphere based *in-situ* gel

Microspheres based *in-situ* ophthalmic gel was prepared using various ratios of HPMC K4M and sodium alginate as polymer and copolymer respectively (Makwana *et al.*, 2016). Different ratios of polymers were dissolved in 50 ml water with heating and continuous stirring (Table 2). Once clear homogeneous polymer solution is obtained the resulting solution cool to room temperature. The CLZ microspheres equivalent to 0.5 gm of CLZ (1%w/v) were dispersed and pH of solution adjusted to 6.5 using 0.1 N HCl.

Evaluation of microsphere based *in-situ* gel

The prepared drug loaded microsphere based *in-situ* gel was evaluated with respect to *in vitro* gelling capacity, clarity, rheological characteristics, pH determination, *in vitro* drug permeation behavior and *in vitro* antifungal activity.

The *in vitro* gelling capacity of the formulated gel was measured by placing a two drops of the prepared formulation in a petri plate containing 10 molar calcium chloride solution and was observed for gelation (Makwana *et al.*, 2016). The gelation behavior of prepared gel was recorded using scoring system as mention in Table 3.

Clarity of formulated gel was observed by visual observation of *in situ* gel against a black and white background, with the continuous swirling action.

The rheological characteristic of *in gel* governs precorneal residence time of drug. Brookfield Viscometer model DVII was used to measure viscosity of formulated gel before and after gelation. The pH of prepared gel was measured using calibrated pH meter.

The permeation of CLZ across goat cornea from the optimized IOG3 formulations was measured out using Franz diffusion cell (Meditech Technologies India Pvt. Ltd.) with 1.76 cm^2 diffusion area. The goat eye was obtained from local slaughterhouse; cornea was removed and used as membrane for assessment of permeation behavior of drug (Khames *et al.*, 2019). The microsphere based gel equivalent to 5 mg of CLZ was placed in donor chamber, and goat cornea was placed in between donor and receptor compartment. The simulated tear fluid of pH 7.4 was selected as a diffusion medium in the receptor chamber. The temperature of diffusion medium was adjusted to 32 ± 0.5°C with the constant stirring at 500 rpm. At periodic intervals the aliquot of diffusion medium was withdrawn and analyzed for drug content using UV spectrometer (V-530, Jasco, Japan) after dilution. The permeation of clotrimazole from IOG 3 formulations were assessed by plotting the graph of cumulative drug permeated (Q) per unit area as a function of time.

The antifungal activity of the prepared microsphere based *in situ* gel formulations was assessed against standard strain of *Aspergillus niger* using agar well diffusion technique. Three samples i.e. Vehicle control (distilled water), marketed CLZ formulation and Prepared CLZ microsphere based gel were selected for antifungal activity. Muller-Hinton agar was used as growth medium for growth of fungus. The petri

plates containing sterile growth medium was prepared and fungal culture was inoculated to produce 1×10^6 CFU/mL. Wells of 12 mm diameter were made in the agar plates after solidification of growth medium. Each well was filled with 200 μL of each sample and incubated at 37°C for 24 h. The inhibition zone diameter was measured using a caliper and compared. The absence of fungal growth around the well indicated fungal growth inhibitory activity of drug.

RESULTS AND DISCUSSION

Preparation and evaluation of CLZ loaded microsphere

The CLZ loaded microspheres were successfully fabricated using solvent evaporation technique. The particle size and zeta potential of drug loaded microsphere were measured using Zetasizer Nano ZS (Malvern, UK). Table 4 displays particle size, zeta potential and encapsulation of drug loaded microspheres. The particle size and zeta potential of formulated microspheres were in the range of 2870-8915 nm and -19.7- -45.6 mV respectively. An increased average particle size was observed with increase in drug: polymer ratio. This could be attributed due to increase in amount of polymer in dispersion medium which could be difficult to wet by stabilizer. The high negative value indicated better stabilization of microspheres in dispersion medium.

The CLZ entrapment efficiency in polymeric microspheres was in the range of 52.71 – 70.89%. The encapsulation efficiency was observed to be increase with increase in polymer concentration. This could be attributed due to availability of more concentration of polymer to encapsulate CLZ. The optimized microsphere dispersion (F3) was used for formulation of *in situ* ophthalmic gel using various ratios of polymer and copolymer. The particle size and zeta potential of optimized formulation (F3) are represented in Figures 1 and 2 respectively.

Evaluation of microsphere based *in-situ* ophthalmic gel

The *in vivo* performance of *in situ* gelling system is governed by two major characteristics i.e. gelling capacity and rheological characteristics (Makwana *et al.*, 2016).

The *in situ* gelling system formulated using different ratios of HPLC and sodium alginate was assessed for gelling capacity and viscosity. Gelling capacity of prepared *in situ* gel formulations are highlighted in Table 5. According that IOG 3 revealed immediate gelation and for extended period of time. Thus formulation IOG 3 with acceptable consistency was considered for further studies. All the prepared batches of *in situ* gel were clear with absence of turbidity and impurities. Newtonian flow before gelling and pseudoplastic flow after gelling was major findings of rheological studies. There was increase in the viscosity after gelling. The pH of *in situ* gel was found to be in range of 6.49–6.60 which was acceptable for ophthalmic drug delivery (Table 5). The formulation IOG3 has pH 6.53, which confer its suitability for ophthalmic administration.

In present study, the permeation of CLZ across goat cornea was assessed to evaluate the effectiveness of the prepared formulation. Effective drug permeation across cornea along with the acceptable antifungal activity are major characteristics of effective ocular antifungal drug delivery system (Khames *et al.*, 2019). The transcorneal permeation study revealed acceptable drug permeation across goat cornea from *in situ* gelling system as represented in Figure 3. The study was performed for 4 hrs due to viability of cornea. The encapsulation of CLZ in PLGA polymer matrix provided sustained drug release. Loading of drug encapsulated microspheres into the *in situ* gelling vehicle represented another factor that could assist in sustained drug release.

The zone of fungal growth inhibition is represented in Figure 4. The marketed CLZ formulation (sample 2) showed zone of inhibition 17 mm whereas microsphere based *in situ* gel (sample 3) showed zone of inhibition 20 mm. Thus developed microsphere based *in situ* gelling system exhibited superior antifungal activity as compared to marketed formulation. However *in-vivo* antifungal activity is necessary to assess in order to prove efficacy of developed formulation.

CONCLUSIONS

Polymeric microsphere based *in-situ* gel of CLZ was successfully developed using solvent evaporation technique to overcome drawbacks associated with conventional ophthalmic drug delivery and for ophthalmic drug delivery for extended period. The selected technique was simple, reproducible and rapid; which led to the formation of microsphere with acceptable drug entrapment efficiency. The diverse drug-polymer ratio revealed varied particle size, zeta potential and entrapment efficiency. Among the all prepared drug loaded microspheres, the F3 formulation was selected for further study on the basis of its superiority in terms of particle size, zeta potential and entrapment efficiency. The microsphere based *in-situ* gel was formulated to improve precorneal residence of drug. The micro-

sphere based gel showed acceptable gelling capacity, clarity, pH and rheological characteristics. The *in vitro* antifungal activity and permeation study revealed promising results. Thus, microsponge based *in-situ* gel developed and investigated in present study was seems to be promising with respect to management of ocular fungal infections and to improve precorneal residence time of CLZ.

Acknowledgement

Authors would like to say thanks to Department of Pharmaceutics, Bombay College of Pharmacy, Mumbai (India) for excellent technical support. We are thankful to Lipoid (Germany), Department of Nanoscience, University of Mumbai (India), Punjab University (India) and Central Institute of Research on Cotton Technology (India) for technical assistance.

Conflict Of Interest

The author declares no conflict of interest.

Source Of Funding

There is no funding source or financial assistance for present study.

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