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Designing of dexamethasone sodium phosphate ocular films for madras eye: *In vitro* and *in vivo* evaluation

Hindustan Abdul Ahad[1]*, Haranath Chinthaginjala[1], Pradeepkumar Bhupalam[2], Rahul Raghav Dasari[1], Bitraganti Samhitha Rao[1] and Ksheerasagare Tarun[1]

[1]Department of Industrial Pharmacy, Raghavendra Institute of Pharmaceutical Education and Research, KR Palli Cross, Ananthapuramu, AP, India

[2]Department of Pharmacology, Raghavendra Institute of Pharmaceutical Education and Research, KR Palli Cross, Ananthapuramu, AP, India

Abstract: The main intention of the current investigation was to fabricate ocular films of Dexamethasone sodium phosphate (DSP) impregnated in rate controlling membrane and to characterize *in vitro* and *in vivo (iv-iv)*. DSP release was regulated by HPMC K4M and ethyl cellulose (EC) and dimethyl sulfoxide (DMSO) as a permeability enhancer. DSP suitability with polymers was observed by DSC and FT Infrared spectroscopic readings. The fabricated DSP ocular films were examined for physicochemical tests, *in vitro* discharge and *in vivo* infusion in rabbits. The improved formulation (F-8) was proved its stability under stressed storage conditions. The fabricated ocular films process acceptable physical characters with DSP release in a controlled manner. The optimized DSP films were intact even in stressed storage situations. It was concluded that the fabricated films effectively hold the DSP at the programmed site for the desired period of time and exhibit expected pharmacodynamics actions.

Keywords: Ocular, film, delivery, controlled.

INTRODUCTION

Dexamethasone sodium phosphate (DSP) is a corticosteroid prescribed to tackle conjunctivitis (madras eye) is used in the present investigation and is of BCS (biopharmaceutical classification system) Class-I with high water solubility and permeation. These drugs easily drain by lachrymal secretions. So, a requisite for the controlled release of the medicament in an appropriate manner (Pepose, *et al.,* 2018).

Old-fashioned ocular delivery viz., drops/ointments have pitfalls like recurrent dose, diminished accessibility, huge and erratic amounts and gutter by nasolacrimal fluids (Tiwari *et al.,* 2018). Ocular films (ophthalmic inserts) are sterile medications of various sizes of solid/semisolid material for the ophthalmic drive (Obiedallah *et al.,* 2018). They majorly contain a supportive polymeric layer incorporated with a drug (s). The essayists in the existing exploration primed DSP ocusert for controlled delivery.

MATERIALS AND METHODS

Materials

Dexamethasone sodium phosphate was gift samples from Torrent Pharmaceuticals, Mumbai, India. HPMC K4M and Ethyl Cellulose were procured from Fischer scientific chemicals, India. Double distilled water (DDW) was used All additional chemicals and solvents were of the AR mark.

**Corresponding author:* e-mail: abdulhindustan@gmail.com

Preparation of Ocular films

The designing of ocular films tangled in 3 diverse paces (Abdul *et al.,* 2011).

Preparation of DSP Film

The polymeric DSP films were made by these steps (fig. 1).

Designing Rate-Controlling Membrane (RCM)

The steps convoluted in making rate modulating membrane was demonstrated in fig. 2.

Sealing

The serial steps in the sealing of films were mentioned in fig. 3.

A sterile condition during this processing was upheld using laminar airflow. The composition of films was denoted in table 1 and revealed in fig. 4.

Evaluation of Polymeric films

Suitability findings

The suitability of DSP with the excipient used was examined by DSC (temperature elevation 10^{o}C/min; Venchal scientific-412105-New York).

Physical Description

The films were assessed for their physical constraints and illustrated below.

Thickness of Film

A Vernier caliper (Mitutoyo 530-312) was employed for gauging the chunkiness of 5 films and mean determined.

pH approximation

The films were allowed to swell in water (1ml) kept in Petri cups at room temperature (27±1°C) for 1h. The swollen film was detached and sited beneath cardinal pH meter (Shimadzu) and surface pH determined (Dang *et al.,* 2018).

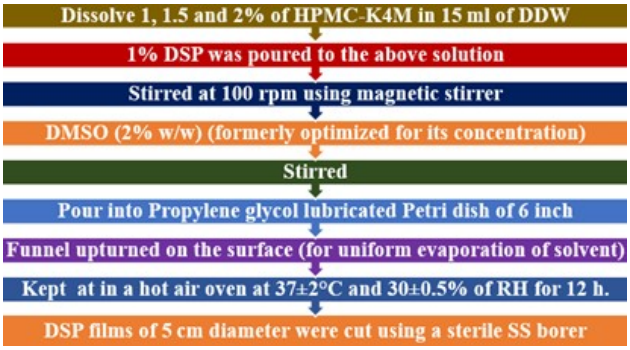

Fig. 1: The steps involved in preparing DSP films

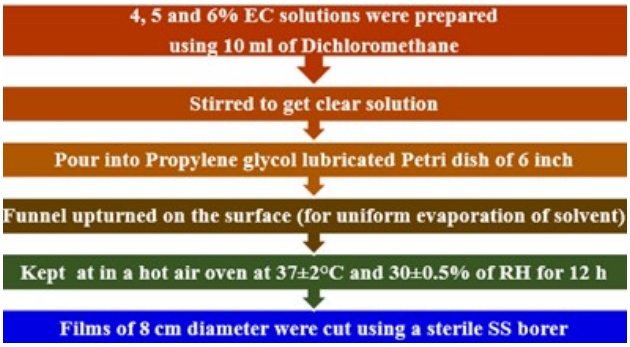

Fig. 2: Steps in making rate-controlling membrane

Uniformity of Weight

An electronic balance (Shimadzu) was employed for measuring the weight of 3 patches (1×1 cm^2) from each formulation.

Folding Endurance

Ocular films were cut evenly cut into strips and independently folded at a single area until it disrupts which indicates the folding endurance (Ahad *et al.,* 2009).

Percentage of Moisture Absorption

The physical integrity of ocular films can be checked by determining moisture absorption (Morrison and Khutoryanskiy, 2014). The prepared DSP films were balanced and located in a desiccator (100ml of saturated Aluminium chloride solution which was maintained at 80.1% RH. 3 days later the DSP films were detached and weighed. The % moisture absorption was measured by the eq. 1.

$$\% \text{ moisture absorption} = \frac{\text{Difference in Final to initial weight}}{\text{Initial weight}} \times 100 \quad (1)$$

% Moisture Loss

The veracity of the ocusert at dry conditions was determined by percent moisture loss. DSP ocuserts were balanced and situated into a desiccator (Anhydrous Ca Cl_2). Later of 3 days, the ocuserts were removed and weighed. The % moisture loss was determined by eq. 2 (Preethi and Prashanth, 2017).

$$\% \text{ moisture loss} = \frac{\text{Difference in Final to initial weight}}{\text{Initial weight}} \times 100 \quad (2)$$

Swelling Index (SI) of the films

The films were coated on the back with EC (to dodge nudging to the dish) later weighed (W_1) and located markedly in Petri dishes of 25 ml distilled water and crammed in a room environment. After 0.5, 1, 2, 3, 4, 5 and 6h, the ocuserts were detached and the surplus water adhered was omitted with blotting paper. The enflamed ocuserts were weighed (W_2) and the % of the bulge was determined by equation 3 (Dixon *et al.,* 2018).

$$\text{SI} = \frac{\text{Difference in Final to initial weight}}{\text{Initial weight}} \times 100 \quad (3)$$

The films used for the approximation of SI were utilized for estimation of its superficial pH with universal pH paper.

Uniformity in DSP Content

The ocular films were placed in pH 7.5PBS (5ml) and were shaken at 50 rpm (orbital shake and incubator) to extract the DSP from the device. Later the above was clarified using a 0.45µ filter, then the filtrate was properly diluted with PBS and the absorbance was restrained at 254 nm (Zhan *et al.,* 2018).

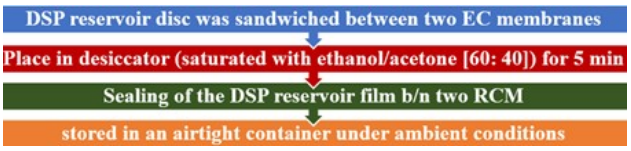

Fig. 3: Steps involved in sealing of ocuserts

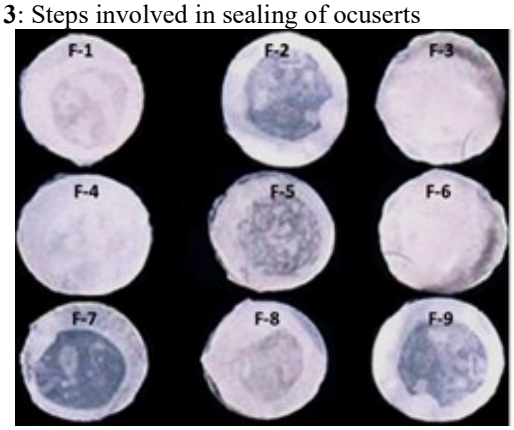

Fig. 4: Various formulations of ocuserts

Microbiological findings

The ocuserts (control &placebo) were assessed for the bacteriological investigation to confirm the anti-bacterial activity. Nutrient agar seeded with the microbes (*S. aureus*) was permitted to coagulate in the Petri plate. An upheaval was detached from the container and sensibly

kept on the agar layer at an appropriate aloofness. The dishes were gestated at 37±0.5°C for 24h. Finally, the zone of inhibition (ZOI) was sedated around the film (Watters *et al.,* 2017).

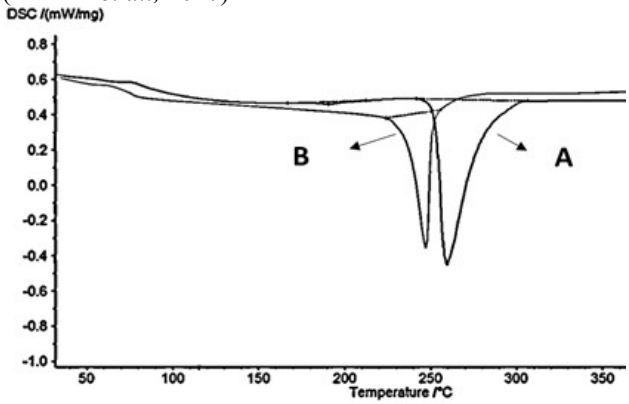

Fig. 5: DSC thermos grams A) DSP B) DSP- excipient blend

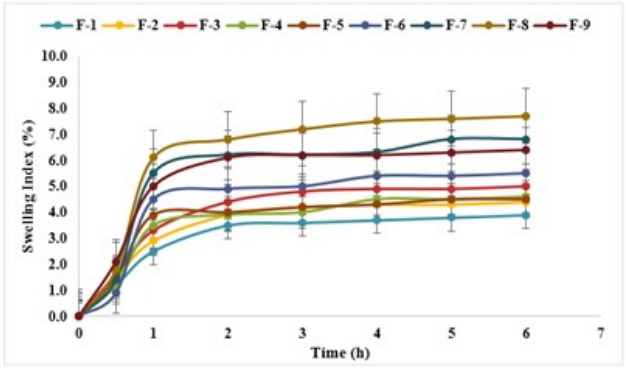

Fig. 6: Swelling behaviour of ocuserts

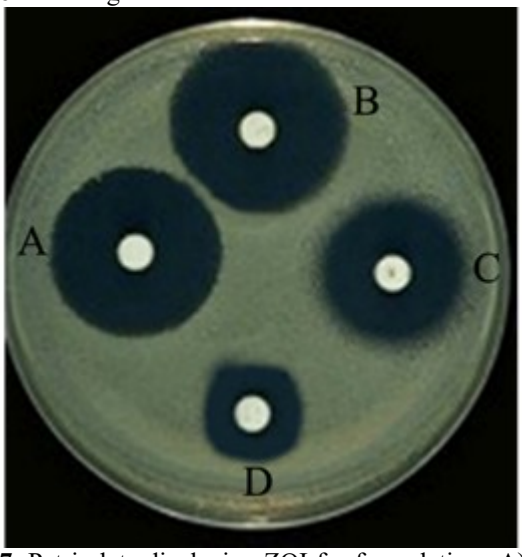

Fig. 7: Petri plate displaying ZOI for formulations A) F-5 B) F-8 C) F-7 D) F-3

Sterilization of prepared Films

These films were sterilized by revealing both sides to UV radiation for 90 min in a LAF and were lastly boxed in pre-sterilized aluminium foil. The absence of microbes was confirmed by the sterility test. The prepared film was aseptically (under LAF) transferred to the fluid. The films were preserved in two different sterile nutrient agar Petri plates. Among them, one is impregnated with *S. aureus* (positive control) and the other un-inoculated (negative control) to test sterility of the medium. These Petri dishes were gestated at 37°C for 24h (Kim *et al.,* 2018).

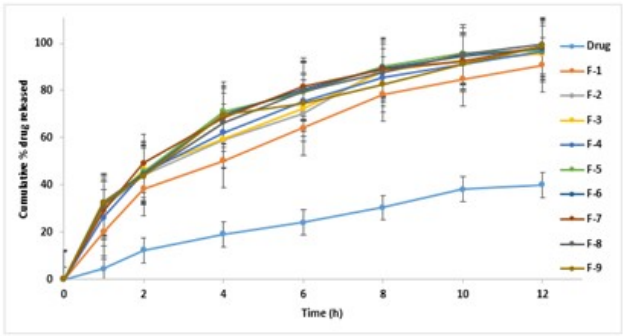

Fig. 8: *In vitro* DSP release

In vitro DSP release

The films were put in 15ml (10ml of pH 7.5PBS) vials. The vials were located in a dithering water bath at 32±1°C with 25 vacillations per min. 1ml of the DSP discharging media was introvert at several time breaks of 0, 2, 4, 6, 8, 10 and 12h by maintaining the sink conditions. Later clarified using 0.45µ membrane filter, diluted with PBS and DSP was estimated by double beam UV-Vis spectrophotometer (Elico SL 210, Mumbai, India) at 254 nm. The acquired data was further assessed by mathematical kinetic modelling (Mahajan and Deshmukh, 2015).

In vivo DSP release study

Patches from F-8 have further analysed pre-clinically based on physical parameters and *in vitro* DSP release. The ocuserts were exposed to UV radiation (1h) before pre-clinical evaluation. After sterilization, the films were moved into polyethylene sack by forceps. Later the films were determined for DSP content and they were endangered to test in rabbits by following strict protocol (CPCSEA Reg. no. 878/ac/05/CPCSEA/004/2015; IAEC #RIPA-18-29).

New-Zealand strain rabbits (weighing 2-2.5kg of both gender) were held on separate coops and adapted to laboratory settings for a day (diet and water were given). The ocular films were kept in the inferior conjunctival cul-de-sac of the 7 rabbits and parallel on the other eye (control).

The films were detached cautiously at 0, 2, 4, 6, 8, 10 and 12 h hand assessed for DSP content as dilution stated in DSP amount uniformity. The DSP residual was deducted from the original DSP of films which gives the quantity of DSP unconfined in the rabbit eye. Any removal of the films during the study was also documented during the experiment. After a week of the washed period, the

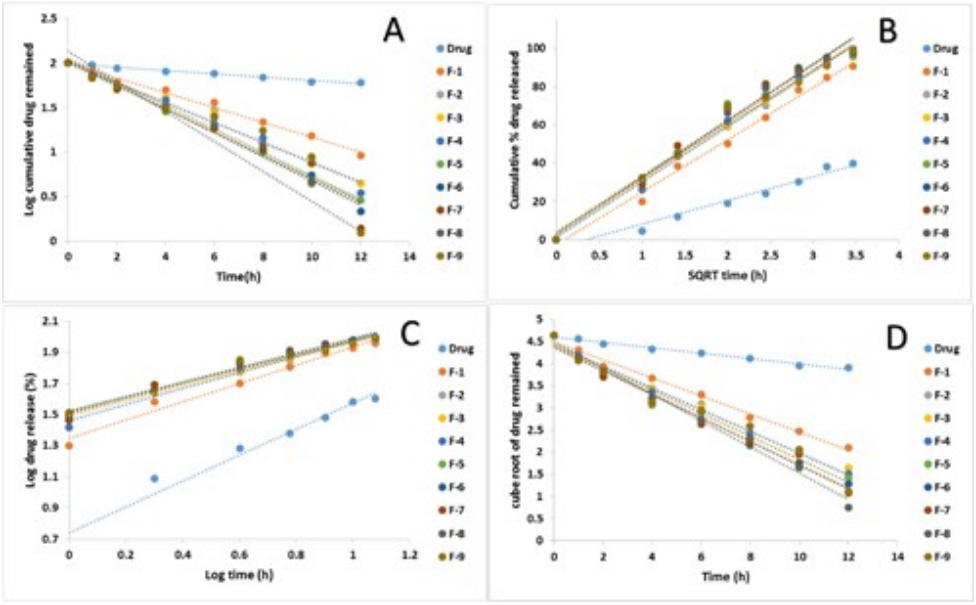

Fig. 9: DSP release plots A) First order B) Higuchi C) Korsmeyer Peppas D) Hixson Crowell

Table 1: Composition of various ocuserts

Ingredients (%w/v)	Formulations								
	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F-9
Dexamethasone sodium phosphate	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
HPMC-K4M	0.25	0.25	0.25	0.50	0.50	0.50	0.75	0.75	0.75
EC	0.25	0.50	0.75	0.25	0.50	0.75	0.25	0.50	0.75
DMSO	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20

Table 2: Physicochemical evaluation of different formulations.

Formulation	Physicochemical parameter						
	Thickness (mm)	pH	Weight (mg)	Folding endurance	Moisture absorption (%)	Moisture loss (%)	Drug content (%)
F-1	0.18±0.004	7.9±0.13	21.2±0.91	85±3	4.5±0.51	7.5±0.25	89.6±1.25
F-2	0.19±0.002	7.4±0.12	25.6±0.84	86±1	4.6±0.12	7.6±0.11	84.5±1.36
F-3	0.20±0.005	7.3±0.28	24.8±0.51	89±3	4.8±0.23	7.8±0.21	91.5±0.56
F-4	0.18±0.007	7.2±0.34	25.1±0.16	78±2	4.9±0.15	7.1±0.03	92.7±1.29
F-5	0.16±0.001	6.7±0.47	21.3±0.35	79±1	4.2±0.21	8.0±0.15	94.5±2.02
F-6	0.17±0.006	7.3±0.19	22.5±0.42	84±4	4.6±0.22	7.6±0.04	88.9±1.68
F-7	0.19±0.005	7.4±0.22	24.9±0.57	91±5	4.7±0.18	7.9±0.21	89.9±0.94
F-8	0.18±0.010	7.8±0.25	22.6±0.68	98±2	4.8±0.26	8.1±0.38	94.9±0.99
F-9	0.19±0.008	7.3±0.33	23.5±0.44	92±1	4.5±0.11	7.9±0.14	91.5±1.94

values in mean ± S.D; trials (n) = 3

testing was recurrent two times as before (Pramanik *et al.*, 2018).

Ocular irritation

The possible ocular annoyance and/or destructive possessions of the films under test were inspected by detecting them for any soreness, swelling, or augmented tear creation. Films were tested on 5 rabbits by placing the ocuserts in the cul-de-sac of the left eye. The rabbit's eyes were checked for any marks of annoyance earlier treatment and pragmatic up to 12h (this study was conducted while testing *in vivo* tribunals) (Verstraelen *et al.,* 2017).

Accelerated stability studies

The generation of the shelf life of the films can be estimated by accelerated stability observations. In the present investigation films of the batch, F-5 & F-8 were selected for selected and crammed in amber-coloured decanters firmly tilled with cotton and stopped (Hindustan

Table 3: Slope and regression values of ocuserts

Formulation	Kinetic order					
	zero	First	Higuchi	Korsmeyer Peppas		Hixson Crowell
	R^2	R^2	R^2	n	R^2	R^2
F-1	0.9269	0.9920	0.9628	0.5884	0.9802	0.9613
F-2	0.8878	0.9809	0.9699	0.4737	0.9908	0.9588
F-3	0.8812	0.9877	0.9704	0.4723	0.9879	0.9397
F-4	0.8795	0.9848	0.9602	0.4693	0.9759	0.9583
F-5	0.8430	0.9929	0.9681	0.5134	0.9779	0.9463
F-6	0.8491	0.9899	0.9616	0.4608	0.9817	0.9231
F-7	0.8416	0.9494	0.9683	0.4776	0.9660	0.9196
F-8	0.8732	0.8915	0.9503	0.4781	0.9901	0.9083
F-9	0.8618	0.8910	0.9152	0.4477	0.9826	0.9269

Table 4: Physicochemical evaluation of different formulations.

Formulation	Folding endurance		Moisture absorption (%)		Moisture loss (%)		Drug content (%)	
	Before	After	Before	After	Before	After	Before	After
F-5	79±1	78±1	4.2±0.21	4.2±0.35	8.0±0.20	8.0±0.15	94.5±2.02	94.4±1.55
F-8	98±2	97±2	4.8±0.26	4.7±0.05	8.1±0.74	8.1±0.38	94.9±0.99	94.9±1.97

values in mean ± S.D; trials (n) = 3

et al., 2016). They were unprotected to stress storage circumstances (40°C/75% RH for 6 months). At fixed intermissions, the films placed 5ml of pH 7.5 PBS and dazed in an orbital shaker incubator (50 rpm) to mine the DSP from the films. After 24 h, the solution was clarified using a 0.45 µ filter and the filtrate was properly diluted with PBS and the absorbance was restrained at 254 nm.

Ethical approval

Animal study was approved by the institutional animal ethical committee of Raghavendra Institute of Pharmaceutical Education and Research and CPCSEA of India. (Ref. No. CPCSEA Reg. No. 878/ac/05/ CPCSEA/004/2015; IAEC #RIPA-18-29).

STATISTICAL ANALYSIS

The confidence and statistical conclusions for getting correct, and precise values were achieved by statistical analysis of the data using MS Excel-2016 software (assessed and expressed by mean ± SD [standard deviation]) for the physicochemical parameters, DSP release kinetics, and physical constraints before and after stability studies. The DSP release kinetics were analysed by fitting the dissolution data to several kinetic models.

RESULTS

The compatibility of DSP and its combination with polymers employed when investigated with DSC analysis (fig. 5). The physicochemical description of DSP films was exemplified in table 2. The swelling of the films was elaborated on fig. 6.

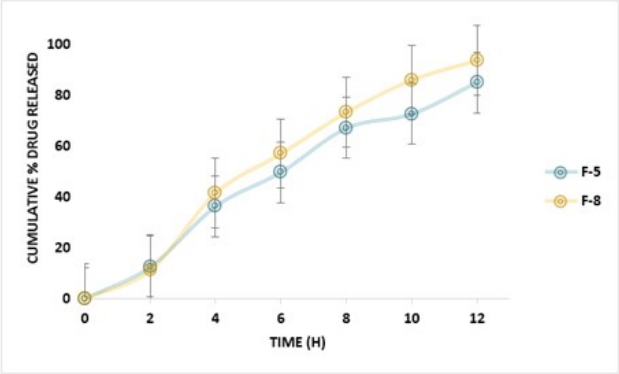

Fig. 10: *In vivo* DSP release in rabbit's eye

Microbiological studies

The optimized film (F-8) presented good antimicrobial properties than the control (fig.7).

In vitro DSP release

Cumulative DSP release for ocuserts was exemplified in fig. 8. The kinetic plots revealed that was clarified in fig. 9. For *in vivo* DSP release, films F-5 and F-8 were as they have uniform DSP content and good *in vitro* DSP release (fig. 10). Stability data exposed that the films were stable and demonstrated in table 4.

DISCUSSION

Compatibility observations

The thermogram of DSP showed one predominant endothermic peak of fusion with a peak maximum of 261.01°C, with onset fusion at 252.09°C. This curve was also found in DSP blend with excipients without any

additional endothermic peak. This gives the impression that DSP is compatible with the excipients used.

Observations of physical characteristics

The chunkiness of the ocuserts was constant (0.16±0.001 to 0.20±0.005 mm) which represents the evenness of all the prepared films. The petite difference was experiential with formulation F-3 owing to the additional amount of RCM. The readings of regularity in mass were observed between 21.2±0.91 to 25.6±0.84 mg which shows the consistency of the fabricated films. The developed ocuserts (F-1 to F-9) revealed decent uniformity in weight without any deviation and were within acceptable limits. Subsequently, the loss of moisture from the films presented no variation in reliability and ranged from 7.1±0.03to 8.1±0.38%, which confirms the ocuserts stability. The gaining of moisture was extended from 4.2±0.21to 4.9±0.15% for films (F-1 to F-9) due to the increased quantity of the HPMC-K4M. The maximum moisture absorption was noticeable from films F-4; this is owing to the existence of medium amounts of the hydrophilic polymer HPMC-K4M and low levels of EC. The folding endurance was ranged between 78±2.31to 98±2 and devoid of crashes was seen in all prepared ocuserts. Films (F-7, F-8 and F-9) exhibited extreme folding fortitude owing to higher amounts of HPMC-K4M. The ocuserts were observed to have evenness in DSP content without any deviation. The prepared films, F-2 showed the least DSP (84.5±1.36%) and films, F-8 showed the uppermost DSP content (94.9±0.99%). The pH on the surface of all the films was observed from 6.7±0.47 to 7.9±0.13, indicates the compatibility of ocuserts to the lachrymal fluids. All these values were illustrated in table 2.

Swelling index

The DSP ocuserts showed immediate swelling in the first hour followed by maintaining the swelling for the next 5h. The swelling is due to increased concentrations of HPMC-K4M.

Sterility test

The microbial growth was observed in positive control and no growth was observed when subjected to incubation, which established that all the apparatus/glassware used in the test were germ-free and clean circumstances were upheld. Later the patches were placed in the -ve control and incubated at the same conditions. There was no development of microbes, which confirms the sterility of the films.

Microbiological Studies

Clear zones of inhibition (ZOI) were achieved in formulation F-3, F-5, F-7 and F-8 against *S. aureus*. Anti-microbiological ability was assessed on the ocusert. As per the observation (fig. 7) in the first 3 days ocusert presented excellent bustle with proper ZOI. Among them ocuserts F-8 showed a good ZOI of 4.5 cm.

In vitro DSP release

Cumulative DSP release for the films F-5 and F-8 were observed to be 96.84% and 97.71% correspondingly at the 12th h. The *in vitro* release of DSP films spoken by Higuchi's model and best fitted to it with chief linearity (R^2) ranged from 0.9152 to 0.9704. Hixson Crowell's plot revealed that the regression values were in the range of 0.9083 to 0.9613. To approve the diffusion appliance, the data were tailored to the Korsmeyer-Peppas equation (fig. 9C), the films disclose good linearity (R^2) ranged from 0.9660 to 0.9908, with a slope (n) values >0.45, representing that diffusion were the leading mechanisms of DSP release from the films. The release profile of DSP from the prepared ocuserts exhibited very poor fitting with the Hixson-Crowell cube root model of drug release (fig. 9D).

In vivo

Based on the uniform DSP content and good *in vitro* DSP release, the ocuserts (F-5 and F-8) were subjected to *in vivo* studies in rabbits. The ocuserts were reserved in the rabbit eye throughout the study. The DSP release of F-5 and F-8 films were found to be 36.52% and 41.84% at the end of 4th h, 65.25% and 72.11% at the end of 8th h and 84.96% and 93.74% at 12th h, which ensures the prolonged release of DSP from the ocuserts and helps in the treatment of conjunctivitis (madras eye).

Stability studies

Stability data revealed that no major changes were noticed in the physicochemical parameters of ocuserts (F-5 and F-8) which ensures the developed films were stable even at stressed storage conditions for 6 months (table 4).

CONCLUSION

The trial made to develop DSP ocuserts was successful for amended bioavailability, evasion of recurrent admin and reduced dose. From the investigational discovery, it can be clinched that HPMC is a good film making hydrophilic polymer and is a gifted agent for ocular delivery. Ethyl cellulose was an acceptable polymeric ingredient to construct the rate regulating membrane of the ocusert system. The amalgamation of DMSO improves the permeability of DSP and thus therapeutic levels can be attained. The kinetic handling of *in vitro* data selected that the ocusert obeyed non-Fickian release. *In vivo* DSP emission represents that DSP release was less associated with *in vitro* DSP discharge and there was ample nonappearance of rabbit's eye annoyance and soreness. An additional future effort will be advanced to launch the therapeutic use of these systems by pharmacological findings.

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