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Novel Nanostructured Lipid Carrier Based Flurbiprofen Loaded Sodium Alginate Inserts for Ocular Drug Delivery

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***SUMMARY*. The aim of the present study was to develop a novel Flurbiprofen (FLB) loaded nanostructured lipid carrier (NLC) based alginate inserts for treatment of ocular inflammation. 0.3% FLB loaded NLCs were prepared by means of high shear homogenization and afterwards 0.75% sodium alginate was added into these NLCs. Glycerin or PEG 400 at 5% concentration was added to NLCs as plasticizers and by using solvent casting evaporation technique, inserts were developed. Inserts were evaluated for diameter, thickness, weight uniformity, drug content, moisture absorption and moisture loss. Also *in vitro* release and stability studies were performed. The characterization properties of inserts were acceptable for ophthalmic application. The inserts developed with the addition of glycerin ($Ins1_{FLB}$) were found as optimum formulation for FLB *in vitro* release. FLB loaded NLC based inserts developed with sodium alginate and glycerin may be offered as appropriate vehicles for ocular delivery.**

RESUMEN. El objetivo del presente estudio fue desarrollar un nuevo vehículo lipídico nanoestructurado (NLC) de flurbiprofeno (FLB) a base de insertos de alginato para el tratamiento de la inflamación ocular. Se prepararon NLCs cargados con 0,3% de FLB por medio de homogeneización de alta cizalladura y después se agregó 0,75% de alginato de sodio dentro de estos NLCs. Se añadió glicerina o PEG 400 al 5% a los NLCs como plastificantes y se desarrollaron los insertos mediante el uso de la técnica de evaporación del disolvente. Los insertos fueron evaluados por diámetro, espesor, uniformidad de peso, contenido de fármaco, absorción y pérdida de humedad. También se realizaron estudios de liberación y estabilidad *in vitro*. Las propiedades de los insertos fueron aceptables para aplicación oftálmica. Los insertos desarrollados con la adición de glicerol ($Ins1_{FLB}$) resultaron la formulación óptima para la liberación *in vitro* de FLB. Los insertos basados en NLCs cargados de FLB desarrolladas con alginato de sodio y glicerina pueden ser ofrecidos como vehículos apropiados para su aplicación ocular.

INTRODUCTION

Ocular drug delivery has been a main challenge for researchers due to its inimitable structure. Most of these challenges are anatomical and physiological barriers that normally protect the eye from detrimental effects. Furthermore, numerous pre-formulation and formulation factors have to be considered while designing an ophthalmic formulation [1]. In clinical applications the anterior chapter of the eye can be treated with topical solutions as the most frequently used dosage formulation in ophthalmic treatment [2]. Unfortunately the eye solutions (eye drops) are quickly drained from the eye surface and, thus, the time for drug absorption is only a few minutes and bioavailability is very low, typically less than 5% [3,4]. Concentration of drug available in the pre-corneal area acts as a driving force for its passive diffusion across cornea. Nonetheless, for effective ocular drug delivery with solutions, high corneal permeation with longer drug/cornea contact time is necessary [5].

In order to overcome anatomical and physiological barriers and improve ocular bioavailability, various conventional and novel drug delivery systems have been developed [5]. Enhanced retention (mucoadhesion) on the eye surfaces, sustained release, and permeation enhancement

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can be considered as novel strategies that have been applied for designing of optimized ophthalmic formulations [6]. Formulations as hydrogels, micro- and nanoparticles, liposomes and collagen shields have been investigated for ocular drug delivery [7,8]. Nanotechnology based systems with an appropriate particle size can be designed to ensure low irritation, adequate bioavailability, and ocular tissue compatibility. Nanoparticles represent a promising candidate for ocular drug delivery because of small size leading to low irritation and sustained release property avoiding frequent administration. It has been shown that ophthalmic drug delivery may benefit from the characteristics of nanotechnology-based drug delivery systems especially solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) [9,10]. However, like aqueous solutions, nanoparticles may be eliminated rapidly from precorneal pocket [11]. Moreover, additional needs in this field are required to improve patient's and doctor's compliance [2]. Ocular inserts are one of the new classes of drug delivery systems, which are gaining worldwide praise for their ability to release drugs at a preprogrammed rate for a longer period by increasing the pre-ocular residence time [12]. In addition each insert can be prepared to contain a definite dose which is fully retained at the administration site, contrary to solutions that can be improperly instilled by the patient and are partially lost after application [13].

One of the most common conditions in ophthalmic problems is the ocular inflammatory disease affecting any part of the eye or the close tissues [14,15]. Ocular inflammation is a common side-effect associated with ophthalmic surgery, producing pain and photophobia in many patients and potentially leading to serious complications including increased intraocular pressure, posterior-capsule opacification, cystoid macular edema, and decreased visual acuity [16,17]. Nonsteroidal anti-inflammatory drugs are a heterogeneous group of compounds with different structural classes, which do not include a steroid nucleus, derived biosynthetically from cholesterol, in their chemical structure. Also known as COX inhibitors based on their mode of action, nonsteroidal anti-inflammatory drugs are important modulators of ocular inflammatory reactions [18]. Flurbiprofen (FLB), a water insoluble drug (2.70 × 10^{-2} mg/mL at 25 °C) with high lipophilicity (log P = 4.24) [6], is currently used as a first choice ophthalmic medication for the inhibition of miosis induced during the course of cataract surgery [7,19]. In addition it is a potent inhibitor of prostaglandin synthesis [20].

The aim of this study was to develop novel FLB loaded NLC based alginate inserts for ocular application for the treatment of ocular inflammation and evaluate their potential for sustained ophthalmic delivery. The physicochemical characterization, *in vitro* release, sterility of these formulations was evaluated. In order to detect destabilization phenomena stability studies were also undertaken.

MATERIALS AND METHODS

Materials

FLB was purchased from Sigma Aldrich (Madrid, Spain). Compritol HD5 ATO was a kind gift from Gattefosse (France). Tween 80 was obtained from Merck (Germany). Sodium alginate, oleic acid, glycerin, PEG 400 and sodium acetate were purchased from Sigma (Germany). High pressure liquid chromatography (HPLC) grade acetonitrile (Sigma, Germany) were used for ultra HPLC (U-HPLC). All the other chemicals and solvents were of analytical or HPLC grade. Ultrapure water was obtained from Sartorius 61316 pro VF, Germany.

Preparation of FLB loaded NLC based inserts

Preparation of NLC

NLCs were prepared with high shear homogenization technique [10]. The aqueous phase (Tween 80 and ultrapure water) was poured into the lipid phase (Compritol and oleic acid) under homogenization at 24000 rpm for 5 min by Ultra Turrax (T25) at 70-80 °C. Subsequently, particles were dispersed in 10 mL ultrapure water at 4 °C. Unloaded NLC consisted of oleic acid (1.47 %), Compritol (0.73 %), Tween 80 (0.73 %) and ultrapure water (97.07 %). For FLB loaded NLC, FLB was added to lipid phase at a concentration of 0.3 % (w/v).

Preparation of inserts

NLC based inserts were prepared with a novel solvent casting evaporation method. Firstly, 60 mL NLC was prepared and then inserts were developed from these NLCs. To obtain inserts, sodium alginate (0.75 % w/v) was added into the prepared NLC formulation and mixed with 5% glycerin (Ins1) or 5% PEG 400 (Ins2) as plasticizers under stirring conditions at room temperature. After proper mixing the mixture was poured on a glass petri dish and allowed to evaporate at 40 °C for 36 h in an oven. The ob-

tained films were cut by a circular molder into circular pieces of definite size. The ocular inserts were then stored in an airtight container (desiccator) under ambient condition.

NLC characterization

The average particle size, polydispersity index (PDI) and zeta potential of NLCs were evaluated by photon correlation spectrometry (Nano ZS, Malvern Instruments, U.K.). The particle size and PDI values were obtained by calculating the average of five measurements at an angle of 173° at 25 °C using disposable cells. The zeta potential was calculated from the electrophoretic mobility using the Helmholtz–Smoluchowski equation under an electrical field of 40 V/cm.

The encapsulation efficiency (EE) of FLB in NLC was determined by measuring the concentration of free drug in the dispersion medium, *i.e.* aqueous phase. The non-encapsulated FLB was separated by filtration/centrifugation. One mL of FLB loaded formulation was placed in the dialysis bags (Spectro/por Dialysis Membrane, molecular weight of 12-14 kDa) and placed in a centrifuge tube having 9 mL ultrapure water inside and centrifuged for 1.5 h, at 55000 rpm (Beckman Coulter L100 XP–USA) at 25 ± 2 °C. The water phase was filtrated through a membrane filter (0.2 µm Nylon, Milipore Millex-GN). The sample was diluted and the amount of the FLB in the aqueous phase was estimated by a U-HPLC method.

The U-HPLC system consisted of a gradient pump, thermo-stable column department and a PDA detector (Thermo Scientific). The column was a Thermo Hypersil Gold C18 column (1.9 µm, 50 × 2.1 mm). The mobile phase consisted of 0.1 M sodium acetate solution (pH 6.3) and acetonitrile (70:30) (v/v). The flow rate was 0.3 mL/min and the column temperature was maintained at 25 °C. The method was validated partially with respect to system suitability for linearity, limit of detection (LOD) and quantitation (LOQ), precision, accuracy and specificity, selectivity and stability at 248 nm.

Insert characterization

Inserts were evaluated for diameter, thickness uniformity, weight uniformity, drug content, moisture absorption and moisture loss.

Diameter/thickness

The inserts have been shaped by the aid of a PVC mold (0.7 cm diameter) to apply into the lower eye lid. The diameters and thicknesses of the inserts were measured with electronic vernier calipers (Mitutoyo – Japan) with a sensitivity of 0.01 mm. Ten measurements were carried out on each insert.

Mass uniformity

Uniformity of prepared inserts was detected by weighing individually of ten randomLy selected inserts from each formulation batch using a digital balance (Sartorius Basic, Germany).

Drug content

To evaluate FLB homogeneity of inserts, drug content studies were performed. Ten inserts were grounded separately in a glass pestle mortar and dissolved in 100 mL acetic acid solution (5 %) and mixed with a horizontal shaker (Schüftelfreguenz Kühner B, Germany) for 12 h. Then this solution was filtered through cellulose acetate membrane (0.45 µm). The drug concentration was determined by U-HPLC. The averages of results were determined. Experiments were performed at 25 ± 2 °C.

Determination of moisture loss

The ocular inserts were weighed accurately and kept in desiccators containing anhydrous calcium chloride. After 3 days, the inserts were taken out and weighed again [21]. The percentage moisture loss of inserts was calculated according to Eq. [**1**]

$$\%\ moisture\ loss = \frac{initial\ weight - final\ weight}{initial\ weight}\ x\ 100 \qquad [\mathbf{1}]$$

Determination of moisture absorption

The ocular inserts were weighed accurately and kept in stability cabinets at 40 °C with relative humidity of 75 %. After 3 days, the inserts were taken out and weighed again [22]. The percentage moisture absorption of inserts was calculated according to Eq. [**2**]

$$\%\ moisture\ absorption = \frac{final\ weight - initial\ weight}{initial\ weight}\ x\ 100 \qquad [\mathbf{2}]$$

Surface imaging

To visualize the surface structure, the surface images of FLB loaded inserts were taken with stereomicroscope (Leica M205C, UK) and were photographed digitally.

***In vitro* release studies**

In vitro drug release experiments were performed for 8 h using the vial method [23]. One insert was placed in 6.5 mL receptor media. The system was held at 32 ± 0.5 °C to mimic conditions of eye surface, stirred continuously with magnetic stirrer at 150 rpm. The samples were

taken at every hour during 8 h. FLB was determined by the validated U-HPLC method at 248 nm. Sink conditions were maintained in the receptor compartment during *in vitro* release studies. To maintain sink conditions of the receptor compartment pH 7.4 PBS/ethanol 70/30 (v/v) was used as receptor phase. The experiment was carried out six times.

Sterilization and evaluation of sterility

Inserts were sterilized under a UV lamp (273.7 nm) (Philips TUV 15w/G15t8 uv, Holland) for 24 h. To find out the presence of viable forms of microorganisms in the preparation step sterility of inserts was checked.

To control the sterility of inserts, they were disintegrated in the brain heart infusion fluid and 100 µL of each formulation was inoculated on eosin methylene blue agar medium and blood agar medium. 100 colony forming units of *E. coli* suspension was prepared by using standard culture of *E. coli* (ATCC 8739). Then this suspension was seeded on the same agar medium plates. All plates were incubated for 48 h at 37 ± 0.5 °C. After 48 h the media were examined and the results were evaluated by bacteria colony counting.

Stability

Stability studies were performed for inserts according to the International Conference on Harmonization (ICH) guidelines. FLB loaded inserts were stored at 4 ± 1 °C in the refrigerator and 25 ± 2 °C (relative humidity 60 %) and 40 ± 2 °C (relative humidity 75 %) for 3 months in the stability cabinets (Nüve ID 300, Turkey). FLB content, physical appearance and sterility were evaluated for inserts. The experiments were repeated five times.

Statistical evaluation

Statistical differences were determined using ANOVA followed by Tukey's test for comparisons between groups. The significance level was taken as 95 % ($P < 0.05$).

RESULTS AND DISCUSSION

Nanoparticles have the ability to deliver ocular drugs to specific target sites and hold potential to revolutionize the treatment of various eye diseases. Especially NLC carrier systems exhibit an excellent tolerability for ocular application [24]. Nevertheless, efforts are still necessary in terms of improving the drug delivery efficiency, especially, for prolonging the retention time of drug on the corneal surface. Therefore in current study, inserts were developed from NLC system to improve patient compliance with an enhanced therapeutic effect of FLB.

Formulation and characterization studies

In this study, compritol and oleic acid were selected as the solid and liquid lipid for NLC preparation that FLB has better solubilization. It was also noted that these liquid and solid lipids were suitable for ophthalmic use [25,26]. In addition the produced NLC system was modified with sodium alginate which was coded as N2 (Table 1).

Sodium alginate, a water soluble biodegradable polymer, is commonly used for ocular applications [27-29]. NLCs were evaluated about particle size and PDI values which points out the homogeneity of particles. The characterization of FLB loaded NLCs ($N1_{FLB}$ and $N2_{FLB}$) are given in Table 1. When N1 was modified with sodium alginate, particle size was increased 59 % and PDI was changed from 0.135 to 0.306. This might be due to increment of viscosity of the nanoparticles. When 0.75 % (w/v) sodium alginate which is a gel forming agent was added to the N1 formulation, the viscosity of the formulation showed an increment as 5.3 cP. Zeta potential values of nanoparticles found -3.7 mV and -8.8 mV. Similarly Jitendra et al. reported that chloramphenicol loaded nanoparticles formulat-

Components	NLCs		Parameter	NLCs	
	N1	N2		$N1_{FLB}$	$N2_{FLB}$
Oleic acid	1.47 %	1.46%	PS (nm)	242 ± 15.8	384 ± 29.61
Compritol	0.73 %	0.72%	PDI	0.135 ± 0.01	0.306 ± 0.01
Tween 80	0.73 %	0.72%	ZP (mV)	-3.7 ± 0.08	-8.8 ± 0.27
Ultrapure water	97.07 %	96.35%	viscosity(cP)	13.9 ± 0.003	19.22 ± 0.02
Sodium alginate	-	0.75%	EE %	86.1 ± 0.15	83.06 ± 0.25

Table 1. Characterization parameters of FLB loaded NLCs.

ed using sodium alginate showed the negative surface charge of -2 mV [30].

Encapsulation efficiencies of FLB loaded NLCs were determined as 83-86 %. For particle homogeneity of NLCs PDI values were checked and these values were determined smaller than 0.3 with narrow size distribution (Table 1).

There has been a growing interest in using bioadhesive inserts in the ocular cul de sac for enhanced or prolonged localized drug delivery [29]. In our previous study NLC based ocular inserts were prepared successfully by solvent casting technique [31], therefore this production method was used in this study. To obtain inserts, sodium alginate (0.75%) was added into this N1 formulation and glycerin (Ins1) or PEG 400 (Ins2) was added at the concentrations of 5% (w/v) as plasticizers under stirring conditions. Then the mixture was poured on a glass petri dish (Fig, 1A) and allowed to evaporate at 40 °C for 36 h. The mixture was dried up to the point where the insert weight was stable. FLB dosage was fixed at 0.3% (w/v).

Sodium alginate has been reported as mucoadhesive, biodegradable, biocompatible polymer and has potential for numerous pharmaceutical and biomedical applications [32]. This polymer is used in a variety of ocular pharmaceutical formulations [32-34]. A novel ophthalmic gel has been developed which is converted to gel in the presence of divalent cations (*i.e.* calcium ion) which are present in the lachrymal fluid [35]. Moreover alginate which has mucoadhesive property is not easily eroded by tear fluid as it transforms into stable gel upon exposure to divalent cations [36]. Sodium alginate has advantages for ocular drug delivery; however alginates are not sufficient enough to increase the retention time of the drug on the precorneal area. Thus a lot of researches used other polymers to combine with alginates to overcome this problem. Bhalerao *et al.* [37] formulated an *in situ* gelling ophthalmic drug delivery system for the treatment of glaucoma. Sodium alginate in combination with hydroxypropyl cellulose (HPC) was used as gelling agent, which also acted as viscosity-enhancer. Other researchers developed a novel *in situ* gum-based ophthalmic drug delivery system of linezolid. Hydroxypropyl guar and xanthan were used as gum with the combination of hydroxyethyl cellulose, Carbopol, and sodium alginate as viscosity-enhancing agents [38]. Zhu *et al.* [39] prepared sodium alginate and chitosan coated nanoparticles for ocular drug delivery. Instead of using another polymer we have decided to make inserts including NLCs inside.

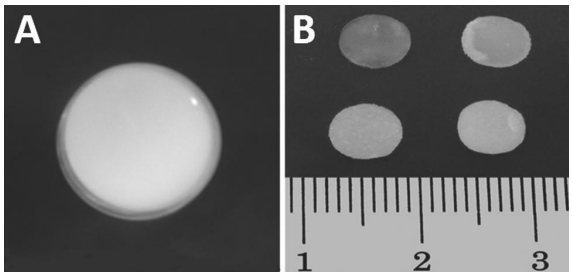

Figure 1. Photographs of inserts before drying in a Petri dish (**A**) and after casting, shaped with a PVC mold (**B**).

After inserts were prepared and dried at 40 °C for 36 h, they had uniform image and it had simply removable property from the petri dishes. The inserts are shown in Figure 1b and after being shaped the diameters of the inserts was found as 6.07 mm (Table 2). Thickness of the inserts was found varing from 0.65 ± 0.01 to 0.88 ± 0.02 mm. Ocusert® is commercially drug system by ALZA, has 0.3 mm thickness value and its dimensions are 5.7 × 13.4 mm on its axes. The other commercial formulation is Ocufit®, its length is 25-30 mm and diameter is 1.9 mm [40]. In this study, prepared ocular inserts have appropriate thicknesses and diameters when compared to the commercial formulations

Parameter	Inserts			
	Ins1	Ins2	$Ins1_{FLB}$	$Ins2_{FLB}$
Thickness (mm)	0.65 ± 0.01	0.81 ± 0.01	0.69 ± 0.03	0.88 ± 0.02
Drug content (mg)	-	-	1.1 ± 0.02	1.17 ± 0.01
Diameter (mm)	6.07	6.07	6.07	6.07
Weight (mg)	29.35 ± 0.4	27.97 ± 0.1	29.78 ± 0.35	28.40 ± 0.42
Moisture absorption %	13.01 ± 0.1	7.15 ± 0.10	12.81 ± 0.20	7.05 ± 0.12
Moisture loss %	7.54 ± 0.02	4.01 ± 0.12	7.14 ± 0.28	4.15 ± 0.09

Table 2. Characterization parameters of developed inserts.

on the drug market. The developed inserts showed appropriate thickness values with low standard deviation.

To evaluate the suitability of preparation technique of inserts, the drug content of inserts is a very important parameter. It has been found that the insert preparation technique is appropriate for developing uniform inserts containing FLB ($Ins1_{FLB}$ and $Ins2_{FLB}$), since FLB content in each insert was found almost the same without any loss (Table 2).

Percentage moisture tests were carried out to check the physical stability of the insert. To evaluate hygroscopicity of developed inserts, they were weighed and retained in desiccators for 3 days. The moisture of the insert was found between 4-7.5 % at the end of the 3rd day. The detected moisture is necessary for inserts because very dry property will affect flexibility and elasticity of the insert. $Ins1_{FLB}$ which contains glycerin, preserved more moisture than $Ins2_{FLB}$ which contains PEG 400. To find out moisture absorption of the ocular inserts, they were weighed precisely and retained in a cabinet with relative humidity of 75% for 3 days. At the end of the 3 days, $Ins1_{FLB}$ absorbed more moisture than $Ins2_{FLB}$. These results were thought to be due to the moisturizing properties of glycerin [41].

Stereomicroscope was used in order to better recognize the surface property of inserts. Pictures showed the homogeneous appearance of $Ins1_{FLB}$ and $Ins2_{FLB}$ (Fig. 2). Surface homogenization is an important factor and it affects the release of drug as a result of erosion and diffusion. Intended controlled release might not be feasible if developed inserts have gaps or cracks.

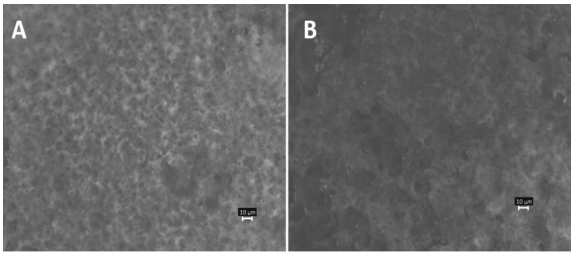

Figure 2. Suface images of $Ins1_{FLB}$ (**A**) and $Ins2_{FLB}$ (**B**) after taken with stereomicroscope (1 × 16).

In-vitro release studies

There are two stage of drug release for degradable inserts: 1) Fast release: tear fluid enters into the formulation thus drug is released; and 2) Slow release: a gel system is occurred on the insert surface thus drug is released for longer time [42]. FLB release from $Ins1_{FLB}$ and $Ins2_{FLB}$ is shown at Fig. 3. $Ins2_{FLB}$ showed slightly faster release at the first 1.5 h which was not different than $Ins1_{FLB}$ significantly ($p > 0.05$). At the end of the 4.5 h, *in vitro* FLB release from $Ins1_{FLB}$ and $Ins2_{FLB}$ was found as 81% and 100%, respectively ($p < 0.05$). At the end of the 6.5 h, *in vitro* FLB release from $Ins1_{FLB}$ was found as 100 %.

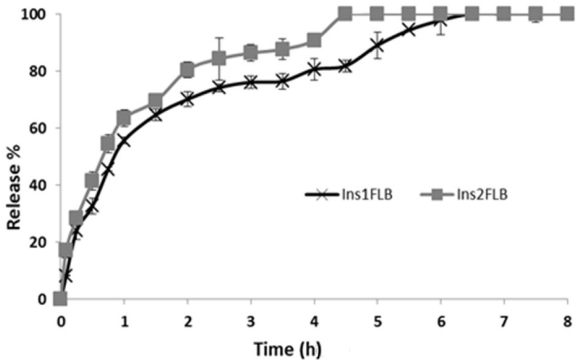

Figure 3. Percentage cumulative release profile of $Ins1_{FLB}$ and $Ins2_{FLB}$ in PBS. Values are means of six experiments ± SD.

Aburahma *et al.* [33] prepared brimonidine loaded inserts, which consisted of 1.5% sodium alginate, 7% polyvinylpyrrolidone K-90 and 5% propylene glycol and the drug release was determined as 80% at 2 h. However for $Ins1_{FLB}$ 80% release was observed at 4th h. The combination of NLC and alginate inserts sustained the drug release almost 2 times than Ins1 insert. This remarkable result can be an advantage for the controlled release of FLB for ophthalmic applications. $Ins1_{FLB}$ is ideal formulation for inflammation of the eye if both first fast- acting and then slow-release was desired.

Evaluation of sterility

The final ophthalmic products must be produced under validated conditions in terms of providing sterility. It is important to sterilize the formulations for ophthalmic applications [31,43]. UV radiation was used for sterilizing the developed ocular inserts. The test for sterility is intended for detecting the presence of viable forms of bacteria, fungi and yeast in sterilized preparations. Eosin metilen blue agar medium and blood agar medium were used to control the suitability of sterilization procedure. There was no appearance of turbidity and hence no evidence of microbial growth; when all formula-

tions were incubated for 48 h at 37 °C. The preparations examined; therefore, passed the test for sterility. It can be concluded that the prepared ocular inserts were detected sterile because there is no microorganism growth detected while the microbial growth was observed in positive control.

Stability

The stability studies of FLB loaded inserts were performed at 4 ± 1 °C, 25 ± 2 °C at 60% relative humidity and 40 ± 2 °C at 75% relative humidity for 3 months. The accelerated stability studies were carried out in accordance with the ICH guidelines. FLB content, sterility and physical characteristics of inserts did not show any significant change for 3 months. These results concluded that developed ocular inserts were physiochemically and microbiologically stable. Compared to solutions, higher stability obtained with inserts is advantageous for drug industry [43]. Nayak *et al.* [44] developed ophthalmic insert of moxifloxacin and evaluated stability of drug content. They found that the inserts were stable for 12 weeks. Since inserts are solid or semisolid dosage forms they do not show the disadvantages of traditional ophthalmic liquid dosage forms.

CONCLUSION

FLB (anti-inflammatory agent) was successfully formulated as NLC and NLC based alginate insert. This study offers a new preparation technique to the ocular inserts by using a combination of NLC and natural polymer sodium alginate. The formulation of FLB as NLC or insert for ophthalmic delivery is achievable. To decrease systemic side effects and increase effective drug concentration in the eye, NLC based ophthalmic inserts could be an alternative approach for ocular drug delivery. The developed NLC based alginate inserts may be useful in clinical practice to maintain mydriasis during cataract or other eye surgical treatments.

Conflict of interest. The authors declare no conflict of interest.

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REFERENCES

1. Gaudana, R., H.K. Ananthula, A. Parenky & A.K. Mitra (2010) *AAPS Journal* **12**: 348-60.
2. Kuno, N. & S. Fujii (2011) *Polymers* **3**: 193-221.
3. Maurice, D.M. & S. Mishima (1984) *Ocular pharmacokinetics', in: Handbook of experimental pharmacology,* (M.L. Sears ed.), 69, Springer Verlag, Berlin-Heidelberg, pp. 16-119.
4. Urtti, A (2006) *Adv. Drug Deliv. Revi.* **58**: 1131–5.
5. Patel, A., K. Cholkar, V. Agrahari & A.K. Mitra (2013) *World J. Pharmacol.* **9**: 47-64.
6. Quinteros, D.A., L.I. Tártara, S.D. Palma, R.H. Manzo & D.A. Allemandi (2014) *J. Pharm. Sci.* **103**: 3859-68.
7. Pignatello, R., C. Bucolo, G. Spedalieri, A. Maltese & G. Puglisi (2002) *Biomaterials* **23**: 3247-55.
8. Le Bourlais, C., L. Acar , H. Zia, P.A. Sado, T. Needham & R. Leverge (1998) *Progr. Retinal. Eye Res.* **17**: 33-58.
9. Gökçe, E.H., G. Sandri, M.C. Bonferoni, S. Rossi, F. Ferrari, T. Guneri, *et al.* (2008) *Int. J. Pharm.* **364**: 76-86.
10. Üstündağ Okur, N., E.H. Gökçe, D. İnci Bozbıyık, S. Eğrilmez, Ö. Özer & G. Ertan (2014) *Eur. J. Pharm. Sci.* **63**: 204-15.
11. Bu, H.Z., H.J. Gukasyan, L. Goulet, X.J. Lou, C. Xiang & T. Koudriakova (2007) *Curr. Drug Metab.* **8**: 91-107.
12. Rao, M.P.C., M. Nappinnai, S. Raju, V.U. Maheshwara Rao & B. Venkateshwara Reddy (2010) *J. Pharm. Sci. Res.* **11**: 693-9.
13. Karthikeyan, D., M. Bhowmick, V.P. Pandey, J. Nandhakumar, S. Sengottuvelu, S. Sonkar, *et al.* (2008) *Asian J. Pharm.* **2**: 192-200.
14. Cronau, H., R.R. Kankanala & T. Mauger (2010) *Am. Fam. Physician* **81**: 137-44.
15. González-Mira, E., M.A. Egea, M.L. García & E.B. Souto (2010) *Colloids Surf. B.* **81**: 412-21.
16. Srinivasan, B.D. & P.S. Kulkarni (1989) *Prog. Clin. Biol. Res.* **312**: 229-49.
17. Araújo, J., E.González, M.A. Egea, M.L. García & E.B. Souto (2009) *Nanomed. Nanotechnol.* **5**: 394-401.
18. Bhattacherjee, P (1989) *Prog. Clin. Biol. Res.* **312**: 211-27.
19. Shaikh, M.Y., J.S. Mars & C.J. Heaven (2003) *J. Cataract Refr. Surg.* **29**: 2372-7.

20. Kumbhat, S (1994) *J. Pharmaceut. Biomed.* **12**: 131-3.
21. Upadhyaya, N., A. Patidar, S. Agrawal & D. Gupta (2011) *Res. J. Pharm. Biol. Chem. Sci.* **2**: 411-20.
22. Gassan, J. & A.K. Bledzki (1997) *Polym. Composite* **18**: 179-84.
23. Dandagi, P.M., F.V. Manvi, M.B. Patil, V.S. Mastiholimath & R. Rathod (2004) *Indian J. Pharm. Sci.* **66**: 309-12.
24. Luo, Q., J. Zhaoa, X. Zhang & W. Pan (2011) *Int. J. Pharm.* **403**: 185-91.
25. González-Mira, E., S. Nikolic, A.C. Calpena, M.A. Egea, E.B. Souto & M.L. García (2012) *J. Pharm. Sci.* **101**: 707-25.
26. Üstündağ Okur, N., E.H. Gökçe, S. Eğrilmez, Ö. Özer & G. Ertan (2014) *J. Ocul. Pharmacol. Ther.* **30**: 319-32.
27. Cohen, S., E. Lobel, A. Trevgoda & Y. Peled (1997) *J. Control. Release* **44**: 201-8.
28. Séchoy, O., G. Tissié, C. Sébastian, F. Maurin, J.-Y. Driot & C. Trinquand (2000) *Int. J. Pharm.* **207**: 109-16.
29. Gilhotra, R.M., K. Nagpal & D.N. Mishra (2011) *Int. J. Pharm. Investig.* **1**: 22-8.
30. Gupta, J., L. Prabakaran, R. Gupta & M. Govind (2011) *Int. J. Pharm. Pharm. Sci.* **3**: 66-70.
31. Üstündağ Okur, N., E. Homan Gökçe, D. İnci Bozbıyık, S. Eğrilmez, G. Ertan & Ö. Özer (2015) *Expert Opin. Drug Deliv.* **12**: 1-17.
32. Shafie, M.A.A. & H.H.M. Fayek (2013) *J. Clin. Exp. Ophthalmol.* **4**: 1-11.
33. Aburahma, M.H. & A.A. Mahmoud (2011) *AAPS Pharm. Sci. Tech.* **12**: 1335-47.
34. Kumar, J.R. & S. Muralidharan (2012) *J. Pharm. Sci. Res.* **4**: 1973-7.
35. Mandal, S., M.K.M.J. Thimmasetty, G.L. Prabhushankar & M.S. Geetha (2012) *Int. J. Pharm. Investig.* **2**: 78-82.
36. Séchoy, O., G. Tissié, C. Sébastian, F. Maurin, J.Y. Driot & C. Trinquand (2000) *Int. J. Pharm.* **207**: 109-16.
37. Bhalerao, A.V. & S.S. Singh (2011) *Int. J. Pharma. Biosci.* **2**: 7-14.
38. Hiremath, S.S., F.S. Dasankoppa, A. Nadaf, V.G. Jamakandi, J.S. Mulla, S.A. Sreenivas, *et al.* (2008) *Sci. Pharm.* **76**: 515-32.
39. Zhu, X., M. Su, S. Tang, L. Wang, X. Liang, F. Meng, *et al.* (2012) *Mol. Vision.* **18**: 1973-82.
40. Gavaskar, B (2010) *Int. J. Pharm. Pharma. Sci.* **2**: 152-65.
41. Shinde, A.J., K.C. Garala & H.N. More (2008) *Asian J. Pharm.* **2**: 265-9.
42. Aldrich, D.S., C.M. Bach, W. Brown, W. Chambers, J. Fleitman, D. Hunt, *et al.* (2013) *USP Expert Panel Stimuli to the Revision Process* **39(5)**: 1-21. Available at <http://www.usp.org/sites/default/files/usp_pdf/EN/meetings/workshops/ophthalmicpreparations.pdf>.
43. Missel, P.J., J.C. Lang, D.P. Rodeheaver, R. Jani, M.A. Chowhan & T. Dagnon (2009) *Design and evaluation of ophthalmic pharmaceutical products, in: Modern pharmaceutics applications and advances* (A.T. Florence & J. Siepmann, eds.), New York: Informa Healthcare, p. 142.
44. Nayak, B.S., S.C. Patnaik, S. Sethy, P. Ellaiah & B.K. Sarangi (2015) *AJPCT.* **3**: 302-12.