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Design and Ocular Tolerance of Flurbiprofen Loaded Nanosuspension

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Abstract: *Background*: Nanosuspension applicability in ocular drug delivery. *Objective*: Development of efficient nano-based ocular delivery is a major challenge. The purpose of this work was to design and evaluate the sustained release flurbiprofen (FB) loaded nanosuspensions for improving the drug availability at the corneal surface. *Methods*: Polymeric nanosuspensions were prepared by solvent displacement method using process variables such as drug to polymer ratio and solvent to non solvent ratio and their influence on particle size, polydispersity index, zeta potential, entrapment efficiency, *in vitro* release and ocular tolerance was investigated. *Results*: The prepared nanoparticles were predominantly spherical in shape having average particle diameter ranging from 107.7±3.8 to 245.0±4.6 nm, with positive zeta potential values from +6.6±2.2 to +19.0±3.1 mV and entrapment efficiency values from 54.67±3.4 to 90.32±3.2%. Drug release from optimized nanosuspension was sustained with approximately 60 % over 12 hrs period, when compared with marketed formulation, Flur eye drops. The release profile of nanoparticles followed zero-order release kinetics. Stability studies revealed that there were no significant change in particle size, entrapment efficiency and drug release even after 6 months storage. *In vivo* experiments showed that, topical instillation of prepared nanosuspension to rabbit's eye found to be non-irritant by Draize's test and also considered safe by hispathological study. *Conclusion*: The above results clearly indicated Eudragit RL 100 loaded FB nanosupension was found to be stable, sustained its drug release and suitability for ocular application.

Keywords: Nanosuspensions, flurbiprofen, ocular tolerance, sustained release, draize test.

INTRODUCTION

Drug targeting to eye is challenging due to the complicated anatomical structure and defensive mechanism of eye. The drug availability at the targeted site is very low especially in the anterior segment of eye, because of precorneal barriers such as nasolacrimal drainage, tear turn over and blinking of eyes which make the drug to reside for very less time at corneal surface, thereby leading to inefficient therapeutic efficiency [1, 2].

The use of nanotechnology provides attractive opportunities for ocular drug delivery, mainly because the availability of drug in nanosize, which allows the drug molecule to enter and interact with specific ocular tissue thereby overcoming ocular barriers and prolonging the drug residence time in targeted region [3]. Furthermore, this technology offers promising solution for formulating poor water soluble drugs in conventional dosage form i.e. eye drops [4].

The ocular inflammatory disease is frequently occurring disorder may be due to natural cause or in ophthalmic therapy especially after cataract surgery [5, 6]. Non-steroidal anti-inflammatory drugs (NSAIDs) are mostly targeted for topical route used in the management and prevention of ocular inflammation and cystoid macular edema (CME) and the maintenance of mydriasis during cataract surgery [7, 8]. Though steroids are considered as the major scope in the treatment of eye diseases since a very long time, their usage has come into a run since two decades [9-13]. Despite of their ability to treat ocular diseases, the steroids have many

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disadvantages like the decreased immunological response to infection, cataract formation, steroid-induced raised intraocular pressure (IOP) and inhibition of re-epithelisation following epithelial denudation [14]. Ocular drug delivery is a suggested route for NSAIDs used in the treatment of ophthalmic diseases as their oral administration may result in gastrointestinal disturbances [15].

Flurbiprofen (FB), a first line drug in the treatment of ophthalmic diseases is an acidic, BCS class II drug. It is mainly used to reduce inflammatory response which is common after cataract surgery [16].

The localized therapy through ocular nano delivery systems improves the efficiency of flurbiprofen as drug of choice. Hence, there is a demand for the development of nano vesicular systems for ocular instillation.

The aim of the study was to design Eudragit loaded FB nanosuspensions, which were produced by solvent displacement method, and to evaluate their potential as nano-based delivery system. These nanosuspensions were optimized by investigating the influence of several key factors including drug to polymer ratio, solvent to non-solvent ratio. The resulting nanoparticles were evaluated for their shape, size, zeta potential and drug release. Selected FB nanosuspension was tested for their ocular tolerance and safety evaluation by Draize's test and histopathology studies. The selected formulation was compared with that of commercially available ophthalmics, Flur.

MATERIALS AND METHODS

Eudragit RL 100 and FB were obtained as a gift sample from FDC Ltd. (Mumbai, India). Acetone, polyvinyl alcohol (PVA) and methanol were purchased from S.D. Fine Chemical Ltd. (Mumbai, India) and were used as received. Syringe filters with a pore size of 0.22 μm were obtained from Millipore Corporation (USA). Dialysis tubing cellulose membrane was purchased from Sigma Chemical Company, USA. All other solvents and reagents used for study were of analytical grade.

Preparation of Nanosuspension

Accurately weighed quantities of flurbiprofen (10 mg) and Eudragit RL 100 (100 mg) were dissolved in organic co-solvents containing acetone and methanol (3:1). This solution was kept aside for half an hour for complete solubilization of drug and polymer, which is an organic phase.

Aqueous phase of polyvinyl alcohol (PVA) solution was prepared by dissolving the required amount of PVA in boiled water. This solution was heated at 90 ^{o}C in a water bath, until complete solubilization of PVA happened and then it was cooled at room temperature.

Polymeric nanosuspension of flurbiprofen (FB) was prepared with Eudragit RL 100 separately by solvent displacement technique [17]. The organic phase was poured drop wise through Millipore microfilter of size 0.22 μm, at a rate of one mL/min under atmospheric pressure into an aqueous medium (40 mL) containing 1% PVA (hydrophilic surfactant) as a stabilizer under moderate magnetic stirring (1000 rpm). After the addition of the organic phase, stirring was continued for one hour at the same speed. Then, it was sonicated (Probe sonicator, Orchid Scientifics) for two minutes to obtain the desired particle size. Later the colloidal dispersion was subjected to heating under reduced pressure at 58 ^{o}C by using rotaevaporator to remove acetone & methanol (organic solvents). The solution concentration depends on the solvent: non-solvent ratio. Formulations containing flurbiprofen with Eudragit RL100 combination were coded as FL as shown in Table **1**.

CHARACTERIZATION OF NANOSUSPENSION

Particle Size and Zeta Potential

Nanoparticle size distribution and zeta potential were determined using photon correlation spectroscopy (Zetasizer, HAS 3000; Malvern Instruments, Malvern, UK). The size distribution analysis was performed at a scattering angle of 90 degrees and at a temperature of 25 °C using samples appropriately diluted with filtered water (0.22μm filter; Millipore), whereas zeta potential was measured using a disposable zeta cuvette.

Entrapment Efficiency

The entrapment efficiency (EE) of FB loaded nanosuspension was assessed indirectly, determining the free FB (un-entrapped) by a UV Spectro-

photometric method by applying the following equation:

$$EE\,(\%) = \frac{\text{Total amount of FB} - \text{Free FB}}{\text{Total amount of FB}} \times 100$$

Free FB was removed by centrifugation technique. The supernatant liquid containing the free drug was withdrawn and analyzed. Total amount of drug incorporated in the nanosuspensions was determined by polymer disruption. All analyses were performed in triplicate.

Table 1. Formulation code and variables used in the preparation of FB nanosuspensions.

Formulation code	Drug-to-polymer ratio	phase volume ratio (solvent-to-nonsolvent)
FL1	1:10	1:2
FL2	1:10	1:3
FL3	1:10	1:4
FL4	1:20	1:2
FL5	1:20	1:3
FL6	1:20	1:4
FL7	1:30	1:2
FL8	1:30	1:3
FL9	1:30	1:4

Morphology

The morphological examination of the nanosuspensions was performed by transmission electron microscopy (TEM, Hitachi 7500, Japan). Samples of the nanosuspension (5-10 µL) were dropped onto Formvar-coated copper grids. After complete drying, the samples were stained using 2% w/v phosphotungstic acid. Digital Micrograph and Soft Imaging Viewer software were used to perform the image capture and analysis, including particle sizing.

In vitro Release Studies

The release of FB from nanosupensions was assessed using a dialysis bag, under sink condition for 24 hrs. The release of FB from nanosuspensions was evaluated by a dialysis system consisting of a Spectrapor membrane (cut-off: 3500 Da), loaded with 3mL of nanosuspension, at room temperature and under slow magnetic stirring. At regular intervals aliquots of 3mL of the dissolving medium were withdrawn, and immediately restored with the same volume of fresh artificial tear fluid. The amount of released drug was assessed by UV analysis at 247nm (Shimadzu UV-1601). The optimized formulation was compared with marketed Flur eye drops.

Infrared (IR) Spectrophotometry

IR spectra of freeze-dried NPs were obtained with a Perkin- Elmer 1600 spectrophotometer (Perkin-Elmer, Waltham, Massachusetts) using the KBr disk technique (about 10 mg samples for 100 mg dry KBr).

Stability Studies

The stability of nanosuspensions in different storage conditions was evaluated. The samples were closed vials and stored at 4±2 ^{o}C and 25±2 ^{o}C for a period of 6 months. The vials kept away from exposure to direct light to avoid photo degradation and at predefined intervals aliquots of samples were withdrawn for particle size, zeta potential and encapsulation efficiency.

In vivo Studies

Ex vivo Permeation Study

Fresh whole goat eyeballs were collected from a local butcher's shop and kept in normal saline until its use. Then Corneas were removed carefully along with 5 to 6 mm of surrounding scleral tissue and stored in freshly prepared artificial tear solution, pH 7.4. The permeation study was carried out in a modified Franz diffusion chamber. The upper chamber served as a donor compartment in which one mL of formulation under study was placed. The excised goat cornea was fixed and clamped between donor and receptor compartments of the Franz diffusion cell in which its epithelial surface faced towards the donor compartment. The lower chamber served as a receiver compartment that filled with freshly prepared artificial tear fluid. About 3.54 cm^2 corneal surface area was exposed to donor compartment cell and was made available for drug permeation. The whole system was maintained at 37±0.5 ^{o}C and rice magnetic bead was

placed in receptor chamber and stirred at 100 rpm. Samples were withdrawn from receptor cell at regular intervals for 24 hrs and analyzed. For hydration study, each cornea (after 24 hrs) was weighed, and soaked in methanol, dried overnight at 90 ^{o}C and was reweighed. The corneal hydration was calculated from the difference in weight. The permeation (%) or *Ex vivo* ocular availability was calculated as follows:

$$\text{Permeation (\%)} = \frac{\text{Amount of drug permeated in receptor}}{\text{An initial amount of drug in donor}} \times 100$$

Ocular Tolerability Test

The potential ocular irritancy was evaluated for formulations to test their damaging effects on cornea according to a modified Draize test using a slit-lamp. The animal studies were carried out as per the approval and guidelines of the Institutional Animal Ethics Committee (Reg No: 516/01/A/CPCSEA). Male albino rabbits weighing 2.0-2.5 kg were used in the studies. Four male albino rabbits (body weight 2 kg) were used in the experiment. They were housed and all procedures in the study conformed to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Aliquot 20µL of the test formulation and was instilled directly into the cornea of right eye for every 30 mins for 6 hrs (12 treatments). Left eyes treated with distilled water served as a control. Condition of the ocular tissue was observed after 10 mins, at 6 hrs, and 24 hrs after the end of the experiments. The congestion, swelling, and discharge of the conjunctiva were graded on a scale from 0 to 3, 0 to 4, and 0 to 3, respectively. Hyperemia and corneal opacity were graded on a scale from 0 to 4.

Safety Evaluation by Histopathological Study

For histopathological examination, healthy male albino rats that were free of any ocular damage were taken for the study. To examine the influence of different formulations on the cornea structure and integrity, evaluation of the prepared flurbiprofen nanosuspensions was carried out on albino rats. Fresh eyes were excised immediately after the rats were sacrificed and the eyes were dissected and rinsed for one minute with 0.9% (w/v) NaCl, and then incubated at 37 ^{o}C for 30 mins in the selected or optimized formulations (FL4). Phosphate buffer saline (PBS 0.1%, w/v) were taken as references. This test procedure reported in literature [18]. After incubation, the eyes were washed with PBS, and immediately fixed with a formalin solution 10% (v/v). The fixed samples were transferred into labeled cassettes and placed in 70% (v/v) ethanol. They were subjected for tissue processing, embedding, sectioning and staining. Each section was paraffin-embedded, bisected into two equal halves and finally mounted in a paraffin block so that a section of each half cut and placed on a single microslide. The slides were stained with haematoxylin and eosine (H & E). The stained corneal sections were imaged using a light microscope for histological modifications.

Statistical Analysis

Multiple group comparison was conducted by one-way analysis of variance (ANOVA). All data are presented as a mean value with its standard deviation indicated (mean±SD), *p*-Values less than 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

The present investigation consists of formulation and evaluation of flurbiprofen nanosuspensions. Flurbiprofen nanosuspensions were prepared by nanoprecipitation or solvent displacement method. Nevertheless, several difficulties were to be overcome to successfully incorporate the drug into the nanosuspension. The difficulty was the selection of an organic phase which was capable of solubilizing both drug and polymer; for which acetone is the solvent of choice (a water soluble, low-boiling point and easily evaporates). However, methanol was ued as co-solvent because of complete solubility of drug in it. The preparation of flurbiprofen-loaded nanosupensions in acetone yielded an amorphous precipitate, may be due to non-associated drug. This problem can be overcomed by using co-solvency technique. Therefore, a co-solvent was utilized to optimize the solubility of both flurbiprofen and polymer. This nanosuspension preparation process appears simple but may involve complex interfacial hydrodynamic phenomena [19]. Only aqueous phase, with or without stabilizer or surfactant can be used in the preparation of nanosuspension. Addition of stabilizers are mostly preferrable due to its enhanced

formulation stability for longer period. In this case PVA was used as stabilizer because of its high water solubility and not only imparts stability but also viscosity to the nanosuspension.

Nanoparticle Size, Polydispersity Index, Zeta Potential and Entrapment Efficiency

The prepared flurbiprofen nanosuspensions are targeted to anterior segment of eye through ocular route. So, particle size and particle size distribution are important parameters for the safe and effective administration of such formulations. Particle size for ophthalmic application should not exceed 10 μm [20].

The size, size distribution, zeta potential and entrapment efficiency data of the formulations FL1 to FL9 are shown in Table **2** and in Fig. (**1**) respectively. All the formulations showed a small mean size, which is suitable for ocular application. The mean particle size for drug loaded formulations varied from 107.7 nm to 245.0 nm. The polydispersity index (PDI) varied in general. The polydispersity index is probably a result which depends on a complex interplay of several phenomena involving the drug, the polymer, the solvents and the stirring rate. The PDI in the range of 0 - 0.05 indicates that the dispersion is monodisperse, if it is less than 0.7 the dispersion is nearly monodisperse and if it is more than 0.7, the dispersion is highly polydispersed [21]. The first case establishes a clear evidence that all the particles have almost same size. The effect of size on the release or penetration of all particles of a single dose can be considered as nil. None of the formulations come under this category. In the second case maximum number of particles have similar size. The formulations FL1 to FL6 and FL9 come under this category which implies that the particles are almost mono disperse and the effect of the particles with in the single unit can be considered as negligible with respect to the release. The FL8 formulation was highly polydispersed having PDI of 0.802. The zeta potential of all the formulations varied in the range +6.6 to +19.0 mVs. The entrapment efficiency of the formulations varied in the range 54.67 to 90.32 % (Fig. **2**).

As the phase volume of the aqueous layer increases, there is the possibility of increased diffusion of water soluble solvent (acetone) into the aqueous phase [22]. Hence the size of the particle might be decreasing. The positive zeta potential of all the formulations might be because the polymers are polycationic in nature. This positive charge

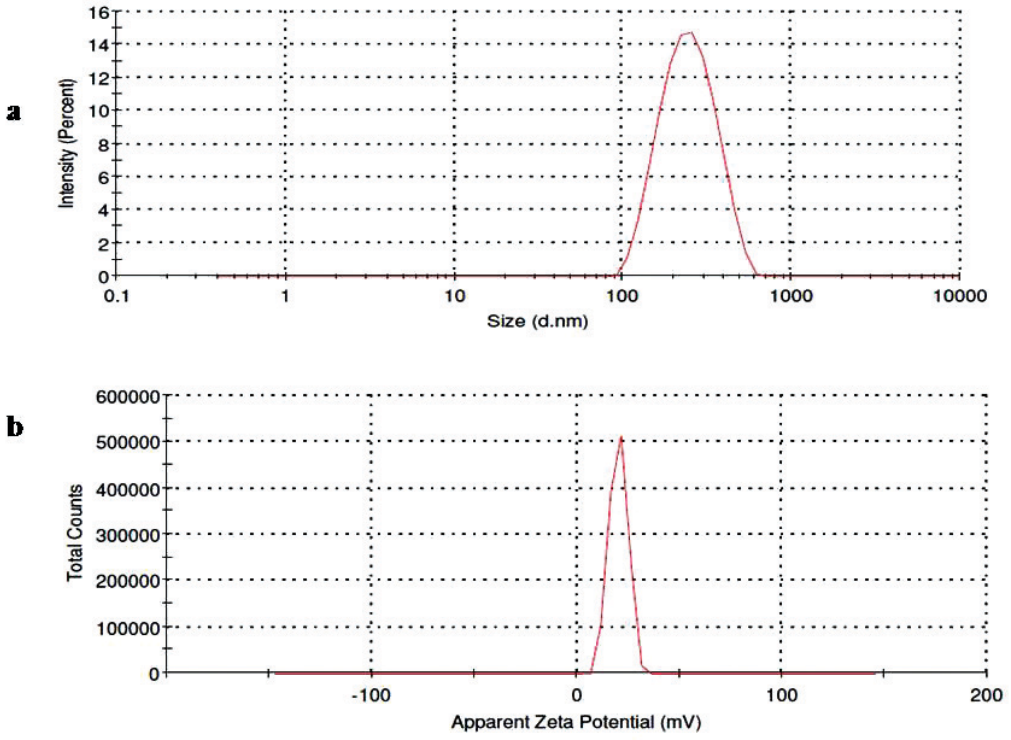


Fig. (1). Graph showing optimized formulations **a**) Particle size distribution and **b**) Zeta potential of FL4 nanosuspensions.

Table 2. Mean particle size, zeta potential and entrapment efficiency of various prepared FB nanosuspensions.

Formulation code	Mean size in (Z ave) nm±SD	Polydispersity index±SD	Zetapotential (mV±SD)	% Entrapment efficiency ±SD
FL1	190.2 ± 3.2	0.336 ± 0.21	+18.6 ± 2.2	79.22 ± 2.2
FL2	182.6 ± 4.6	0.386 ± 0.30	+17.4 ± 1.2	66.07 ± 4.1
FL3	167.2 ± 3.9	0.498 ± 0.27	+16.6 ± 3.8	54.67 ± 3.4
FL4	205.0 ± 4.5	0.293 ± 0.10	+19.0 ± 3.1	80.27 ± 3.6
FL5	150.5 ± 5.7	0.321 ± 0.12	+13.2 ± 3.2	87.37 ± 2.1
FL6	130.4 ± 3.3	0.530 ± 0.20	+18.8 ± 2.7	62.09 ± 3.5
FL7	245.2 ± 4.6	0.357 ± 0.28	+9.6 ± 3.4	90.32 ± 3.2
FL8	215.7 ± 5.2	0.802 ± 0.25	+6.6 ± 2.2	70.52 ± 4.3
FL9	107.7 ± 3.8	0.610 ± 0.22	+18.3 ± 3.6	54.96 ± 2.9

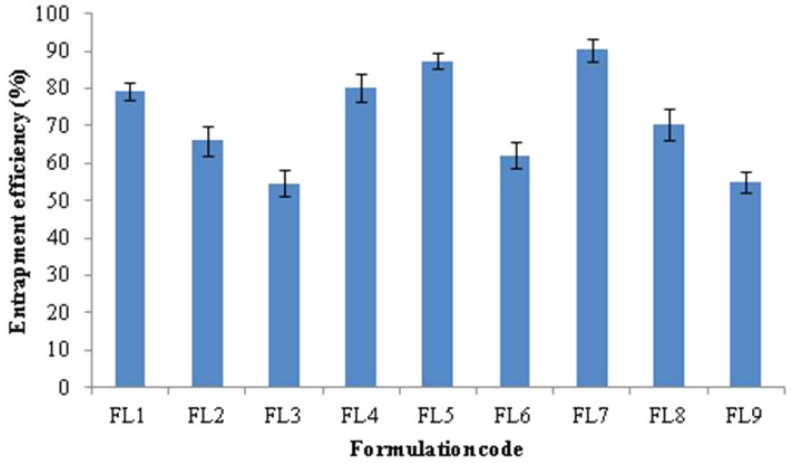


Fig. (2). Graph showing the entrapment efficiency of various nanosupensions.

would facilitate effective adhesion to the cornea surface, consequent interaction with negatively charged mucosa of the conjunctiva and anionic mucin present in the tear film and consequent prolonged time of the formulation in the eye. Increase in polymer concentration in organic phase might be leading to increased viscosity of organic phase leading to increased resistance to the diffusion of the drug molecule from the organic to the aqueous phase. This might be leading to greater entrapment efficiency. In most of the formulations, it is observed that the entrapment efficiency increased with increasing organic phase volume.

Surface Morphology

Transmission electron micrographs of flurbiprofen loaded nanosuspension showed that the shape of prepared nanoparticles are of spherical and smooth surface, which are uniformly distributed (Fig. **3**).

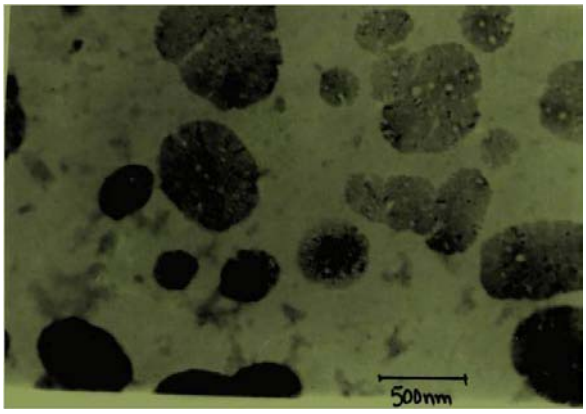


Fig. (3). Transmission electron micrograph of flurbiprofen loaded nanoparticle (FL4).

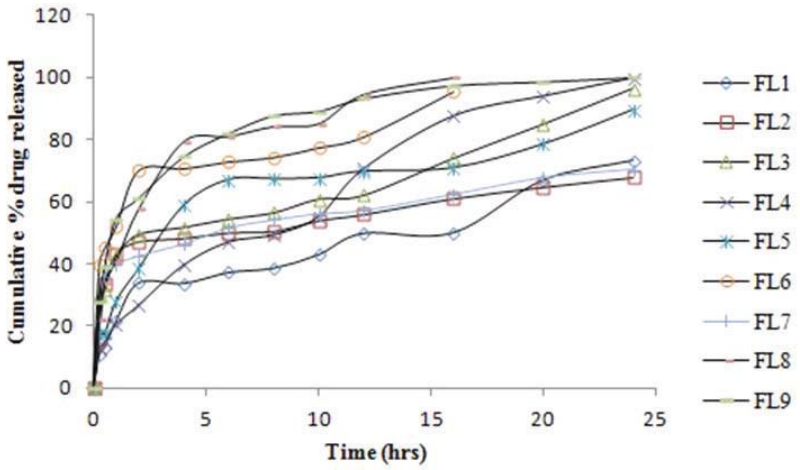


Fig. (4). Dissolution profiles of various flurbiprofen nanosuspension.

In vitro Drug Release

Drug releases from Eudragit nanosuspensions was found to be slow and spread over extended periods of time with all the formulations as shown in the (Fig. **4**). The drug release is biphasic, with fast release in the first two hours and slow and gradual release over the next 24 hrs. In spite of drug loading and processing, Eudragit has not lost its positive surface charge.

The quick release of the drug in the first two hours is probably from the pure unentrapped drug adsorbed on the polymer. The drug release rate and the time taken for total drug release are results which depend on the interplay of factors like drug:polymer ratio, phase volume ratio, particle size, polydispersity index, drug properties like solubility and nanoparticle properties such as the polymer network and the diffusion mechanism. Factors affecting dissolution rate include (a) rate at which dissolution fluid penetrates the polymer matrix, and (b) rate at which polymer matrix swells. Studies on Eudragit RS and RL microparticles, showed the drug release is a complex phenomenon which results from co-existing dissolutive and diffusional phenomena [23-25]. Pignatello *et al*, reported that an increase of drug release was observed as a function of drug:polymer ratio [26]. They reported that such a fast release is because of a progressive saturation of polymer ammonium group by drug molecules occurring at a higher drug to polymer ratio, which increases the dissolutive nature of the drug. All the formulations follow zero order kinetics. The *n* value is an empirical parameter characterizing the release mechanism [27]. The *n* value was 0.583 for optimized formulation, indicating non-Fickian diffusion. Thus the mechanism similar to dissolution rate is dependent on factors like concentrations of drug, polymer and volume of aqueous phase. The drug release from formulations is taking place by a mechanism of diffusion (since Higuhi's fit shows high correlation coefficient values) but also the concentrations of drug, polymers and solvents are playing a significant role. Drug release from Flur was fast, and was spread over 6 hrs. It was slow, gradual and was spread over 24 hrs for FL4 formulation.

FT-IR Studies

The principle IR absorption peak at flurbiprofen at 3367 cm^{-1} (-OH-), 3076 cm^{-1} and 1553 cm^{-1} for (=C-H) and (-C=O) and other peaks at 1482, 1314,1145 and 694 were all observed in the spectra of flurbiprofen and optimized formulation of nanoparticles. Eudragit has characteristics IR absorption frequency at 3434.04 cm^{-1} (OH stretch), 2952.30 cm^{-1}(*sp*3 CH stretch), and at 1736.01 cm^{-1}(CO stretch). The nanosuspension formulation showed a shifting of peak from 3367 to 3385 cm^{-1} indicating the hydrogen bond is enhanced. The peaks of 2975 & 2940 cm^{-1} shifted a to 2976 & 2952 cm^{-1} in formulation, indicating the presence of (C-H) stretching in both as shown in Fig. (**5**). The observation of these spectra also indicates that there is no chemical interaction between flurbiprofen and polymer in the formulation.

Stability Studies

Effect of storage time and conditions on different parameters on optimized nanosupensions was

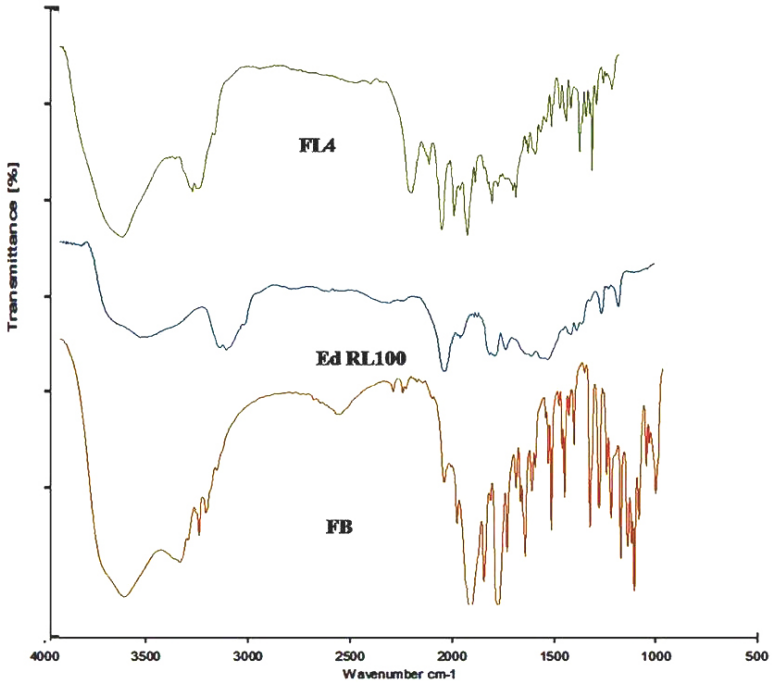


Fig. (5). The Infra Red (IR) Spectrum of (FB) flurbiprofen, (Ed RL100) Eudragit RL100 and (FL4) optimized flurbiprofen nanosuspension.

Table 3. Stability studies of FL4 nanosuspensions.

Parameter		At 27 ± 1 °C (RT)		At 2-6 °C	
Time	**Initial**	**3 months**	**6 months**	**3 months**	**6 months**
Drug content (%)	97.34 ± 2.8	96.45 ± 3.3	94.47 ± 2.9	98.34 ± 2.7	97.54 ± 2.6
pH	7.4 ± 1.2	7.4 ± 1.8	7.7 ± 0.9	7.4 ± 0.9	7.2 ± 1.3
Particle size (nm) n=3	205.0 ± 4.5	223 ± 3.2	240.8 ± 4.3	207 ± 4.3	218.9 ± 1.1
Zeta potential (mV)	+19.0 ± 3.1	+18.4 ± 1.4	+16.3 ± 1.2	+18.9 ± 5.2	+18.1± 1.4
Entrapment efficiency (%)	80.2 ± 3.6	82.3 ± 2,2	87.2 ± 3.2	83.1 ± 1.5	86.8 ± 4.6
Particle aggregation	No	No	No	No	No
Settling of Particles	No	No	Few particles	No	No
In vitro release (24 hrs) (%)	99.8 ± 0.01	92.3 ± 0.23	87.4 ± 0.34	94.3 ± 0.65	91.4 ± 0.04

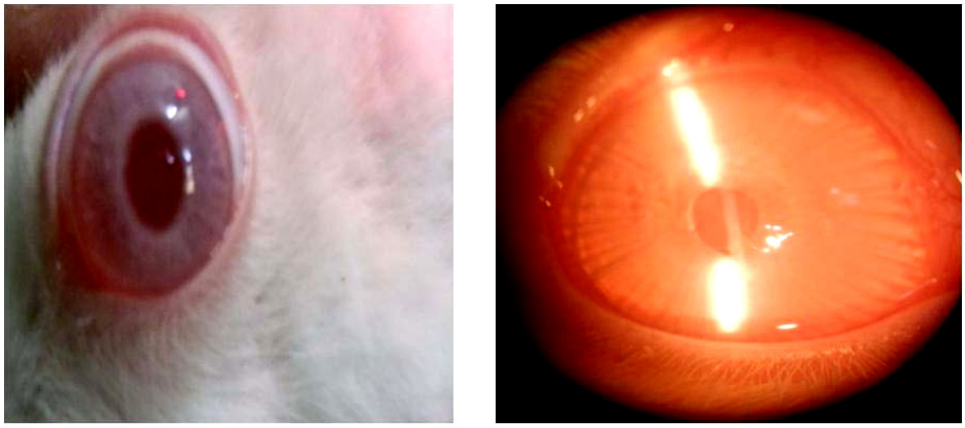
RT- Room temperature.

shown in the Table **3**. During 6 months of storage in refrigerator (2 - 6 °C) and at room temperature (27±1°C), particle size, zeta potential & entrapment efficiency of FL4 nanosupensions before and after storage under varying conditions were compared to evaluate the stability studies. Stored nanosuspensions were tested for turbidity, particle aggregation and particle settling to the bottom of the vials.

Increase in Particle size may be due to particle agglomeration [28]. The small decrease in zeta potential might be due to a reduction of repulsive forces between the particles. This might also have caused agglomeration of particles and consequent small increase in particle size. The nanosuspensions showed positive charge after storage. Positive charge helps to facilitate effective adhesion of the nanosuspension to the corneal surface. Even after storage for six months, the electrophoretic behavior did not change significantly. Increased drug entrapment upon storage might be due to the reabsorption of the dissolved drug onto the particle

Table 4. Permeation of flurbiprofen from optimized nanosuspensions (FL4) and marketed formulation (Flur) through freshly excised goat cornea.

F.code	Cumulative amount permeated (mg) (±SD,n=3)	% Permeation (±SD,n=3)	Permeability coefficient (P_{app} cm/hr)	Steady state flux (J_{ss} mg/cm^{2}hr)	Corneal hydration (%) (±SD,n=3)
Flur	0.295±0.13	98.23±0.24	0.109±0.34	0.033±0.03	80.34±0.79
FL4	0.710±0.24	94.60±0.06	0.230±0.22	0.173±0.07	81.23±0.52



A **B**

Fig. (6). Picture showing **A**) normal picture of rabbit eye after instillation **B**) observed eye through slit lamp for ocular irritation test.

surface. Baudoun *et al*., (2004) reported studies with similar systems showed that the drug remained adsorbed onto the particle surface even after several months of storage at low temperatures. Decreased percentage drug release over 24 hrs may be due to higher amount of drug deposition on the polymer coat. The constancy in pH over the storage period and easy redispersibility suggest the stability of nanosuspensions to ophthalmics. No macroscopic properties were observed. Thus from above result, it may be concluded that nanosupensions showed good stability.

Ex vivo Corneal Penetration Study

Percent permeation of Flur is more than that of the optimized formulation (FL4) as in Table **4**. The corneal hydration percentages were in the range of 80.32±0.79 to 81.23±0.52%. Flurbiprofen is a small molecule (MW=244), and its permeability is probably high and is independent of the formulation effect. The role of nanosuspensions is in prolonging the drug release rather than in enhancing permeation. There was binding of nanoparticles to the corneal surface by both electrostatic force and hydrogen bonds, which consequently facilitate the drug absorption into the cornea. In addition to this, the polycationic Eudragit could improve the permeability of cornea by opening the tight junctions of corneal epithelial cells. Consequently, the drug penetration was significantly enhanced by the nanosuspension. The normal level for cornea hydration is of 76-80% [29]. Corneal hydration level, 3-7% units or more over the normal value indicates damage of the corneal epithelium and/or endothelium [30]. All the experiments showed corneal hydration within the range showing no corneal damage. Hence, it can be concluded that the prepared formulations have good permeability and do not damage the corneal integrity.

Ocular Irritation Test (Modified Draize Test)

The ocular irritation test were measured and graded with scores by observing under slit lamp as shown in Fig. (**6**). There was no difference between control and test formulation, especially for corneal opaqueness and conjunctival congestion as shown in Table **5**. There is slight iris irritation, conjunctival discharge and swelling after 6 hrs, however these symptoms were gradually disappeared after 12 hrs.

Despite of conjunctival redness observed for test formulation until 12 hrs, the degree of redness

Table 5. Data for Draize's eye irritation test* of FL4 nanosuspension.

	6 hours		12 hours	
	Control	**Test**	**Control**	**Test**
Cornea				
Opaqueness	0	0	0	0
Iris				
Irritation value	0	1 ± 0.20	0	0
Conjunctiva				
1. Degree of flare	0.3 ± 0.51	0.9 ± 0.08	0.3±0.07	0.2 ± 0.06
2. Degree of swelling	0	0.3 ± 0.08	0	0
3. Degree of redness	0.1 ± 0.09	1	0	1
4. Congestion	0	0	0	0
5. Secretion (discharge)	0	1	0	0

*Irritation and corneal opacity were graded on a scale from 0 to 4. Congestion, flare, swelling discharge, and redness of the conjunctiva were graded on a scale from 0 to 3, 0 to 4, 0 to 3 and 0 to 3 respectively.

Values are mean ± SD (n=3).

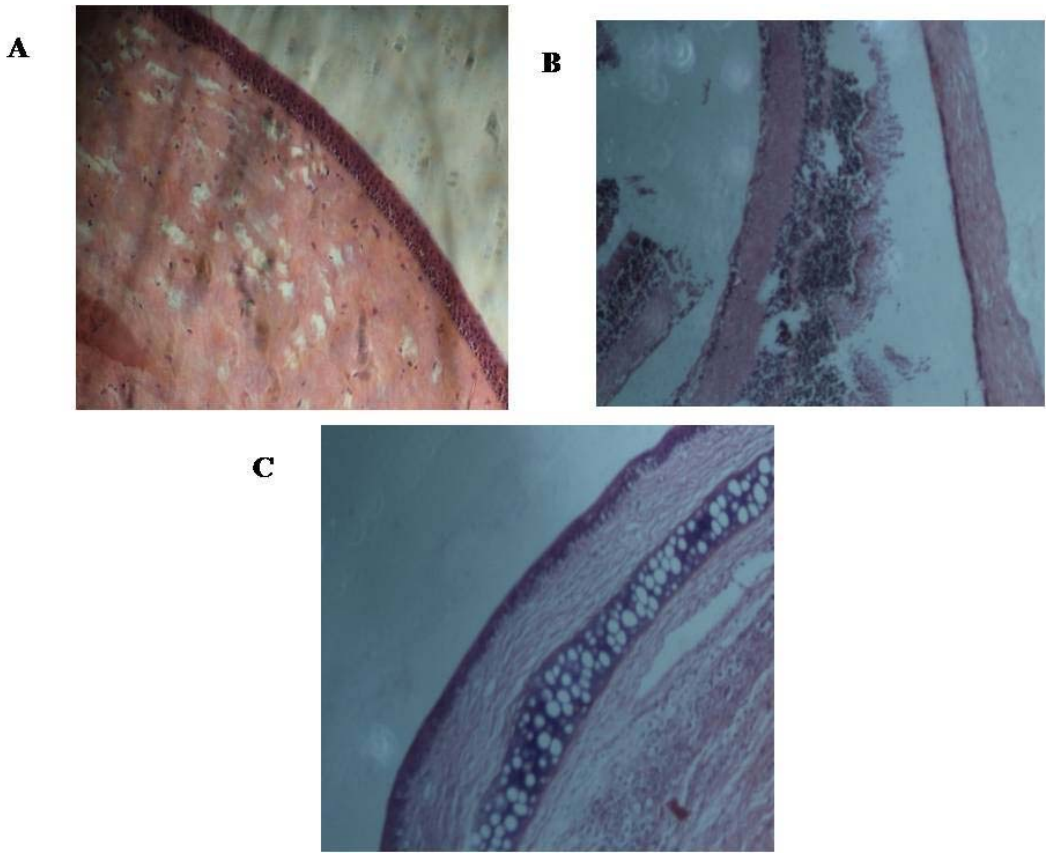


Fig. (7). Histologic cross sections of excised rat cornea showing epithelium (EP) and stroma (ST), stained with hematoxylin-eosin after incubation at 37 ^{o}C (**A**) PBS, pH 7.4 (**B**) sodium dodecyl sulfate (SDS) 0.1% (**C**) FL4 pH 7.0.

was within the limit (<2). A score greater than or equal to 2 in any condition (Opaqueness, redness, swelling etc.) or a total added score greater than 4 are considered as an indicator of "non-tolerance".

In this case, all the score were within the specified limit indicating the suitability and ocular tolerance of prepared formulation.

Safety Evaluation by Histopathological Examination on Albino Rats

Cross sections of freshly excised rat corneas after incubating in different media were as shown in Fig. (**7**). There was no disturbance of epithelial and stromal structure after incubation of eye balls in PBS pH 7.4 (Fig. **7A**). In Fig. (**7B**), corneal layers were detached and no structural integrity was maintained, indicating irritancy and unsafe nature of 0.1% sodium dodecylsulfate (SDS). Figure **7C**, showed that intact corneal layers without any detachment of epithelial and stroma cells in agreement with control suggesting the ocular compatibility. Thus, the prepared nanosuspension is safe and non-irritant on ocular application.

CONCLUSION

Eudragit polymer is most efficiently used polymer for ocular administration, mainly for poorly soluble drugs. Solvent displacement method, by using Eudragit RL 100 is a simple, easy and reliable method for the preparation of flurbiprofen nanoparticles. Eudragit RL 100 has a good permeability character and also bears positive charge, which helps in charge interaction with corneal mucin to retain the drug for prolonged period of time; thereby drug release was slow and gradual through diffusion. The prepared formulation can store not only in refrigerator but also stable at room temperature. *In vivo* studies revealed that that the flurbiprofen nanosuspension has good ocular tolerance and safe. EUDRAGIT RL 100 nanosuspention should be recommended as a good vehicle for ophthalmology suspension.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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