Extended levobunolol release from Eudragit nanoparticle-laden contact lenses for glaucoma therapy

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Abstract

Background: Majorly, the reason for the permanent loss of vision is glaucoma. But the currently available common treatment methodologies such as eye drops have various disadvantages like patient incompliance due to repeated administration and poor (1–5%) bioavailability leading to poor efficiency. The objective of this research was to formulate Eudragit-based nanoparticles of levobunolol incorporated into a contact lens to obtain sustained ocular delivery of levobunolol at the therapeutics level. Eudragit nanoparticles of levobunolol were formulated by nanoprecipitation methodology utilizing different ratios of Eudragit S100 and polyvinyl alcohol. The prepared nanoparticles were evaluated and optimized by efficiency of entrapment, particle size, morphology of surface and zeta potential. The optimized nanoparticles were then entrapped into the matrix of the contact lens by the soaking method which were then characterized and compared for optical clarity study, equilibrium swelling study, shelf life and in vitro drug release in simulated tear fluid followed by ex vivo transcorneal permeation study.

Results: Formulation F3 was obtained as optimized nanoparticle formulation with 102.61 nm ± 3.92 of particle size, −22.2 mV ± 2.76 of zeta potential and 86.995% ± 1.902 of efficiency of entrapment. The equilibrium swelling index and transmittance of nanoparticle incorporated into contact lenses showed better results when compared to drug solution-loaded lenses. In vitro release indicated more sustained drug profiles (84.33% ± 0.34 of drug release over a period of 12 days) as compared to drug solution-loaded lenses (89.282% ± 0.900 of drug release over a period of 3 days). Ex vivo transcorneal permeation studies showed more permeation (6.75% ± 0.170) through contact lenses as compared to marketed eye drops (3.03% ± 0.088).

Conclusion: This research demonstrates the remarkable results of drug-laden contact lenses to serve as a great medium for the continued delivery of ocular drugs without affecting the physical and optical characteristics of the lens content.

Keywords: Levobunolol, Eudragit S100, Polyvinyl alcohol, Nanoparticle, Contact lens, Sustained delivery

Background

Glaucoma is the world’s number one cause of permanent loss of vision. Glaucoma impacts over 70 million people globally, with around 10% becoming bilaterally blind, leading to permanent visual impairment. Glaucoma is associated with asymptomatic symptoms until it becomes severe, creating a high probability that the people most affected will be much larger than the number reported to possess it. Glaucoma is a very serious and complicated ophthalmic condition that affects approximately 70% of people over 40 years of age [1–3]. Glaucoma management has no permanent cure, so its treatment regimen is very long and it is also very difficult for the patient to remember to instil eye drops every day. Conventional eye drops are the most common eye disease management approaches, but they are highly

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ineffective due to a poor bioavailability of less than 1–5%. As the drug is maintained in the eye for a relatively short duration, eye drops are instilled 3 to 4 times every day, often in large doses. Frequent eye drop administration leads to inconvenience in patients and causes sensitivity reactions, pigmentation of the cornea and mechanical injury of the conjunctiva and eyelid. The currently available anti-glaucoma drug formulations are not very effective as the concentration of the drug increases initially but it drops below the effective concentration very soon. As a result, the intraocular pressure rises and declines. Therefore, ocular therapies with such classes of drug can be significantly improved by interventions with the ophthalmic drug delivery system [4, 5].

Contact lenses are being used for medication delivery into the eyes from the past few years. Such lenses are primarily used for the correction of eye refractive errors. Simultaneous correction of the refractive error would provide a prominent complement to the use of contact lenses and thus receive drug treatment simultaneously. Several studies have suggested lenses for controlled delivery of drugs as a medical aid. Since it shows bioavailability of far more than 50% relative to the eye drop treatment, the utilization of contact lenses as a system for delivery of medications has a growing interest in enhancing the effectiveness of eye care. Contact lenses lead to dose reduction, increased bioavailability, increased residence time and reduced side effects [6, 7].

The major drawback of contact lens soaked with a drug is shorter drug diffusion which is unable to obtain sustained delivery of the drug. Thus, to overcome this, it has been suggested that formulations based on vesicular carriers can maintain the medication in cold storage during storage as well as provide prolonged release after incorporation into the eyes as recorded in different studies. Sustained and controlled release of the drug was obtained from microemulsion-, microparticle-, and nanoparticle-based formulations [8, 9]. Also, medication-loaded microparticles and nanoparticles into the matrix contact lens can retain its optical and physical characteristics [10, 11].

Levobunolol (LB) is an effective non-selective beta-adrenergic blocker utilized for the treatment of glaucoma effectively. Levobunolol HCl (hydrochloric acid) is more than 60 times more potent in its beta-blocking activity than its dextro-isomer (bunolol). Many studies have shown that the levobunolol shows the same efficiency as of timolol in beta-blocking activity with better absorption of the ocular and 10 times greater elimination. Levobunolol lowers mean intraocular pressure (IOP) from baseline by around 30 to 40% in glaucoma patients with elevated IOP [12–14]. For the diagnosis of open-angle glaucoma as a first-line medication, Betagan® eye drop (containing 0.5–1% of levobunolol) is recommended [15].

The purpose of this research was to formulate therapeutic contact lenses loaded with levobunolol nanoparticles for the effective treatment of glaucoma. Eudragit S100 (ES 100) was utilized for the development of Eudragit-based nanoparticles incorporating levobunolol. The Eudragit S100 has dissolution behaviour that shows characteristic release above pH 7.0. Hence, it was utilized to provide pH-dependent sustained drug release from the matrix of nanoparticles at the pH of tear fluid, i.e. 7.4 [16, 17]. Due to the positioning of lenses upon the cornea segregated by a thin fluid layer called post-lens tear film (POLTf), ophthalmic drugs can be administered very easily through the contact lens, as shown in Fig. 1. The time of residence of the drug in the POLTf is around 30 min, that is, substantially greater than those of eye drop drugs (5 min). Once these therapeutic contact lenses are positioned on the surface of the eye, the medication gradually diffuses from the matrix of the nanoparticle, passes via the lens structure and reaches the post-lens and pre-lens tear film, with far more long retention time compared to eye drops [18–20].

The prepared nanoparticle formulation was evaluated and optimized utilizing parameters such as entrapment efficiency, zeta potential and particle size. Furthermore, levobunolol nanoparticle (LB-NP)-loaded contact lens was evaluated for physical appearance, surface pH, transmittance, equilibrium swelling study, etc. In vitro studies with LB-ES 100 nanoparticle-loaded contact lenses were conducted and matched with the drug permeated through marketed eye drop formulation of the same drug (i.e. Betagan®). Similarly, ex vivo transcorneal permeation studies were also conducted using goat cornea as a membrane. Hence, the proposed research work was concerned to establish the potential advantage of sustained action of LB-NP-laden contact lenses for the delivery of drugs into the ocular system for the effective treatment of glaucoma.

Methods
Materials
Levobunolol and Eudragit S100 were procured as a gift sample from the Piramal Healthcare Pvt. Ltd., Mumbai, India, and Evonik India Pvt. Ltd., Mumbai, India, respectively. Polyvinyl alcohol was purchased from Central Drug House, Lab Reagents, Delhi, India. Aqualens Slip-on® (Omaxilcon A) contact lenses manufactured by Aqua Lens Pvt. Ltd., Haryana, India, were used in this study. Fresh goat eyes were purchased from a local butcher’s shop. 0.5% eye drop of levobunolol was purchased from a local pharmacy. All other solvents and chemicals were of analytical grade.
Drug excipient interaction study
Fourier transform infrared analysis (FTIR) spectra were obtained on a PerkinElmer FTIR spectrometer, USA. Powdered sample spectra were obtained using the method of potassium bromide discs. In each case, the spectra were collected in the area 400–4000 cm\(^{-1}\) to study the interaction between the drug's and polymer's physical mixture.

Formulation of LB-loaded Eudragit S100 nanoparticles
The encapsulated LB nanoparticles were formulated by using the nanoprecipitation methodology as shown in Fig. 2. Different concentration ratios (1:1, 2:1, 1:3 and 3:2) of Eudragit S100 and polyvinyl alcohol (PVA) were taken for nanoparticle formulation. An aqueous solution of PVA and LB (5 mg/mL) was prepared, and then Tween 80 (1%) was added into it. Separately, an organic

Fig. 1 Schematic diagram of drug-eluting therapeutic contact lens containing LB-loaded polymeric nanoparticles

Fig. 2 Schematic representation of various steps involved in ES 100 nanoparticle preparation
solution of Eudragit S100 in ethanol was prepared and then added to the aqueous solution dropwise under continuous magnetic stirring at 800 rpm at 25 °C. Tween 80 was added in the aqueous phase in order to avoid polymer aggregation. Through evaporation, the organic phase is completely removed, leaving the nanoparticles suspended in the aqueous phase. After the organic solvent evaporates, the prepared nanoparticles were centrifuged at 18,000 rpm, 4 °C for 30 min, and nanoparticles obtained after centrifugation were washed with distilled water several times and kept at 4 °C [21–23].

The following mentioned ratios of ES100 and PVA as shown in Table 1 are used for the LB-NP preparation. The final selection of optimized particles for loading in contact lenses was determined on the basis of the results of particle size distribution, zeta potential and efficiency of entrapment of all the formulations.

**Characterization and optimization of Eudragit S100 nanoparticles**
The formulated nanoparticles were characterized and optimized on the basis of the parameters listed below.

**Physiochemical evaluation**
Particle size, zeta potential and polydispersity index of ES 100 nanoparticles were obtained by utilizing Nicomp® N3000 PSS sizing system (USA) which uses the principle of dynamic light scattering (DLS). The prepared nanoparticles were diluted 5 times with distilled water and sonicated for 10 min. Triplicate readings were determined [24, 25].

**Drug entrapment study**
The entrapped LB in ES 100 nanoparticles was obtained by the centrifugation method, which involves separation of the entrapped drug from nanoparticles. For this, 1 mL of each formulation was taken which was centrifuged at 15,000 rpm for 30 min at 4 °C by cooling centrifuge (R-8C, Remi instruments, Mumbai, India). The supernatant was obtained and evaluated at 257 nm by an ultraviolet-visible spectrophotometer (UV 1800, Shimadzu, USA) for LB concentration. The same procedure was repeated with all ratios of formulation prepared taking \( n = 3 \) [26, 27]. The percentage entrapment efficiency of LB was calculated using the equation given below:

\[
\text{Entrapment efficiency} = \frac{\text{Calculated drug content}}{\text{Theoretical drug content}} \times 100
\]

**Transmission electron microscopy (TEM)**
The prepared Eudragit nanoparticles were characterized by TEM at 300 kV (JEOL 2100F, USA) to determine size and surface morphology. A drop of prepared dispersion was stratified over a carbon film-covered copper grid for approximately 15 min and then negatively stained with 0.1% phosphotungstic acid. The sample was allowed to air dry and examined with a Hitachi H-7500 transmission electron microscope (Hitachi Ltd., Tokyo, Japan) at an accelerating voltage of 80 kV.

**Development of levobunolol nanoparticle-loaded contact lens**

**Selection of contact lenses**
As the present study involves continuous lens wear for few days, it is very important that the contact lenses selected for the study should not interfere with the normal vision of the patient, should be comfortable to wear and provide sufficient water content and oxygen permeability to prevent hypoxia and should have minimal chances of causing any eye infections [28]. Five types of contact lenses were purchased from various manufacturers in India for the study and were checked for their water content and oxygen permeability.

**Loading of LB-NPs into the matrix of contact lenses**
The soaking method was employed for the loading of LB-NPs into the matrix of contact lenses. Primarily, the contact lenses were soaked for 10 min in 30 mL of

| Formulation code | Ratio ES 100:PVA | ES 100 (%) | PVA (%) | Tween 80 (%) | Drug (mg) |
|------------------|-----------------|-----------|---------|--------------|-----------|
| F1               | 1:1             | 0.5       | 0.5     | 1            | 50        |
| F2               | 2:1             | 1         | 0.5     | 1            | 50        |
| F3               | 3:2             | 1.5       | 1       | 1            | 50        |
| F4               | 1:2             | 0.5       | 1       | 1            | 50        |
| F5               | 1:3             | 0.5       | 1.5     | 1            | 50        |
| F6               | 2:3             | 1         | 1.5     | 1            | 50        |
| F7               | 3:1             | 1.5       | 0.5     | 1            | 50        |
Millipore water. They were then submerged in 10 mL of ethanol for 10 min, resulting in swelling and an increase in the contact lens thickness, which would further improve the drug uptake by the lenses. Then, each lens was then soaked in 5 mL of LB-NPs (5 mg/mL) solution for 24 h. After loading, the lenses were blotted to remove any residual surface drug solution by using clean filter paper (without lint) and then used for further studies [29].

**Therapeutic dose estimation**

The prescribed dose of Betagan® eye drop (0.5 % w/v solution of LB solution) is 1 drop 2 times daily for glaucoma therapy with 50 μL of volume remained in the eye. Thus, a drop administered twice a day would yield 500 μg of levobunolol per day but levobunolol bioavailability obtained by eye drops is just about 5%. Levobunolol’s clinical requirement from the contact lens is about 25 μg per day as its bioavailability is 50% [15, 30, 31].

**Characterization of prepared LB-NP-loaded contact lenses**

**Physical appearance**

The physical appearance of NP-loaded contact lenses was judged visually for transparency and change in colour.

**Optical clarity studies**

LB-NP contact lenses’ optical properties should not alter after nanoparticle loading. Thus, the percentage transmittance through contact lenses was determined. For clear vision, more than 90% of transmittance should be achieved. The control contact lens was hydrated for 24 h by soaking in simulated tear fluid, and placed in a quartz cuvette and scanned in a UV spectrophotometer (UV 1800, Shimadzu, Japan) at wavelengths ranging from 200 to 1000 nm. Similarly, transmittance of LB-NP contact lenses has been noted and results have been compared with the standard to obtain any significant changes in optical clarity that may affect normal vision compared to the marketed contact lens [24]. The values of absorbance of the subsequent lenses were determined and the values of transmission (%) were obtained using the formula:

\[ A = 2 \log_{10} \%T \]

where \( A \) = absorbance and \( \%T \) = percentage transmittance

**Equilibrium swelling study**

Equilibrium swelling percentage was determined using the mass balance method. This study was carried out by placing contact lenses in LB nanoparticles until the equilibrium was achieved. After 24 h of swelling, the tissue was utilized to dry the lenses and weighed \((W_{\text{wet}})\) in air utilizing sensitive balance weighing (Sartorius). After this step, the lenses were allowed to dry and the initial dry weight \((W_{\text{dry}})\) was determined. The ratio of swelling at equilibrium was determined by the equation:

\[
\text{Equilibrium swelling index} = \frac{W_{\text{wet}} - W_{\text{dry}}}{W_{\text{wet}}} \times 100
\]

The swelling index of LB-NP-loaded contact lenses was then matched to that of the contact lenses marketed in order to understand any major variables in the study due to loading [26, 31].

**In vitro drug release in simulated tear fluid**

The LB-NP-loaded contact lenses were positioned in 3 mL of simulated tear fluid (STF pH 7.4) at the room temperature in 5-mL glass tubes and kept in a shaking incubator at 100 rpm for the release study. The release medium volume was selected to be 3 mL roughly equivalent to the in vivo conditions of normal tear turnover of the human eye. The simulated tear fluid was substituted with the equal volume (1 mL) of fresh simulated tear fluid at each interval (every 24 h), in order to obtain perfect sink conditions. The dynamic concentration of the drug in tear fluid was estimated using a UV-visible spectrophotometer (UV 1800, Shimadzu, Japan) to measure the absorbance at 257 nm. Levobunolol release profile was assessed by plotting graphs of cumulative drug release (μg) versus time. The experiments were conducted in triplicates [32, 33].

**Determination of drug release kinetics**

To determine the kinetics of release of medication from LB-NP-laden contact lenses, the in vitro release data was fitted in Higuchi models, Peppas, first order and zero order [34].

**Ex vivo transcorneal permeation study**

The ex vivo transcorneal permeation studies demonstrate the effect of the prepared LB-loaded contact lenses on the corneal permeation. Goat cornea excised freshly, free of adhering sclera was first fixed between donor and acceptor compartments of modified Franz diffusion cell in such a way that the epithelial surface faced the donor compartment and the endothelial surface faced the receptor section. The region available for diffusion on the cornea was 0.50 cm². Simulated tear fluid (pH 7.4) of volume 10 mL was added to the receptor compartment. The contact lenses loaded with the LB-NPs were then placed on the top of the cornea surface. A Teflon-coated magnetic stirred bead was used to keep the receptor fluid at 37 °C. One milliliter of the sample was removed from the receiver compartment and replaced by the
Fig. 3 FTIR spectra of the drug

Fig. 4 FTIR spectra of Eudragit S100
Fig. 5 FTIR spectra of PVA

| Sample Name | Description | Quality Checks |
|-------------|-------------|----------------|
| divya 19(13)| Sample 019 by divya Date Friday, January 31 2020 | The Quality Checks give rise to a Weak Bands warning for the sample. |

Fig. 6 FTIR spectra of physical mixture
same volume of simulated tear fluid for maintaining sink conditions. All samples were analysed in a UV-visible spectrophotometer (UV 1800, Shimadzu, Japan) for LB content by determining absorbance at 257 nm. Likewise, LB solution-loaded contact lenses were also used to study transcorneal permeation through goat cornea to achieve comparative results. The permeation was studied for 6 h, and all the experiments were undertaken in triplicate [35, 36]. Percentage transcorneal permeation has been measured as:

\[
\text{Permeation} \% = \frac{x}{\frac{\text{Drug uptake by contact lens}}{C2}} \times 100
\]

**Table 2** Data showing particle size distribution, zeta potential and entrapment efficiency in different nanoparticle formulation

| Formulation code | Particle size (nm) ± SD | Zeta potential (mV) ± SD | PDI ± SD | Entrapment efficiency (%) ± SD |
|------------------|-------------------------|--------------------------|---------|-------------------------------|
| F1               | 235.87 ± 6.87           | −29.9 ± 2.13             | 0.341 ± 0.039 | 67.701 ± 1.902 |
| F2               | 135.04 ± 6.93           | −22.3 ± 4.72             | 0.485 ± 0.074 | 80.564 ± 2.938 |
| F3               | 102.61 ± 3.92           | −22.2 ± 2.76             | 0.415 ± 0.027 | 86.995 ± 1.902 |
| F4               | 304.62 ± 15.56          | −18.3 ± 5.95             | 0.372 ± 0.055 | 63.152 ± 1.245 |
| F5               | 233.55 ± 5.920          | −23.8 ± 5.39             | 0.338 ± 0.060 | 70.524 ± 2.414 |
| F6               | 150.04 ± 7.74           | −29.7 ± 2.02             | 0.368 ± 0.030 | 77.113 ± 2.591 |
| F7               | 168.61 ± 13.56          | −26.18 ± 3.55            | 0.387 ± 0.032 | 68.172 ± 2.121 |

**GAUSSIAN SUMMARY:**

- Mean Diameter = 102.4 nm
- Variance (P.I.) = 0.560
- Std. Deviation = 76.6 nm (74.8%)
- Chi Squared = 15.105
- Norm. Stnd. Dev. = 0.748
- Baseline Adj. = 0.000 %
- (Coeff. of Var’n)
- Z-Avg. Diff. Coeff. = 4.54E-008 cm2/s

**Fig. 7** DLS report for the particle size of the optimized nanoparticle formulation
Shelf life study of LB-NP-loaded contact lenses

Shelf life was performed to evaluate the drug leached from the nanoparticle-loaded contact lenses in the packed liquid. The drug-incorporated lens was stored in the packaging solution for a period of 3 months at room temp. Then, the lenses were removed and the packed solution was analysed for LB content leached in packed solution for a period of 3 months using a UV-vis spectrophotometer (UV 1800, Shimadzu, Japan) [31].

Results

FTIR analysis

The infrared spectrum of levobunolol showed a C–O bond due to a strong band seen at 1239 cm$^{-1}$. Other characteristic bands seen in the spectra which authenticate it to be of the LB are C=C strong stretching band of benzene ring observed as a doublet at 1475 which is characteristic of the benzene skeletal. C–N band at 1264 cm$^{-1}$ further confirms the drug. A weak C–H stretching band at 2805 cm$^{-1}$ shows the presence of alkane. An N–H stretching band at 3368 cm$^{-1}$ was also observed. Hence, all the bands were found to be in correlation with the chemical structure of LB. Thus, this characterizes the drug to be LB as shown in Fig. 3. Eudragit S100 showed peaks at 1446.14 cm$^{-1}$, 1723.02 cm$^{-1}$ due to CH$_3$ bond and the presence of C=O ester respectively as shown in Fig. 4, confirming the structure of Eudragit S100. PVA showed the FTIR spectrum of PVA showed the stretching vibrations of CH2 asymmetric stretching at 2980.82 cm$^{-1}$ and C–O stretching vibrations at 1087.49 cm$^{-1}$ as shown in Fig. 5. No significant peak shift was observed in the physical mixture of levobunolol hydrochloride (1238 cm$^{-1}$, 1479 cm$^{-1}$, 1264.82 cm$^{-1}$, 2806 cm$^{-1}$, 3368.34 cm$^{-1}$), Eudragit S100 (1457 cm$^{-1}$, 1676.16 cm$^{-1}$) and PVA (2979.05 cm$^{-1}$, 1056 cm$^{-1}$) as shown in Fig. 6.

Characterization of Eudragit S100 nanoparticles

Physiochemical evaluation

Particle size, zeta potential and polydispersity index (PDI) of ES 100 nanoparticles are reported in Table 2. The size formulated by nanoprecipitation methodology was obtained in Nano range, indicating its suitability. From Table 2, it is clearly seen that the minimum particle size was found at 3:2 and 2:1 ratios of Eudragit S100 and polyvinyl alcohol (Fig. 7). The PDI obtained by nanoprecipitation methodology indicates the uniform size distribution of prepared nanoparticles. The highest PDI values were found at 3:2 and 2:1 ratios of Eudragit S100 and polyvinyl alcohol. It was observed that as the Eudragit S100 concentration increased, the particle size of nanoparticles decreased.

The zeta potential on Eudragit S100 nanoparticles was found negative for all nanoparticle’s batches (Table 2), although there was really no perceptible variation between the values of zeta potential of the various nanoparticles. The optimum value of zeta potential was found to be $-22.2 \pm 2.76$ mV at the 3:2 ratio of Eudragit S100 and polyvinyl alcohol. Eudragit S100 has characteristic dissolution behaviour at pH 7.0. So at pH 7.0, it became highly ionized resulting in high zeta potential which can provide an electric repulsion to avoid particle aggregation.

Table 3 FDA-approved silicon contact lenses and their properties

| Commercial contact lens | Iconnect | Slip-on | Optima 38 | Acuvue Moist | Softlens |
|------------------------|----------|---------|-----------|--------------|---------|
| Manufacturer           | Bausch & Lomb | Aquawell | Bausch & Lomb | Johnson & Johnson | Bausch & Lomb |
| Hydrogel material      | Hilafilcon B | Omafilcon A | Polymacon | Etafilcon A | Hilafilcon B |
| Percentage of water content | 48       | 58      | 38.6      | 58            | 56      |
| Oxygen transmissibility| 1.0      | 28–36.7 | 8.5–24.3  | 23.8–28      | 22      |
| FDA group              | II       | II      | III       | IV            | II      |
| Water content          | High     | High    | Low       | High          | High    |
|                        | Non-ionic | Non-ionic | Ionic     | Ionic         | Non-ionic |
Drug entrapment study
The distribution of percentage entrapment efficiency among different formulations of Eudragit S100-based LB-loaded nanoparticles is shown in Table 2. Maximum entrapment of LB is found at the 3:2 ratio of Eudragit S100 and polyvinyl alcohol. It was observed that as the concentration of Eudragit increased, keeping concentrations of PVA constant, there was an increase in entrapment efficiency.

It is clearly seen that the formulation (F3) having 3:2 ratio of ES 100 and PVA is the optimized formulation, depending on the findings of size, zeta potential and efficiency of entrapment [37].

Transmission electron microscopy (TEM)
A transmission electron microscope was used for the visualization of optimized Eudragit nanoparticle formulation (F3). The nanoparticles were found to be spherical in shape, and they appeared dark in bright surroundings. The droplet size was approximately 178 to 246 nm in TEM images, and this was consistent with our previous results obtained by using a size analyser (Fig. 8).

Development of levobunolol nanoparticle-loaded contact lens
Selection of contact lenses
For this study, five types of contact lenses are purchased from various manufacturers in India for the study and checked their water content and oxygen permeability as mentioned in Table 3. From the table, it is clearly seen that AquaLens® “Slip-on” contact lens containing Omafilcon hydrogel material possesses the maximum water contact and highest oxygen transmissibility while the other four contact lenses did not possess significant oxygen transmissibility and water content [38].

Drug uptake by contact lenses
After 24 h of drug loading, the lenses were removed from LB-NPs as well as from LB solution and soaked in 10 mL of ethanol for 24 h to extract the absorbed drug from the matrix of hydrogel contact lens. Then, the ethanolic solution was obtained by a UV spectrophotometer at 257 nm. The drug uptake by the contact lenses from both the loading solution is mentioned in Table 4 [39, 40]. No further drug uptake was observed by the contact lens.

Characterization of the prepared LB-NP-loaded contact lenses
Physical appearance
The NP-loaded contact lenses were found to be transparent, and there was no change in colour as compared to the control lenses when inspected visually as shown in Fig. 9.

Optical clarity study
The optical clarity was evaluated by noting the transmittance of control as well as nanoparticle-loaded contact lens at 630 nm in a UV-visible spectrophotometer, and the lens was found to be clear and exhibited maximum transmittance of 97.15% in the UV range as evident from Table 5. Both the control and NP-loaded contact lenses show similar percentage transmittance; this is due to nanoparticles loading to the contact lens matrix.

| Table 4 Drug uptake by contact lenses after 24 h of soaking period |
|---------------------------------------------------------------|
| From LB solution-loaded contact lenses (n = 3) | From LB-NP-loaded contact lenses (n = 3) |
| 0.6851 mg ± 0.23 | 0.5676 mg ± 0.024 |

Fig. 9 Visual appearance of the control contact lens and LB-NP-loaded contact lens
Equilibrium swelling studies

The swelling index of LB-NP-loaded contact lenses is compared with that of drug solution-loaded contact lenses to identify any significant changes in results due to loading as shown in Table 5. The NP-loaded contact lenses swell slightly less than the control contact lens.

This suggests that the presence of Eudragit nanoparticles lead to increased crosslinking in the contact lenses. Increased crosslinking will also lead to slow and sustained release of the drug.

In vitro drug release in simulated tear fluid

The cumulative drug release in fresh simulated tear fluid from LB-NP-based lenses incorporated with 0.5676 mg of LB is shown in Fig. 10. In the release studies, an initial release was burst as obtained by all batches, which can be due to the drug’s low molecular weight and high solubility of LB in STF. Then, sustained release for up to 12 days was achieved with a percentage of 82.33 ± 0.34. Also, during the entire test, the lenses looked translucent, suggesting the existence of medication at nanosize following dissolution of Eudragit S100, whereas around 91.12% ± 0.900 of the LB is released in 3 days from the contact lenses showing high burst release.

Determination of drug release kinetics

Table 6 shows the slope and $r^2$ values obtained from the zero order, first order, Peppas and Higuchi models. It was observed that the release of the drug from the developed contact lenses was considered to be following zero order kinetics as it showed the highest $r^2$ value and follows the Peppas model for drug release. The plot’s $n$ value was 1.39, indicating that the mechanism for releasing drugs is super case II transport.

Ex vivo transcorneal permeation studies

Goat cornea excised freshly was being carefully dissected from the eyeball along with the 2–4 mm of encircling sclera tissue and rinsed with saline water to remove any adhering pigments as shown in Fig. 11. The results obtained for percentage cumulative drug release from optimized LB-NP-loaded contact lenses and LB solution-incorporated contact lenses are shown in Fig. 12.

As the 5676 μg ± 0.024 amount of drug was loaded in the lens, LB-NP-loaded contact lenses showed the higher permeation across the freshly excised goat cornea (6.756% ± 0.170) in 6 h as compared with the LB solution-loaded contact lenses (3.033% ± 0.088).

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**Table 5** Percentage transmittance and ESI of the contact lens

| % transmittance of contact lenses | Equilibrium swelling index (%) |
|-----------------------------------|--------------------------------|
| Control contact lens ($n = 3$)    | NP-loaded contact lens ($n = 3$) |
| 97.923 ± 0.189                    | 97.15 ± 0.261                  |
| Drug solution-loaded lenses ($n = 3$) | LB-NP-loaded lenses ($n = 3$)    |
| 27.016 ± 0.310                    | 25.47 ± 0.520                  |

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**Fig. 10** Cumulative drug release profile of LB solution- and LB-NP-loaded contact lenses
Shelf life study of LB-NP-loaded contact lens
The drug release from NP-loaded contact lenses after 1, 2 and 3 months was obtained as 1.210% ± 0.109, 1.894% ± 0.161 and 2.381% ± 0.091 of leaching, respectively. Also, the drug content as measured by the UV spectrophotometer after 1, 2 and 3 months was obtained as 98.84% ± 0.41, 98.21% ± 0.15 and 97.88% ± 0.63, respectively. This data suggests that considering 5676 μg±0.024 of loading, the drug loss during the storage period was not significant. The Eudragit nanoparticles therefore prevented the drug loss in the packed solution during the shelf life of the therapeutic contact lenses.

Discussion
The presented work successfully revealed the potential advantages of delivering levobunolol from Eudragit nanoparticles through contact lenses for glaucoma management. The DLS report confirmed the uniform distribution of nanoparticles and their nanosize.

The formulation batch was found to be optimized with the 3:2 ratio of ES 100 to PVA. Optimized nanoparticles showed particle size and zeta potential of 102.42 nm ± 15.56 and −22.2 mV ± 2.76. The maximum entrapment efficiency was found to be 86.99% ± 1.90.

Depending on the water contact and oxygen transmissibility [38], Aqua Lens Slip-on contact lens containing Omafilcon hydrogel material was selected for the study.

The maximum drug uptake by the contact lenses was 0.5676 mg after 24 h of the soaking period [39, 40]. The LB-NP-loaded contact lenses exhibit 97% UV transmittance in the UV region, which shows that the nanoparticle loading did not interfere with the contact lenses’ optical and physical properties. The decrease in the swelling index of LB-NP-loaded contact lens data indicates that the presence of nanoparticles contributes to increased crosslinking in the gels due to the use of polymers in nanoparticle preparation [41].

The Eudragit S100 NPs prevented the loss of drug in the packaging solution during the shelf life of 3 months.

The in vitro drug release study from LB-NP-based lenses demonstrates initial burst release, followed by a sustained therapeutic release for up to 12 days. This indicates the controlled release of LB from pH-sensitive Eudragit S100 nanoparticles. Eudragit nanoparticle dissolution led to the development of Nano cavities, which regulated the rate of drug release from hydrogels. It also was found that within the nanoparticles, some portion of the medication stayed indefinitely bound while 91% of the drug from LB solution-Incorporated lens was released within 3 days because no layer was formed on the lens surface which may provide for the medication slow release.

The drug release from the developed contact lenses was found to follow zero order kinetics with an \( R^2 \) value of 0.9961. Fickian diffusion with swelling was the exact real mechanism behind drug release from LB-NP contact lenses.

The ex vivo permeation data suggest that there is more than 40–50% permeation achieved with LB-NP-loaded contact lenses as compared to marketed eye drops due to the presence of POLTF. This increase in the permeation through nanoparticle-loaded contact lenses across the cornea is due to the presence of

Table 6 Release kinetics

| Model       | Slope (n value) | \( R^2 \) |
|-------------|----------------|-----------|
| Zero order  | 6.9537         | 0.9961    |
| First order | −0.0018        | 0.9955    |
| Higuchi model | 25.67      | 0.8979    |
| Peppas model | 1.39           | 0.9052    |

Fig. 11 Images showing the a freshly excised goat cornea and b dissected goat cornea with adhering sclera
POLTF from which the medication is administered gradually into the precorneal region [36].

**Conclusion**

The utilization of contact lenses as a system for delivery of medication is significantly important in improving the efficiency of ocular treatment to overcome the limitations of poor bioavailability, patient incompliance and reduced efficacy associated with conventional eye drop formulations. Loading of the medication into the contact lenses enhances the release duration of the drug from several hours to several days. The presented work successfully revealed the potential advantages of delivering levobunolol from Eudragit nanoparticles through contact lenses for glaucoma management. The optimized nanoparticle formulation was discrete and spherical. The equilibrium swelling index and transmittance of nanoparticle incorporated into contact lenses showed better results when compared to drug solution-loaded lenses. The in vitro release of the drug study demonstrates burst release initially, preceded by a sustained therapeutic release for up to 12 days. This indicates the controlled release of LB from pH-sensitive Eudragit S100 nanoparticles. The ex vivo permeation data suggest that there is more than 50% permeation with LB-NP-loaded contact lenses as compared to marketed eye drops due to the presence of POLTF. On the basis of the results, it is inferred that effective glaucoma treatment is possible through the development of contact lenses loaded with LB-NPs, which releases the drug in a controlled way over an extended period of time.

**Authors’ contributions**

NK contributed to the drafting, design and analysis of the data. RA contributed to the drafting, design and analysis of the data. MKC contributed to draft revision and data interpretation. All “authors” have read and approved the final manuscript.

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**Availability of data and materials**

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Not applicable

**Competing interests**

The authors declare that they have no competing interests.

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