Preparation and Characterization of Tacrolimus-Loaded SLNs in situ Gel for Ocular Drug Delivery for the Treatment of Immune Conjunctivitis

Kexin Sun
Ke Hu

Department of Ophthalmology, The First Affiliated Hospital of Chongqing Medical University, Chongqing Key Laboratory of Ophthalmology, Chongqing Eye Institute, Chongqing, People’s Republic of China

Background: The aim of this study is to develop a novel in situ gel of tacrolimus-loaded SLNs (solid lipid nanoparticles) for ocular drug delivery.

Methods: The optimal formulation was characterized by surface morphology, particle size, zeta potential, entrapment efficiency, drug loading and in vitro release behavior. In vivo studies were also conducted to evaluate the pharmacokinetic and pharmacodynamic results.

Results: In this study, TAC-SLNs ISG were prepared using homogenization followed by probe sonication method. The average particle size of TAC-SLNs ISG was observed to be 122.3±4.3 nm. Compared with TAC-SLNs, in situ gel did not increase particle size, and there was no significant difference between them. The results of viscosity measurement showed that TAC SLNs-ISG were typical of pseudo plastic systems and showed a marked increase in viscosity as temperature increased and ultimately formed a rigid gel (32°C). In vitro and in vivo studies illustrated the sustained release model of the drug from TAC-SLNs ISG. Animal model showed that TAC-SLNs ISG had good pharmacodynamics when compared with eye drops and SLNs.

Conclusion: Our results demonstrated that TAC SLNs-ISG had the potential for being an ideal ocular drug delivery system.

Keywords: tacrolimus, in situ gel, SLNs, ocular drug delivery

Introduction

Immune mediated inflammatory anterior segment diseases (IIAODs) are a kind of common ophthalmic diseases, including conjunctivitis and anterior uveitis. This kind of disease may cause some serious complications and sequelae, which is one of the main causes of blindness. Conjunctivitis requires antibiotic treatment for 5–7 days, which may lead to poor compliance with conventional dosage forms because of the higher frequency of administration, ie 2 drops per day. The reason for that is in the process of ophthalmic administration, due to the physiological constraints of eye protection mechanism, the drug absorption rate is low and the duration of treatment effect is short. When the drug solution drops into the eye, effective tear drainage and blinking can reduce the drug concentration by 10 times in 4–20 minutes.1 We can improve the ocular therapy by improving the precorneal residence time of drugs. In order to slow down drug elimination and to prolong the resident time on the ocular surface, several new preparations have been developed for ocular use, such as liposomes, nanoparticles and nanocapsules.2–8

Many agents available for the treatment of conjunctivitis and tacrolimus (TAC) are the widely used novel macrolide immunosuppressants. However, TAC is
Materials and Methods

Materials

TAC was purchased from Kerui Pharmaceutical Co., Ltd (Fujian, China). Compritol® 888 ATO (glyceryl behenate) and glyceryl monostearate (GMS) were purchased from Gattefossé. Pluronic® F-68 (Poloxamer 188), Pluronic® F-127 (Poloxamer 407) and Tween® 80 were obtained from Sinopharm Chemical Reagent (Shanghai, China). All the other reagents used were of analytical grade and were used without further purification.

Preparation of TAC SLNs-ISG

TAC SLNs-ISG were prepared by using homogenization coupled with ultra-probe sonication method. Briefly, Compritol® 888 ATO (0.25%, w/v) and GMS (2%, w/v) were dissolved in the dichloromethane (2 mL) and were heated to 80 °C, after that TAC (0.1%, w/v) was added under magnetic stirring until a clear drug-lipid phase was obtained. The organic solvent was evaporated in vacuum at 45 °C to form the lipid film, which was further dried in the vacuum drying oven at 37 °C to remove the residual organic solvent. Simultaneously, the aqueous phase, consisting of Tween-80 (0.5%, w/v) and glycerin (2%, w/v) in distilled water, was heated (30 °C) and transferred to the molten drug-lipid mixture under constant stirring, to form a pre-mixture. The mixture was homogenized with a probe-type ultrasonicator (S220, Covaris, USA) in the ice bath for 10 cycles to get the TAC SLNs. Then TAC SLNs-ISG was obtained by mixing TAC SLNs and Poloxamer 188 (12%, w/v)/Poloxamer 407 (26%, w/v) solution in the ice bath.

Characterization of System

Particle Size and Zeta Potential

Intensity-mean particle size, zeta potential and polydispersity index of TAC SLNs-ISG were determined using a Zetasizer NanoZS (Malvern Instruments Ltd.). All measurements were performed under automatic mode at 25°C. The hydrodynamic diameter was calculated from autocorrelation function of the intensity of light scattered from particles with the assumption that the particles have a spherical form.

Entrapment Efficiency

The entrapment efficiency (% EE) was determined by measuring the concentration of unentrapped drug in the lipid dispersion. Briefly, the SLNs-ISG dispersion was subjected to
centrifugation for 30 min, 4 °C at 6000 rpm and the amount of TAC in supernatant was determined by dissolving supernatant in acetone and 0.1% acetic acid (1:1) mixture by HPLC. The amount of free drug in the supernatant was determined spectrophotometrically at 210 nm. The entrapment efficiency was determined in triplicate and calculated as follows.

\[ EE\% = \frac{W_{\text{TAC}}}{W_{\text{SLNs-ISG}} + W_{\text{TAC}}} \times 100\% \]

\( W_{\text{TAC}} \) represents the amount of TAC loaded in the SLNs-ISG, \( W_{\text{SLNs-ISG}} \) represents the weight of the TAC SLNs-ISG.

**Viscosity Experiments**

The sol-gel transition temperature of TAC SLNs-ISG sol in water was measured by the tube transformation method. A vial containing 20 mL TAC SLNs-ISG sol was immersed in an oil bath at different temperatures to achieve equilibrium. When the flow rate was no longer visually observable within 30 s by inverting the vial, the temperature was increased by 2°C per step, and the sample was regarded as a “gel”. The viscosity of TAC SLNs-ISG in solution or gel was then measured using a rotating viscometer (60 rpm, rotor 2) with suitable samples (20 mL). The appropriate number of spindles was used for measurement at different speeds. The viscosity was read directly from the viscometer display.

**In vitro Release**

The in vitro release studies were conducted by a dynamic dialysis method. Briefly, TAC SLNs-ISG containing 20 mg were placed into dialysis bags, which were then placed into an end-sealed vial. The release medium was 2000 mL simulated tear fluid (STF; made with sodium chloride-0.67g, sodium bicarbonate-0.20g, calcium chloride dihydrate −0.008g in distilled water q.s 100 mL). The sealed vials were maintained at 37°C and 75 rpm in a gas bath thermostatic oscillator. Aliquots (1 mL) were withdrawn at intervals of 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 16 h, and the same replacement volume was supplied as the release medium. The collected samples were determined by HPLC. An equal amount of free TAC and TAC SLNs was used for comparison.

**Stability Studies**

Stability studies were carried out on TAC SLNs-ISG according to ICH (International Conference on Harmonization) Guidelines at 40°C, 75% relative humidity. Three packs of formulations were subjected to these stability studies. The samples were withdrawn at the 0th, 1st and 3rd month. Every time 0.1 mL of samples were withdrawn and analyzed for the assay with HPLC for the drug content. At the same time, some other stability parameters were also determined.

**In vivo Kinetics**

In vivo ocular disposition studies of TAC SLNs-ISG and TAC SLNs in comparison with TAC eye drops, were carried out in New Zealand rabbits, weighing between 2 to 3 kg. The rabbits were allowed to acclimatize to the new surroundings for one week, and the ocular disposition studies were then performed. A formulation of 50 μL of the TAC SLNs-ISG, TAC SLNs and TAC eye drop (0.1% w/v) was dripped into the cul-de-sac of the right eye while the left eye served as the control. Approximately 5 microliters of tear were collected from the cul-de-sac of the test eye, using a micropipette, at 15th, 30th min, 1st, 2nd, 4th, 5th, 6th, 8th and 10th h. Ten hours later, the rabbits were euthanized by intravenous injection of pentobarbital. Rinse eyes with cold DPBS and remove them immediately. The eye tissues were carefully separated and stored at −80°C until further analysis. Tear samples were collected in acetone: 0.1% acetic acid = (1:1) and analyzed by HPLC. The ocular tissue samples were extracted using acetone precipitation method and analyzed using LC-MS/MS system. Separation was achieved on a Genesis C18 column with a gradient mobile phase elution. Ammonium-adduct ions formed by a Turbo Ionspray in positive ion mode were used to detect each analyte. The MS/MS detection was traced by monitoring the fragmentation of 807.5 → 772.4 (m/z) for TAC on a triple quadrupole mass spectrometer (Sciex API 3000). The above method was simple, fast, and specific. The calibration curve of the LC assay for TAC in neat solution has good linearity (r²=0.996) over the range of 10–10,000 ng/mL. The lower limit of the quantitation of TAC in the LC-MS/MS assay was found to be 0.1 ng/mL. These results indicated that the assays provided good linearity and sensitivity for their specific applications.

**Pharmacodynamic Evaluation**

BALB/c mice were used to investigate the pharmacodynamic effect of TAC SLNs-ISG in this study. The grouping information was as follows: A: Normal (without any treatment, positive control); B: PBS (negative control); C: 0.1% (w/v) TAC eye drop; D: 0.1% (w/v) TAC-SLNs; E: 0.1% (w/v) TAC-SLNs ISG. ① Sensitization stage: BALB/c mice were intraperitoneal injected with Ovalbumin (OVA, 100 μg and 35 μg Al(OH)3 in 200 μL PBS) on immun-day 1. ② Immune intervention stage: on immun-day 7, the eyes were challenged with different formulations three times
a day for 5 days of the B, C, D, E group. ③Stimulation stage:

Ova (10 μL, 5 mg/mL) was dissolved in PBS (pH=7.0) after drug immune intervention treatment. The mice in groups B, C, D, E were injected with a micro sampler to induce the clinical symptoms of immune conjunctivitis. The clinical symptoms of immune conjunctivitis were observed and scored once a day for one week. Then the eyes were examined under microscope, and the scoring was performed at the same time every day and carried out once daily from day 1 to day 7. As shown in Table 1, mice were examined biomicroscopically based on 3 independent parameters. Each parameter was ascribed 0 (none) to 3+ points (serious) and was summed to yield a maximum score of 9+. ④After OVA challenge induced immune conjunctivitis, mice in groups B, C, D, and E were treated with PBS, 0.1% TAC eye drop, 0.1% TAC-SLNs, 0.1% TAC-SLN-ISG one time a day for 5 days. Scores of various systems in different groups were calculated and graphed. After pharmacodynamic study, OVA-s IgE, IFN-γ and IL-4 concentration in different treatment groups of mice were determined, respectively.

Meanwhile the pathological sections of corneal tissue of all groups were also observed. All animal experiments were performed in accordance with institutional guidelines, following the protocol approved by the Ethics Committees of Chongqing Medical University (19A2040). Strictly follow the National Institutes of health laboratory animal care and use guidelines.

Permeability Studies
Permeability studies were performed on the corneas isolated from rabbit whole eyes. The eyes were stored in Hanks’ balanced salt solution under ice-cold condition and shipped overnight. Immediately upon their receipt, the corneas were carefully separated, and used for the permeability studies. The isolated corneas were washed in ice-cold Dulbecco’s phosphate buffer saline (DPBS) solution, pH 7.4. The tissues were then mounted on Valia-Chien diffusion cells with the epithelial surface towards the donor chamber. The temperature of the diffusion cells was maintained at 32 °C with a circulating water bath, throughout the studies.

TAC concentration in eye drops, SLNs and SLN- ISG were kept at 0.1% w/v, and about 1 mL of the formulation was added to the donor chamber of the respective diffusion cells. Five milliliters of DPBS with 5% w/v hydroxyl propyl beta cyclodextrin (HPβCD) solution was used as the receiver medium and stirred continuously with magnetic stirrer. Samples (500 μL) were withdrawn from the receiver chamber at the predetermined time points up to 2 h and replaced with an equal amount of DPBS-5% HPβCD solution to maintain sink conditions. The samples were stored at −80 °C until further analysis by HPLC. The analyses for all the samples were carried out in triplicate.

The cumulative amount of TAC was calculated as per the equation:

$$M_n = V_r C_{r(n)} + \sum_{x=1}^{n} V_{s(x-1)} C_{r(x-1)}$$

where n is sampling time point; V_r and V_s are the volume in the receiver chamber (mL) and the volume of the sample collected at the nth time point (mL), respectively; and C_r(n) is the concentration of the drug in the receiver chamber medium at nth time point (µg/mL).

The rate of TAC transported across rabbit cornea was calculated using the slope of the cumulative amount of TAC transported versus time plot. The steady state flux of TAC was determined using the following equation:

Flux(J) = (dM/dt)/A

where M is the cumulative amount of drug transported and A is the surface area of the cornea (0.625 cm²).

The transcorneal permeability of TAC was calculated by the following equation:

Permeability = Steady state flux/Donor concentration.

### Table 1 Scoring of Conjunctivitis Signs and Symptoms

| Conjunctivitis Symptoms (Score) | None (0) | Slight (1) | Medium (2) | Serious (3) |
|-------------------------------|---------|-----------|-----------|------------|
| Conjunctival congestion       | -       | Pink      | Red       | Dark red   |
| Blepharoptosis                | -       | Edema of lower eyelid only | The upper and lower eyelids are edema, and the eyelids are partially closed | The eyelid is ectropion, edema is obvious, and the eyelids are closed |
| Secretion                     | -       | Mucilaginous liquid | The hair around the eyelids is moist | The hairs on the eyelids and around them are moist and sticky |
Statistical Analysis
The results were analyzed by extreme value analysis and ANOVA. SPSS software was used for statistical analysis. The difference was significant when $p < 0.05$.

Results and Discussion
Preparation and Characterization
The main purpose of the study is to develop SLNs and its corresponding in situ gel to retard the clearance of TAC in the eyes and increase drug absorption. According to the solubility of the drug in the lipid phase (using GMS, and Compritol 888 ATO as the lipid phase), Tween80 and glycerol were selected as the aqueous phase to design, prepare and optimize the SLN formulation.

TAC-SLNs ISG were prepared using homogenization followed by probe sonication method. The physicochemical properties including particle size, encapsulation efficiency (EE%) were listed in Table 2. The average particle size of TAC-SLNs ISG was observed to be 122.3 ± 4.3 nm. Compared with TAC-SLNs, in situ gel did not increase particle size, and there was no significant difference between them. Some researchers think that PDI value in the range of 0.01–0.5 represents a sufficiently narrow distribution range. In the case of TAC SLNs ISG, the PDI value of 0.21 was in this range, so it was considered to be a more uniform high-throughput and high permeability formulation, as shown by in vitro release data.25

Viscosity Experiments
The residence time of the ophthalmic formulation at the precorneal surface is affected by its viscosity that is of importance for enhancing the residence time. In context to ocular physiology, the range of shear rate experienced during relative movement of eyelids and globe is extremely wide ranging from 0.03 to 0.14 s$^{-1}$ during inter blinking period to 4250–28,500 s$^{-1}$ during a blink. Thus the viscosity of formulation should not be such that it disturbs the pseudoplastic behavior of tear film in the eye.26 Hence it is advisable to use a polymer with pseudoplastic character.27 Many literatures have confirmed that the gel prepared by poloxamer has the characteristics of pseudoplastic fluid.28,29 In order to study the effect of temperature on gelation, the viscosity of the gel was determined. The results of viscosity measurement showed that TAC-SLNs-ISG was a typical pseudoplastic system. With the increase of temperature, the viscosity increased obviously, and finally the rigid gel was formed. The gelation temperature of the prepared thermogelling gels was 32°C (Figure 1). At the same time, the viscosity of TAC-SLNs system did not increase with the change of temperature, and it always maintained at a relatively low viscosity level.

In vitro Release
The in vitro drug release curve of TAC SLNs-ISG, TAC SLNs and free TAC were shown in Figure 2. At the end of the experiment, nearly 80% of the drug was released in TAC SLNs group, while only 56% in TAC SLNs-ISG group did so. The release curve of TAC SLNs-ISG was a two-phase model. TAC SLNs-ISG released quickly at the early stage (about 30% TAC was released within 1 hour), and then released slowly. This phenomenon is mainly caused by the release of drugs dispersed in the gel. In the late stage, the sustained release was due to the fact that with the dissolution and diffusion mechanisms on the lipid matrices and polymer material, the solubilized or dispersed drug can only be released slowly. As was shown in Table 3, the Higuchi equation: $Q = 9.287t^{1/2} - 2.263$ ($r=0.994$) was the best fit well with the release kinetic model of TAC SLNs-ISG. Therefore, it was speculated that the sustained-release characteristics of TAC SLNs-ISG may enhance the ocular absorption of TAC.

Stability Studies
There were negligible alterations in the initial values of viscosity of the formulations over a storage of 3 months. The samples were also analyzed for drug content by HPLC analysis (data not shown). Again the drug degraded to a negligible extent, and the percentage of drug degradation is < 5%. Other stability parameters are shown in Table 2.

Table 2 The Characteristics of TAC-SLNs ISG and TAC-SLNs

| Parameters          | TAC-SLNs | TAC-SLNs ISG |
|---------------------|----------|-------------|
|                     | 0 m      | 1 m         | 3 m         |
| Particle size (nm)  | 121.5 ± 3.8 | 122.3 ± 4.3 | 125.5 ± 5.1 | 127.4 ± 5.4 |
| Encapsulation efficiency (%) | 84.6±6.2 | 85.9±7.2 | 84.4±6.7 | 82.6±4.3 |
| Polydispersity index | 0.24 | 0.21 | 0.24 | 0.26 |
| Zeta potentials (mV) | 23.7±3.8 | 26.8±3.4 | 28.4±4.1 | 29.3±4.5 |

Note: (n=3).
In vivo Kinetics

The concentration in tear-time plots, in rabbits after ophthalmic administration of test formulations are shown in Figure 3 and the AUC parameters are

Table 3 Release Kinetic of TAC SLNs-ISG

| TAC SLNs-ISG | Equation                                                                 | Correlation Coefficient (R) |
|--------------|---------------------------------------------------------------------------|------------------------------|
| Zero-order   | \( Q = 4.312t + 0.217 \)                                                  | 0.931                        |
| First-order  | \( \ln(1–Q) = -3.287t + 0.673 \)                                         | 0.971                        |
| Weibull      | \( \ln\ln(1/(1–Q)) = 4.938 \ln t + 1.332 \)                              | 0.954                        |
| Higuchi      | \( Q = 9.287t/1/2 - 2.263 \)                                             | 0.994                        |

Note: (n=6).
Table 4 Ocular Tear Pharmacokinetic Parameters of TAC in Tear versus Time Profiles in 600 Min for Various Formulations

| Formulation       | Cmax (ng/mL) | Tmax (h) | MRT (h) | T1/2 (h) | AUC0–t (ng min/mL) | AUC 0–t Ratio |
|-------------------|--------------|----------|---------|----------|--------------------|--------------|
| TAC eye drops     | 4657.7±412.2 | 30.2±4.3 | 1.28±0.45 | 1.84±0.76 | 222,382.5 ± 20,192.5 | –            |
| TAC-SLNs          | 1892.6±206.4 | 33.6±8.9 | 2.07±0.92 | 3.72±0.88a | 339,555.4 ± 31,927.2 | 1.53         |
| TAC-SLNs-ISG      | 2132.3±241.6 | 120.6±11.6 | 2.82±1.21a | 3.92±0.94a | 590,355.9 ± 57,281.7 | 2.61         |

Notes: Each value represents the mean ± SD of three determinations. *p<0.05 (compared to TAC eye drops). (n=6).

drug tabulated in Table 4. The Tmax was 30 min and the Cmax was 4657.7 ng/mL after ophthalmic administration of TAC eye drops. However, the time to achieve maximum concentration of TAC was delayed in the form of ISG. For TAC-SLNs, highest drug concentration of 1892.6 ng/mL was observed at the 30th min, whereas TAC-SLNs-ISG showed maximum concentration (2132.3 ng/mL) at the second hour. The Cmax of TAC-SLNs and TAC-SLNs-ISG was significantly (p < 0.05) lower than that obtained within the TAC eye drops. The AUC0–t of TAC-SLNs-ISG was 590,355.9 ng·min/mL, 2.65 folds higher than AUC0–t of 222,382.5 ng·min/mL for TAC eye drops, clearly defining performance superiority of in situ gels over drops. SLNs are believed to be actively engulfed by the corneal and conjunctival cells. This is considered to be the main advantage of colloidal dispersion. In addition to passive diffusion out of the SLNs, the lipases in the tear film and in the epithelial cells are also responsible for the controlled release of TAC. Release occurs both in the tear as well as in the corneal matrix. Therefore, in vivo kinetics studies can show the slow release characteristics of drugs from gels.

Figure 3 Concentration-time curve of TAC in different formulations (TAC eye drops, TAC SLNs and TAC SLNs-IGS). (n=6).

Figure 4 The pharmacodynamic scores in different groups. Group A: Normal (untreated, positive control); Group B: PBS (negative control); Group C: 0.1% TAC eye drop; Group D: 0.1% TAC-SLNs; Group E: 0.1% TAC-SLNs ISG. (n=6).

Figure 5 OVA-s IgE, IFN-γ and IL-4 concentration of different treatment groups in mice after pharmacodynamic study. Group A: Normal (untreated, positive control); Group B: PBS (negative control); Group C: 0.1% TAC eye drop; Group D: 0.1% TAC-SLNs; Group E: 0.1% TAC-SLNs ISG. (n=6). *p<0.05, Group E vs Group B; **p<0.05, Group E vs Group C; ***p<0.05, Group E vs Group B.
Pharmacodynamic Evaluation

The establishment of the animal model in this study was completely successful, and there were significant differences between the positive control group and the negative control group (Figure 4).

In the untreated group (Group A), there were almost no symptoms, so the score remained at a relatively low level during the observation period. The symptoms of the positive control group (Group B) were more obvious, and the score was always at a high level. In this experiment, the unified administration time of each group was morning, and the unified time of scoring statistics was afternoon, so on the first day after treatment, there was a significant difference between the three groups. The scores of the three experimental groups (Group C, D, E) showed different degrees of decline from the first day. The effect of group E was the most obvious, and the improvement of symptoms was the most obvious on the fifth day of the experiment. The reason for the relatively short retention time of the eye drops and TAC-SLNs is that they are not completely observed by the naked eye. Through the detection of three biochemical indexes (OVA-s IgE, IFN-γ and IL-4), Group E has significant curative effect on conjunctivitis compared with other control groups (p<0.05, Figure 5). The mechanism of TAC’s action is to inhibit the activation and degranulation of conjunctival mast cells, inhibit the release of inflammatory mediators by mast cells, and down regulate IL-4 in serum, thus inhibiting the antibody response of B cells from IgM to IgE and reducing

![Image of histopathological studies of corneal tissue of different groups.](https://www.dovepress.com)
the synthesis of IgE. Through up regulating IFN-γ in serum, the proliferation of Th2 cells and the function of IL-4 are inhibited, and the transformation of Th1 to Th2 is inhibited, so that Th1 and Th2 are in a dynamic balance state. Through this mechanism, type I allergy can be controlled and immune conjunctivitis can be treated. Through the optimization of dosage forms (SLNs or in situ gel), the therapeutic effect is further expanded. Corneal histopathology of showed that TAC-SLNs-ISG had good safety (Figure 6).

Permeability test

The permeability coefficient and flux for TAC in eye drops, SLNs and SLNs-ISG is calculated in Table 5. The transcorneal permeability and flux of TAC from SLNs was significantly higher compared to that of the eye drops. This indicates that the lipid nanoparticles enhance the permeation of the drug through intact corneal tissues. The slightly lower flux and permeability of TAC from the SLNs-ISG compared with SLNs indicates the controlled release of the drug from the higher viscosity formulation.

Table 5 The Result of Permeability and Flux of Different TAC Formulation

| Formulation    | Permeability (*10^-4 cm/sec) | Flux (μg/min/cm²) |
|----------------|-------------------------------|-------------------|
| TAC eye drops  | 0.9±0.08                      | 0.06±0.007        |
| TAC-SLNs       | 9.15±1.34*                    | 0.12±0.04*        |
| TAC-SLNs-ISG   | 8.26±0.73*                    | 0.065±0.004       |

Notes: Each value represents the mean ± SD of three determinations. *p<0.05 (compared to TAC eye drops). (n=6).

Acknowledgments

We wish to express our thanks to Dr. Wei ZHU (Department of Pharmaceutical, School of Pharmacy, Fudan University, Shanghai, China) for his help in the study design.

Disclosure

The authors report no conflicts of interest for this work.

References

1. Maurice DM. Kinetics of topical applied drugs. In: Saettoni MS, Bucci P, Speiser P, editors. Ophthalmic Drug Delivery: Biopharmaceutical, Technological and Clinical Aspects. Padova: Liviana Press; 1987:19–26.
2. Pleyer U, Lutz S, Jusko W, et al. Ocular absorption of topically applied FK506 from liposomal and oil formulations in rabbit eye. Invest Ophthalmol Vis Sci. 1993;34(9):2737–2742.
3. Bochat A, Fattal E, Grossiord JL, Puisieux F, Couverpeur P. Characterization of a new ocular delivery system based on a dispersion of liposomes in a thermosensitive gel. Int J Pharm. 1998;162:119–127. doi:10.1016/S0378-5173(97)00419-5
4. de Campos AM, Diebold Y, Carvalho EL, Sanchez A, Alonso MJ. Chitosan nanoparticles as an ocular drug delivery system: in vitro stability, in vivo fate, and cellular toxicity. Pharm Res. 2004;21(5):803–810. doi:10.1023/B:PHAM.0000026452.75781.cb
5. Nayak K, Misra M, Nayak K, et al. A review on recent drug delivery systems for posterior segment of eye. Biomed Pharmacother. 2018;107:1564–1582. doi:10.1016/j.biopharma.2018.08.138
6. Bachu RD, Chowdhury P, Al-Saedi ZHF, et al. Ocular drug delivery barriers-role of nanocarriers in the treatment of anterior segment ocular diseases. Pharmaceutics. 2018;10(1):28. doi:10.3390/pharmaceutics10010028
7. De Campos AM, Sánchez A, Gref R, Calvo P, Alonso MJ. The effect of PEG versus a chitosan coating on the interaction of drug colloidal carriers with the ocular mucosa. Eur J Pharm Sci. 2003;20(1):73–81. doi:10.1016/S0928-0987(03)00178-7
8. Lakhani P, Patil A, Majumdar S, et al. Recent advances in topical nano drug-delivery systems for the anterior ocular segment. Ther Deliv. 2018;9(2):137–153. doi:10.4155/tde-2017-0088
9. Ferraboschi P, Colombo D, De Mieri M, et al. Evaluation, synthesis and characterization of tacrolimus impurities. J Antimicrob Chemother. 2012;65(7):349–354. doi:10.1093/jac/djs228
10. Honbo T, Kobayashi M, Hane K, Hata T, Ueda Y. The oral dosage form of FK-506. Transplant Proc. 1997;5(1 Suppl):17–22.
11. Arima H, Yunomae K, Miyake K, Irie T, Hirayama F, Uekama K. Comparative studies of the enhancing effects of cycloexodrin on the solubility and oral bioavailability of tacrolimus in rats. J Pharm Sci. 2001;90(6):690–701. doi:10.1002/jps.1025
12. Gao S, Sun J, Fu D, Zhao H, Lan M, Gao F. Preparation, characterization and pharmacokinetic studies of tacrolimus-dimethyl-β-cycloexodrin inclusion complex-loaded albumin nanoparticles. Int J Pharm. 2012;427(1–2):410–416. doi:10.1016/j.ijpharm.2012.01.054
13. Kwon M, Yeom D, Kim NA, et al. Bioequivalence of tacrolimus formulations with different dynamic solubility and in-vitro dissolution profiles. Arch Pharm Res. 2015;38(1):73–80. doi:10.1007/s12272-014-0343-3
Drug Design, Development and Therapy

Publish your work in this journal

Drug Design, Development and Therapy is an international, peer-reviewed open-access journal that spans the spectrum of drug design and development through to clinical applications. Clinical outcomes, patient safety, and programs for the development and effective, safe, and sustained use of medicines are a feature of the journal, which has also been accepted for indexing on PubMed Central. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.

Submit your manuscript here: https://www.dovepress.com/drug-design-development-and-therapy-journal

14. Skytte DM, Jaroszewski JW, Johansen KT, et al. Some transformations of tacrolimus, an immunosuppressive drug. *Eur J Pharm Sci*. 2013;48(3):514–522.

15. Loftsson T, Mullertz A, Siepmann J. For the special IJP issue “Poorly soluble drugs”. *Int J Pharm*. 2013;453(1–2). doi:10.1016/j.ijpharm.2013.05.056

16. Xiao P, Dudał Y, Corvini P, Spahr P, Shahgaldian P. Synthesis and characterization of fluoroquinolone-imprinted polymeric nanoparticles. *React Funct Polym*. 2012;72:287–293. doi:10.1016/j.reactfunctpoly.2012.02.006

17. Müller RH, Mäder K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery—A review of the state of the art. *Eur J Pharm Biopharm*. 2000;50(1):161–177. doi:10.1016/S0939-6411(00)00087-4

18. Mehnert W, Mäder K. Solid lipid nanoparticles. production, characterization and applications. *Adv Drug Deliv Rev*. 2001;47(2–3):165–196. doi:10.1016/S0169-409X(01)00105-3

19. Khames A, Khaleel MA, El-Badawy MF, El-Nezhawy AOH. Natamycin solid lipid nanoparticles - sustained ocular delivery system of higher corneal penetration against deep fungal keratitis: preparation and optimization. *Int J Nanomedicine*. 2019;14:2515–2531. doi:10.2147/IJN.S190502

20. Takte A, Dudhipala N, Janga KY, et al. In situ gel of triamcinolone acetonide-loaded solid lipid nanoparticles for improved topical ocular delivery: tear kinetics and ocular disposition studies. *Nanomaterials (Basel)*. 2018;9(1):33. doi:10.3390/nano9010033

21. Balgari SP, Adelli GR, Majumdar S. Topical ophthalmic lipid nanoparticle formulations (SLN, NLC) of indomethacin for delivery to the posterior segment ocular tissues. *Eur J Pharm Biopharm*. 2016;109:224–235. doi:10.1016/j.ejpb.2016.10.015

22. Yadav M, Schiavone N, Guzman-Aranguez A, et al. Atorvastatin-loaded solid lipid nanoparticles as eye drops: proposed treatment option for age-related macular degeneration (AMD). *Drug Deliv Transl Res*. 2020;10(4):919–944. doi:10.1007/s13346-020-00733-4

23. Narendar D, Kishan V. Candesartan cilexetil loaded solid lipid nanoparticles for oral delivery: characterization, pharmacokinetic and pharmacodynamic evaluation. *Drug Deliv*. 2016;23(2):395–404. doi:10.3109/10717544.2014.914986

24. Yu L, Zhang Z, Zhang H, Ding J. Biodegradability and biocompatibility of thermoreversible hydrogels formed from mixing a sol and a precipitate of block copolymers in water. *Biomacromolecules*. 2010;11(8):2169–2178. doi:10.1021/bm100549q

25. Rakesh K, Sinha VR. Solid lipid nanoparticle: an efficient carrier for improved ocular permeation of voriconazole. *Drug Dev Ind Pharm*. 2016;42(12):1956–1967. doi:10.1080/03639045.2016.1185437

26. Giannavola C, Bucolo C, Maltese A, et al. Influence of preparation conditions on acyclovir- loaded poly-d, l-lactic acid nanoparticles and effect of PEG coating on ocular drug bioavailability. *Pharm Res*. 2003;20(4):584–590. doi:10.1023/A:1023290514575

27. Srividya B, Cardoza RM, Amin PD. Sustained ophthalmic delivery of ofloxacin from a pH triggered in-situ gelling system. *J Control Release*. 2001;73(2–3):205–211. doi:10.1016/S0168-3659(01)00279-6

28. Moreira TS, de Sousa VP, Pierre MB. Influence of oleic acid on the rheology and in vitro release of lumiracoxib from poloxamer gels. *J Pharm Pharm Sci*. 2010;13(2):286–302. doi:10.18433/J34880

29. Perez AP, Mundifia-Weilenmann C, Romero EL, Morilla MJ. Increased brain radioactivity by intranasal P-labeled siRNA dendrimers within in-situ-forming mucoadhesive gels. *Int J Nanomedicine*. 2012;7:1373–1385. doi:10.2147/IJN.S28261