# SUPPORTING INFORMATION

# An antibiotic efficacy comparative study between market-prescribed and liposomal-based fluoroquinolone ophthalmic nanoformulation against conjunctivitis

**Rakesh P. Patel[a,*], Bijit Saha[a], Tripti Halder[b] and Nitin Gupta[c]**

[a]Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Mehsana- 384012, Gujarat, India.

[b]Institute of Pharmacy, Nirma University, Ahmedabad- 382481, Gujarat, India.

[c]School of Nano Sciences, Central University of Gujarat, Gandhinagar- 382030, Gujarat, India.

**(*) Address for correspondence**

Prof. Rakesh P. Patel

Head, Pharmaceutics & Pharmaceutical Technology Department

Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Mehsana- 384012, Gujarat, India.

M. +91- 9879106580

Email: rakesh_patel@ganpatuniversity.ac.in

## Methodology

### Release Kinetics:

The release kinetics of BSF DS from optimized BSF@SLNPs ophthalmic DP was identified by applying various release kinetics equations such as zero-order release kinetics, first-order release kinetics, and the Higuchi model on BSF drug release profile. The released obtained data were calculated by using various parameters. The parameters "*n*" and time component "*k*" the release rate constant, and "*r*" the regression coefficient was determined by the Korsmeyer-Peppas equation to understand the release mechanism.

### Result:

The release kinetic results of optimized BSF@SLNPsA9 ophthalmic DP in different equations exhibited different mechanisms. The release constants, the correlation coefficients ($r^2$) and exponents (*n*) values of different formulations are shown in **Table S7**. The release model of BSF DS from BSF@SLNPsA9 ophthalmic DP is well fitted with a zero-order model ($r^2$: 0.992), first-order model ($r^2$: 0.975), Hixon-Crowell ($r^2$: 0.967), Higuchi's model ($r^2$: 0.987) and Korsmeyer-Peppas model ($r^2$: 0.991) and (*n*: 1.163). This revealed that the release of the drug was dependent on the encapsulated drug concentration.

### Discussion:

The quantitative results obtained *in vitro* release assay are converted by using the following mathematical models [1]. The released data were plotted according to the following equations [2,3].

#### i. Zero-order model

The equation explains the collective amount of drug release is directly proportional to time. The equation for the zero-order model is mentioned below:

$$C_t = C_0 + K_0 t$$

In the above Equation, where $C_t$ represents the amount of active agent released during the time *t*; $C_0$ is the initial concentration of active released (generally, $C_0 = 0$); and $K_0$ is the zero-order constant.

ii. **First-order model:** The drug release behaviour of the first-order equation presented by the remaining drug (as log cumulative percentage) versus time. The equation for the first-order model is mentioned below [4].

$$log\ Q1 = log\ Q0 + \frac{k1t}{2.303}$$

In the above equation, where $Q_1$ is the amount of active agent released on time *t*; $Q_0$ is the initial amount of drug dissolved; and $K_1$ is the first-order rate constant.

iii. **Hixson-Crowell model:** This model depicts the drug release from formulations for the changing diameter of particles and surface area. The equation of the Hixson-Crowell model is mentioned below [5].

$$Q_0{}^{1/3} - Q_t{}^{1/3} = K_{HC}$$

In the above equation, $Q_t$ is the drug released amount in t time, $Q_0$ is the drug amount at an initial time and $K_{HC}$ is the rate constant.

iv. **Higuchi model:** The Higuchi model is explained as drug released in cumulative percentage versus square root of time. The equation of the Higuchi model is mentioned below [6].

$$Q=K\sqrt{t}$$

In the above equation, *Q* is the drug released amount in t time and "K√t" is the rate constant.

v. **Korsmeyer-Peppas model:** The dissolution results can be fitted to the Korsmeyer-Peppas equation which is used to explain the drug release pattern from the polymer-contained system. The equation of the Korsmeyer-Peppas model is mentioned below:

$$\log (Mt/Mf) = \log k + n \log t$$

In the above equation, $M_t$ is the drug released amount in t time; $M_f$ is the drug release amount at an infinite time; *k* is the rate constant; n is the diffusion exponent indicative of the mechanism of drug release [7].

**References:**


[1] Kumar P, Ganure AL, Subudhi BB, Shukla S. Design and comparative evaluation of in-vitro drug release, pharmacokinetics and gamma scintigraphic analysis of controlled release tablets using novel pH-sensitive starch and modified starch-acrylate graft copolymer matrices. Iran J Pharm Res 2015;14:677–91.

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[3] Bruschi MLBT-S to M the DR from PS, editor. Mathematical models of drug release. Strategy to Modify Drug Release from Pharm. Syst., Woodhead Publishing; 2015, p. 63–86. https://doi.org/10.1016/b978-0-08-100092-2.00005-9.

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[5] Hixson AW, Crowell JH. Dependence of Reaction Velocity upon Surface and Agitation: I—Theoretical Consideration. Ind Eng Chem 1931;23:923–31. https://doi.org/10.1021/ie50260a018.

[6] Higuchi T. Rate of release of medicaments from ointment bases containing drugs in suspension. J Pharm Sci 1961;50:874–5. https://doi.org/10.1002/jps.2600501018.

[7] Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. Mechanisms of solute release from porous hydrophilic polymers. Int J Pharm 1983;15:25–35. https://doi.org/10.1016/0378-5173(83)90064-9.

# List of Supporting Information Tables

**Table S1:** Physico-chemical properties of Besifloxacin hydrochloride drug substance.

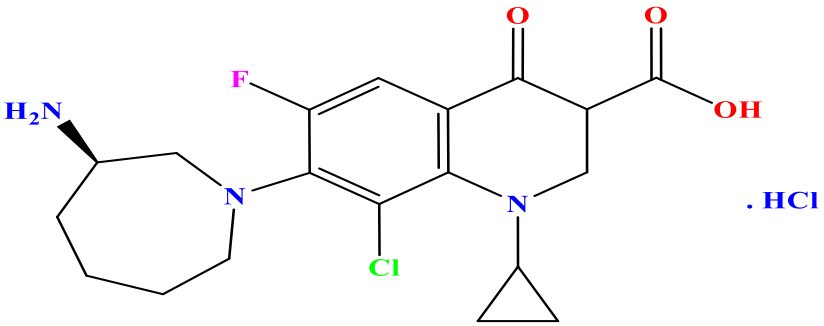
| | |
|---|---|
| Drug substance Name | Besifloxacin hydrochloride |
| IUPAC name | 7-[(3*R*)-3-aminoazepan-1-yl]-8-chloro-1-cyclopropyl-6-fluoro-4-oxoquinoline-3-carboxylic acid; hydrochloride |
| Pharmacological Classification | Anti-Bacterial Agents |
| Molecular Weight | 430.30 |
| Molecular structure |   |
| Molecular Formula | $C_{19}H_{22}Cl_2FN_3O_3$ |

**Table S2:** Details of approved drug product of Besivance (Besifloxacin ophthalmic suspension 0.6%) available on electronic Orange Book.

| **Marketed status** | **Rx.** |
|---|---|
| Active ingredient | Besifloxacin hydrochloride |
| Proprietary Name | BESIVANCE |
| Application Number | N022308 |
| Dosage Form | Suspension/Drops |
| Route of Administration | Ophthalmic |
| National Drug Code (NDC) | 24208-446-05 |
| Strength | EQ 0.6% BASE (6 mg/mL) |
| Reference Listed Drug | Yes |
| Reference Standard | Yes |
| TE Code | None |
| Product Number | 001 |
| Approval Date | May 28, 2009 |
| Applicant Holder Full Name | BAUSCH AND LOMB INC |
| Marketing Status | Prescription |
| Storage | Store at 15°C to 25°C (59°F to 77°F). Protect from light. |

**Table S3:** Composition details and process parameters for manufacturing and optimization of besifloxacin hydrochloride drug substance-loaded solid lipid nanoparticles (BSF@SLNPs).

| **Batch no.** | **SLNPs-1** | **SLNPs-2** | **SLNPs-3** |
|---|---|---|---|
| **Ingredients** | **Quantity (mg/mL)** | | |
| Besifloxacin HCl | 6.6 | 6.6 | 6.6 |
| Glyceryl monostearate | 3.3 | - | - |
| Glyceryl palmitostearate | - | 3.3 | - |
| Glyceryl Behenate | - | - | 3.3 |
| Polysorbate 80 | 0.5 | 0.5 | 0.5 |
| Poloxamer 188 | 0.5 | 0.5 | 0.5 |
| Purified water | 1 | 1 | 1 |
| **Process parameters** | | | |
| HSH | 10000 RPM for10 min during the mixing lipid phase | | |
| HSH | 10000 RPM for10 min during the mixing lipid phase | | |
| HPH | 5 Nos. cycles at 1000 bar pressure | | |
| Centrifugation | 10000 RPM for 10 min | | |

**Table S4:** $3^2$ factorial designs of besifloxacin hydrochloride drug substance-loaded solid lipid nanoparticles (BSF@SLNPs).

| **Factors (independent variables)** | **Actual Levels used** | | |
|---|---|---|---|
| | **Low (-1)** | **Medium (0)** | **High (+1)** |
| $X_1$: Ratio of the drug: Lipid | 1:1 | 1:0.75 | 1:0.5 |
| $X_2$: HPH cycles at 1000 bar pressure | 5 | 10 | 20 |
| **Response (dependent variables)** | **Limits** | | |
| Response 1 ($Y_1$): $M_D$ (nm) | 200 ≤Y1≤ 500 | | |
| Response 2 ($Y_2$): ZP (mV) | -20 ≤Y2≤ -60 | | |
| Response 3 ($Y_3$): % EE | 40≤Y3≤ 80 | | |

**Table S5:** Composition and process optimization of besifloxacin hydrochloride drug substance-loaded solid lipid nanoparticles (BSF@SLNPs) drug product.

| **Batch no.** | **SLNPs-4** | **SLNPs-5** | **SLNPs-6** |
|---|---|---|---|
| **DS and lipid ratio** | **1:1** | **1:0.75** | **1:0.5** |
| **Ingredients** | **Quantity (mg/mL)** | | |
| BSF | 6.6 | 6.6 | 6.6 |
| GMS | 6.6 | - | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | - | | | 4.95 | | | - | | |
| | - | | | - | | | 3.3 | | |
| Polysorbate 80 | 0.5 | | | 0.5 | | | 0.5 | | |
| Poloxamer 188 | 0.5 | | | 0.5 | | | 0.5 | | |
| WFI | 1 mL | | | 1 mL | | | 1 mL | | |
| **Sub-batch no** | **SLNPs-4a** | **SLNPs-4b** | **SLNPs-4c** | **SLNPs-5a** | **SLNPs-5b** | **SLNPs-5c** | **SLNPs-6a** | **SLNPs-6b** | **SLNPs-6c** |
| **Process parameters** | | | | | | | | | |
| HSH | 10,000 RPM for 10 mins during the mixing lipid phase and mixing of the aqueous to lipid phase. | | | | | | | | |
| No. of HPH cycles at 1000 bar pressure | 5 | 10 | 20 | 5 | 10 | 20 | 5 | 10 | 20 |
| Centrifugation | 10000 RPM for 10 mins | | | | | | | | |

**Abbreviation:** BSF: Besifloxacin hydrochloride; GMS: Glyceryl monostearate ($C_{21}H_{42}O_4$); HPH: High-pressure homogenization; HSH: High-speed homogenization; SLNPs: Solid lipid nanoparticles.

**Table S6:** Composition and its quantity for preparation of optimized BSF@SLNPs ophthalmic drug product.

| Sr. No. | Ingredients | Quantity (mg/mL) |
|---|---|---|
| 1. | Besifloxacin Hydrochloride (BSF) | 6.6 |
| 2. | Glyceryl monostearate (GMS) | 3.0 |
| 3. | Gellan Gum (Gelrite) | 3.00 |
| 4. | Benzalkonium Chloride | 0.10 |
| 5. | Mannitol | 10.00 |
| 6. | Poloxamer 188 | 0.5 |
| 7. | Polysorbate 80 | 0.5 |
| 8. | Sodium Chloride (NaCl) | 5.00 |
| 9. | Di Sodium Edetate | 1.00 |
| 10 | Sodium Hydroxide (NaOH) | Q.s. to adjust pH |
| 11. | Water for injection (WFI) | Q.s. to 1 mL |

**Table S7:** Result of in vitro release kinetics result for model fitting of optimized BSF@SLNPsA9 ophthalmic drug product into simulated tear fluid.

| Batch no. | Zero-order | | First order | | Hixon-Crowell | | Higuchi | | Korsmeyer-Peppas | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Ko** ($h^{-1}$) | $r^2$ | **$K_1$** ($h^{-1}$) | $r^2$ | **$K_{HC}$** ($h^{-1/3}$) | $r^2$ | **$K_H$** ($h^{-1/2}$) | $r^2$ | ***n*** | $r^2$ | **$K_{KP}$** ($h^{-n}$) |
| BSF@ SLNPsA9 | 0.213 | 0.992 | -35.113 | 0.975 | -5.049 | 0.967 | 0.046 | 0.987 | 1.163 | 0.991 | 0.066 |

**Table S8:** Various ingredients, their concentration and pH of the media used for the preparation of agar media for testing of antimicrobial activity against the *S. aureus* bacterium.

| Sr. No. | Ingredients | Concentration (g/liter) |
|---|---|---|
| 1. | Casein enzymic hydrolysate | 15.00 |
| 2. | Peptic digest of soya bean meal | 5.00 |
| 3. | Agar | 15.00 |
| 4. | Sodium chloride | 15.00 |
| 5. | pH at 25 ºC | 7.4 ± 0.2 |