INTRODUCTION

Solid dispersion is an effective way of improving the dissolution rate of poorly water-soluble drugs and their bioavailability. Drug absorption from the gastrointestinal (GI) tract can be limited by various factors. The most significant contributors are poor aqueous solubility and/or poor membrane permeability of the drug molecule. Therefore, a drug with poor aqueous solubility will typically exhibit a dissolution rate with limited absorption, and a drug with poor membrane permeability will typically exhibit a permeation rate with limited absorption. Solid dispersion is defined as dispersion of one or more active ingredients (hydrophobic) in an inert carrier (hydrophilic) at solid state prepared by melting (fusion) method, solvent, or melting solvent method (Allawadi et al., 2013; Kumar, 2017).

When the solid dispersion comes in contact with the aqueous medium, the inert carrier dissolves and the drug is released, the increased surface area produces a higher dissolution rate thus increasing the bioavailability of the poorly soluble drug (Kumar, 2017).

Tobramycin is a new aminoglycoside antibiotic with a broad antibacterial spectrum in vitro, and pharmacokinetic properties (Dhondikubeer et al., 2012; Kumar & Kumar, 2017). The molecular weight of tobramycin is 467.52 g/mol, and its log P is 6.5. Tobramycin is slightly soluble in water and is usually administered in an encapsulated form. Tobramycin is absorbed when given orally. Peak plasma concentration is achieved in 6-8 h. The oral absorption of Tobramycin is only about 23-47%, leading to low bioavailability of the compound; it is administered as a standardized extract (70-80% Tobramycin), and the other components of Tobramycin are rapidly conjugated with sulphate and glucuronic acid in the liver and excreted through the bile (Hill et al., 2019).

MATERIALS AND METHODS

Tobramycin was purchased from Yarrow Chem Pvt Ltd, Mumbai (INDIA). All other chemicals used were of analytical reagent grade. Benzalkonium chloride (Yarrow Chem Pvt Ltd, Mumbai), HPMC (Fisher Scientific, Mumbai), PVP K30 (Nice Chemicals Pvt. Ltd., Kerala) and Beta cyclodextrin (Medilise Chemicals, Kerala).

Preparation of Tobramycin Solid-dispersion

Solid dispersions of Tobramycin were prepared using PVP-K30, HPMC and Beta cyclodextrin by solvent evaporation technique. This solid dispersion was formulated as an eye drop using sodium chloride, EDTA and benzalkonium chloride. Totally 9 formulations were prepared. The in-vitro dissolution profile of optimised formulation (F6) showed 85.1% drug release at the end of 2nd hour. The optimized formula was evaluated which showed no microbial growth, and drug content uniformity was 80.12% to 85%. The optimized formulation was evaluated for clarity, pH, isotonicity, microbial growth, stability and in vitro diffusion studies and all these showed acceptable results.

KEYWORDS: Solid dispersion, Tobramycin, HPMC, PVP-K30, beta cyclodextrin
stirring. The solvent was then completely evaporated at 45°C with continuous stirring to obtain dry mass. The dried mass was pulverized passed through a 44 mesh sieve and stored in a desiccator until used for further studies (Saha et al., 2002). Beta cyclodextrin, PVP-K30 and HPMC were taken in a ratio of 1:1, 1:2, and 1:3 respectively (Sharma & Jain, 2010).

**FTIR**

The compatibility of the drug and polymer was analysed using an FTIR spectrophotometer. In this technique, 1mg of the sample and 100mg of potassium bromide (KBr) (1:100 ratio) were finely ground using mortar and pestle. A few mixtures were placed for 2 minutes under a hydraulic press compressed at 7 kg/cm² to form a transparent pellet. The pellet was kept in the sample holder and scanned from 4000 cm⁻¹ to 400 cm⁻¹ in the Shimadzu FT-IR spectrophotometer. Samples were prepared for the drug-polymer and physical mixture of drug and polymers. The spectra obtained were compared and interpreted for the functional group peaks (Kamal et al., 2009; Raj & Kumar, 2018).

**Differential Scanning Calorimetry**

A differential scanning calorimeter was used for the analysis of tobramycin and excipients. The API sample was weighed directly under the pierced DSC aluminium pan and scanned in the temperature range of 40-270°C (Kim et al., 1985; Dash & Suryanarayan, 1991).

**Drug Content Determination**

The amount of drug contained in the Ocular drop was determined by dissolving 1 mL of the formulation in 9 mL of water and the volume was made up to 100 mL with water. The mixture was analysed by a UV-Visible spectrophotometer at 204 nm against water as a blank (Deshpande et al., 2013; Schwartz et al., 2013).

**In Vitro Dissolution of Tobramycin Solid Dispersion**

The dissolution study was carried out using USP XXIII apparatus type-II. The dissolution medium was 900 ml including Tobramycin of solid dispersion in Phosphate buffer of pH 7.3, kept at 37±1°C. The drug or solid dispersions was taken in a suitable medium and kept in the basket of the dissolution apparatus; the basket was rotated at 50 rpm. Samples of 1ml were withdrawn at specified time intervals and analysed spectrophotometrically at 204 nm using Shimadzu-1700 UV-visible spectrophotometer; the samples withdrawn were replaced by fresh buffer solutions. Each preparation was tested in triplicate and then means values were calculated (Miller et al., 2017; Rosasco et al., 2018).

**Preparation of Eye Drop from Tobramycin Solid Dispersion**

Tobramycin solid dispersion prepared using polymer Beta cyclodextrin, PVP-K30, and HPMC were added individually into Sodium chloride, benzalkonium chloride, and EDTA solution and adjusted to a final volume of 10mL with purified water (Table 1). Formulations were stored in eye drop container bottles, and protected until used (Mingeot-Leclercq et al., 1999).

**EVALUATION OF TOBRAMYCIN EYE DROP**

**Visual Appearance**

The colour was checked by the naked eye after the formulation.

**Clarity**

Clarity examination involves the visual assessment of formulation in suitable lighting on white and black background (Shantier et al., 2012; Baranowski et al., 2014).

**pH**

The pH of the formulation was measured using a digital pH meter. The determination of the pH is done by using the glass electrode. The glass electrode is dipped into the solution and notes down the reading shown in the display (Patil et al., 2019; Patere et al., 2020).

**Isotonicity Testing**

Formulation (1 mL) was mixed with a few drops (4 drops) of blood and observed under a microscope at 45X magnification and the shape of the blood cell was compared with the standard marketed ophthalmic formulation (Gupta & Reddy, 2015; Vyas et al., 2015; Kumar & Singh, 2018).

**In-Vitro Diffusion Studies of Tobramycin Eye Drops**

An in vitro diffusion study of eye drop was carried out using Franz diffusion cell. The formulation was placed in the donor compartment and freshly prepared simulated tear fluid was in the receptor compartment. Between donor and receptor compartment dialysis membrane is placed (0.22 μm pore size). The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at 37°C ± 0.5°C. 1mL of sample is withdrawn at the predetermined time interval of 15min for 2hrs and the same volume of fresh medium is replaced. The withdrawn samples are diluted to 10 mL in a volumetric flask with respective solvent and analysed by UV spectrophotometer at 204 nm using reagent blank. The drug content is calculated using the equation generated from the standard calibration curve. The % cumulative drug release (%CDR) was calculated (Kurniawansyah et al., 2018; Sebastian-Morello et al., 2020).

**Microbiological Assay**

The microbiological assay of Tobramycin was carried out by the cup plate method. The potato dextrose agar medium was prepared, sterilized and inoculated with Candida albicans.
micro-organism at a temperature of 27°C and immediately pored the inoculated medium into Petri plates to give a depth of 4 to 5 mm uniformly and kept aside for solidification. Small cavities of 10 mm diameter were made on solidified agar Petri plates by using a sterilized cylinder-shaped borer. 500 μL of the prepared standard solutions and sample solutions (i.e., equivalent to 1 μg/mL and 5 μg/mL drug concentration) were added into each cavity. These Petri plates are left for 1 to 4 hours at room temperature as a preincubation diffusion to minimize the effects of variation in time between different solutions. Prepared petri plates were incubated for 48 hours at 27°C and measured the diameter of circular inhibited zones. The test is also conducted for microbiological studies to test the significant difference between standard and Tobramycin of solid dispersion formulations (Pooja et al., 2016; Rajia et al., 2020).

Stability Study

The optimized sterile formulation was subjected to stability testing. Sterile optimized ophthalmic formulation was filled in glass vials, closed with grey butyl rubber closures and sealed with an aluminium cap. The vials containing the optimized formulation were kept in stability chamber, maintained at 40 ± 2°C and 75 ± 5% RH for one month. Samples were withdrawn and estimated for drug content, pH, visual appearance, in vitro drug release (Curti et al., 2017; Kurniawansyah et al., 2021).

RESULTS AND DISCUSSION

IR Spectroscopy of Drug

The IR spectrum of Tobramycin was recorded by FT-IR spectrophotometer. From the peaks observed, it was seen that the functional group peak frequencies resembled the standard range values of Tobramycin (Figure 1). Thus, the presence of Tobramycin can be confirmed. The functional groups of Tobramycin are clearly seen in IR Spectra of Tobramycin and excipients which confirms that there is no interaction between drug and excipients (Figure 2).

Differential Scanning Calorimetry

DSC thermogram showed a sharp endothermic peak at 204.11°C which is corresponding to the melting point of the drug. The drug mixture also shows the peak in the same region confirms the compatibility between them (Figure 3).

In-Vitro Dissolution Study

The dissolution data of tobramycin with solid dispersion and PVP K30, HPMC and Beta cyclodextrin systems are given in Figure 4. The pure drug shows only 43.17% of release at the end of two hours. Solid dispersion of the drug shows release

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**Table 1: Formulation chart of eye drop with Tobramycin solid dispersion.**

<table>
<thead>
<tr>
<th>Sl no</th>
<th>Ingredients</th>
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<th>F2</th>
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<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
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<tbody>
<tr>
<td>1</td>
<td>Tobramycin (mg)</td>
<td>0.6</td>
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<td>2</td>
<td>PVP K30 (mg)</td>
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<td>3</td>
<td>Beta cyclodextrin (mg)</td>
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<td>12</td>
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<td>4</td>
<td>HPMC (mg)</td>
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<td>6</td>
<td>12</td>
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<td>5</td>
<td>Sodium chloride (mg)</td>
<td>0.09</td>
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<td>6</td>
<td>Benzalkonium chloride (ml)</td>
<td>0.02</td>
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<td>7</td>
<td>EDTA (mg)</td>
<td>0.018</td>
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<td>8</td>
<td>Sodium hydroxide (mg)</td>
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<td>9</td>
<td>Distilledwater (ml)</td>
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**Figure 1: IR Spectra of Tobramycin**
between 68.54-81.33% which clearly indicates the enhancement of solubility of the drug.

**Physical Appearance, Clarity, Isotonicity and pH**

The eye drops formed were in solution form and transparent/translucent in appearance. The maintenance of the shape of RBC cells without rupturing indicates all the formulations were isotonic in nature. The pH of the freshly prepared 1% aqueous solution of Tobramycin was found to be 7.0 to 7.3.

**In Vitro Diffusion Study**

*In vitro* diffusion study was performed for the optimised formulation ie F6. The release was found to be 93.12% at the end of 2hrs.

**Antimicrobial Study**

The zone of inhibition was better with pseudomonas aeruginosa (gram positive micro-organism) and E. coli (gram negative micro-organism) for the formulation and marketed product (Figure 5). The zone of inhibition of the prepared formulation was found to be almost similar. The result indicates that eye drops of Tobramycin retained their antimicrobial efficacy when formulated as a solid dispersion eye drops system.

**Sterility Study**

All the formulations were subjected to direct inoculation using fluid thioglycolate medium and there was no microbial growth seen (Table 2). The zone of inhibition was better with E. coli.
Table 2: Test for sterility Fluid Thioglycolate medium

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<tr>
<th>Formulation</th>
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(gram negative micro-organism) for the formulation. The zone of inhibition of prepared formulation was studied.

Where “-” sign indicates the no growth of micro-organism

CONCLUSION

From the present study, tobramycin solid dispersion eye drop was found to be good stability and showed no microbial growth. The drug content was uniform in all the formulations prepared. Infrared spectroscopic and Differential Scanning Calorimetry studies indicated that the drug is compatible with the polymers and excipients. The use of an effervescent agent, and polymer in combination had its own advantages of eye drop properties. For proper solid dispersion duration and in vitro release, the polymer must be used in the proper ratio. The prepared tobramycin eye drop has a good solid dispersion lag time thereby enhancing its solubility properties and leading to its increased bioavailability. Administration of conventional drops of Tobramycin has been reported that its dissolution almost ceases because of the low solubility and degradation in eye laceration pH. The eye dropping bioavailability of Tobramycin is 30-50%. The Advantage of solid dispersion of Tobramycin will surely enhance the patient compliance and help the Anti-bacterial treatment for a long time and improve its bioavailability.

AKNOWLEDGEMENT

The authors are thankful to the Management of T John College of Pharmacy, Bengaluru, India for providing facilities to carry out this work.

CONFLICT OF INTEREST

Authors have no conflicts of interest.

REFERENCES


Rajia, S., Hasan, I., Ahmmed, B., & Islam, A. U. (2020). Development and Validation of a Microbiological Assay for the Quantification of


