Research in Pharmacy 2022, 12: 5-10
doi: 10.25081/rip.2022.v12.7557
https://updatepublishing.com/journal/index.php/rip

Research Article

ISSN: 2231-539X

Formulation and evaluation of ocular drops containing solid dispersion of tobramycin

R. Panner Selvam*, Ankith Kumar Pandey, Sahab Uddin, Vineeth Chandy

Department of Pharmacy, T. John College of Pharmacy, Bengaluru – 560083, Karnataka, India

Received: March 02, 2022
Revised: November 28, 2022
Accepted: December 01, 2022
Published: December 14, 2022

***Corresponding author:**
R. Panner Selvam,
pannerselvam.r9@gmail.com

ABSTRACT

Tobramycin is a potent antimicrobial aminoglycoside that can be used to treat ocular infections. Its solubility and bioavailability are limited, so its solid dispersion was 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

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, Beta cyclodextrin and HPMC (Mingeot-Leclercq *et al.*, 1999). The method used for the preparation was solvent evaporation method (Saha *et al.*, 2002; Nikghalb *et al.*, 2012).

Solvent Evaporation Method

The required amount of Tobramycin and the carrier were dissolved in a sufficient volume of methanol with continuous 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) ware finely ground using mortar and pestle. A few mixtures were placed for 2 minutes under a hydraulic press compressed at 7 kg/cm^2 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 thermos gram showed a sharp endothermic peak at 204.11ºC 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

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

Sl no	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Tobramycin (mg)	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
2	PVP K30 (mg)	6	12	18						
3	Beta cyclodextrin (mg)				6	12	18			
4	HPMC (mg)							6	12	18
5	Sodium chloride (mg)	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
6	Benzalkonium chloride (ml)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
7	EDTA (mg)	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018
8	Sodium hydroxide (mg)	Q. S	Q. S	Q. S	Q. S	Q. S	Q. S	Q. S	Q. S	Q. S
9	Distilledwater (ml)	Q. S	Q. S	Q. S	Q. S	Q. S	Q. S	Q. S	Q. S	Q. S

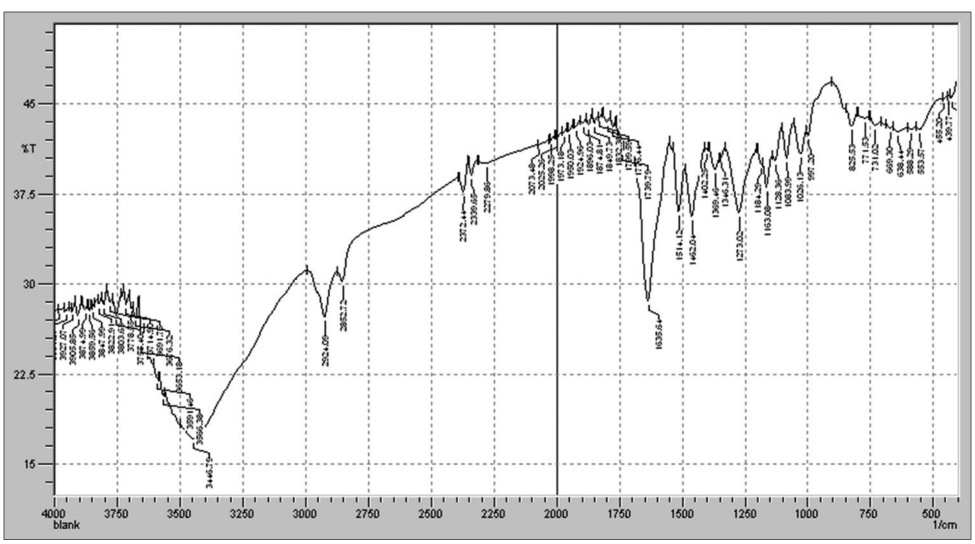

Figure 1: IR Spectra of Tobramycin

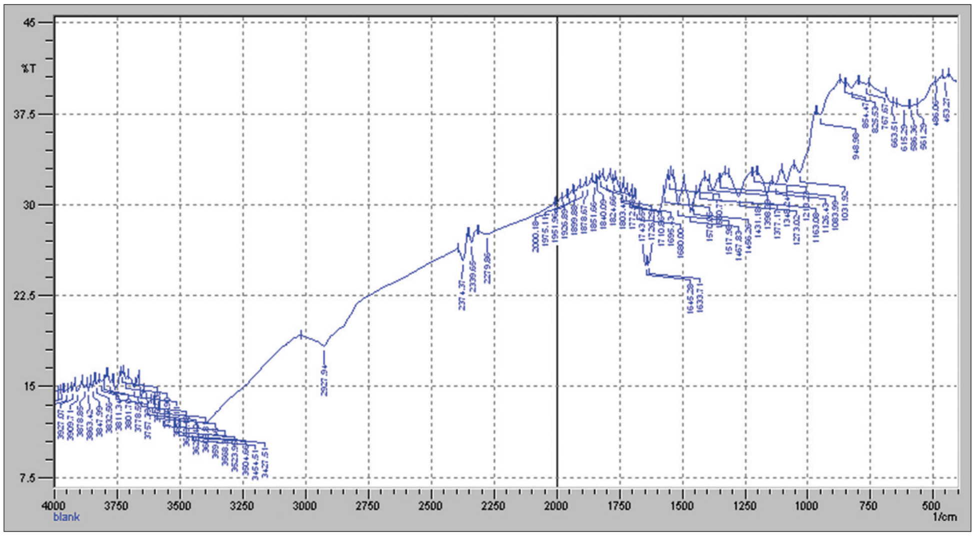

Figure 2: IR Spectra of Tobramycin and excipients

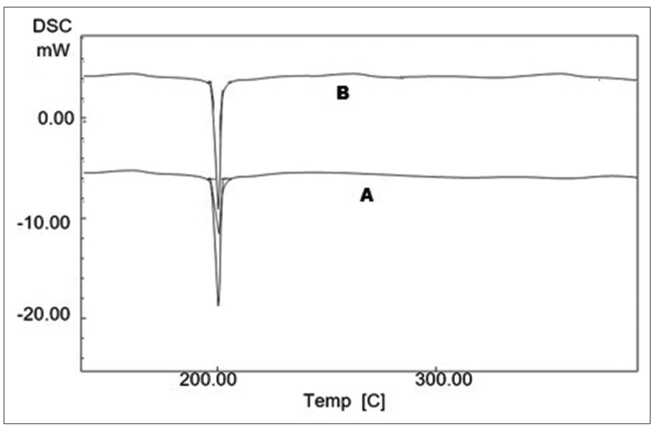

Figure 3: DSC of Tobramycin (A) and Tobramycin mixture (B)

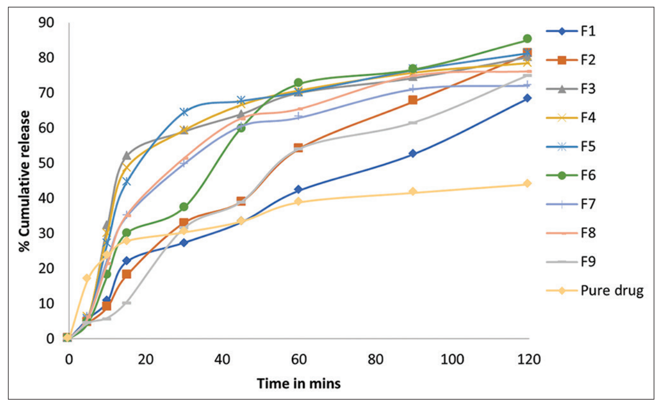

Figure 4: *In vitro* drug dissolution studies

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: Antimicrobial study

(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 in 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

Formulation	Days of incubation						
	1	2	3	4	5	6	7
F1	-	-	-	-	-	-	-
F2	-	-	-	-	-	-	-
F3	-	-	-	-	-	-	-
F4	-	-	-	-	-	-	-
F5	-	-	-	-	-	-	-
F6	-	-	-	-	-	-	-
F7	-	-	-	-	-	-	-
F8	-	-	-	-	-	-	-
F9	-	-	-	-	-	-	-

(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

Allawadi, D., Singh, N., Singh, S., & Arora, S. (2013). Solid Dispersions: A Review on Drug Delivery System and Solubility Enhancement. *International Journal of Pharmaceutical Sciences and Research*, *4*(6), 2094-2105. https://doi.org/10.13040/IJPSR.0975-8232.4(6).2094-05

Baranowski, P., Karolewicz, B., Gajda, M., & Pluta, J. (2014). Ophthalmic Drug Dosage Forms: Characterisation and Research Methods. *The Scientific* World Journal, 2014, 861904. https://doi.org/10.1155/2014/861904

Curti, C., Lamy, E., Primas, N., Fersing, C., Jean, C., Bertault-Peres, P., & Vanelle, P. (2017). Stability studies of five anti-infectious eye drops under exhaustive storage conditions. *Die Pharmazie – An International Journal of Pharmaceutical Sciences, 72*(12), 741-746. https://doi.org/10.1691/ph.2017.7089

Dash, A. K., & Suryanarayan, R. (1991). Solid-State properties of Tobramycin. *Pharmaceutical Research, 8*, 1159-1165. https://doi.org/10.1023/a:1015858503031

Deshpande, J., Shah, P. B., & Bhandari, A. (2013). Design and Development of pH-monitered in situ Gel of Lomefloxacin. *Journal of Pharmaceutical Science and Bioscientific Research, 3*(1), 10-15.

Dhondikubeer, R., Bera, S., Zhanel, G. G. & Schweizer, F. (2012). Antibacterial activity of amphiphilic tobramycin. *The Journal of Antibiotics, 65*, 495-498. https://doi.org/10.1038/ja.2012.59

Gupta, N. V., & Reddy, G. V. (2015). A Comparative study of quality control tests for eye preparations as per IP, BP and USP. *International Journal of Drug Development and Research, 7*(1), 61-68.

Hill, M., Cunningham, R. N., Hathout, R. M., Johnston, C., Hardy, J. G., & Migaud, M. E. (2019). Formulation of antimicrobial tobramycin loaded PLGA nanoparticles via complexation with AOT. *Journal of Functional Biomaterials, 10*(2), 26. https://doi.org/10.3390/jfb10020026

Kamal, D., Kavita, P., Sharma,V. K., Singh, U. V., & Ramana M. V. (2009). Applications of solid dispersion & determination of Solid dispersion technology. Retrieved from www.pharmabiz.com

Kim, K. H., Frank, M. J., & Henderson, N. L. (1985). Applications of differential scanning Calorimetry to the study of the solid drug dispersions. *Journal of Pharmaceutical Sciences, 74*(3), 283-289. https://doi.org/10.1002/jps.2600740312

Kumar, A., & Kumar, K. (2017). Solid Dispersion- Strategy to Enhance Solubility and Dissolution of Poorly Water-Soluble Drugs. *Universal Journal of Pharmaceutical Research, 2*(5), 54-59. https://doi.org/10.22270/ujpr.v2i5.RW4

Kumar, B. (2017). Solid Dispersion- A Review. *Pharma Tutor, 5*(2), 24-29.

Kumar, P., & Singh, P. (2018). Formulation and Evaluation of Eye Drop for Dry Eye Syndrome. *International Journal of Pharma Research and Health Sciences*, 6(2), 2410-2412.

Kurniawansyah, I. S., Rusdiana, T., Abnaz, Z. D., Sopyan, I., & Subarnas, A. (2021). Study of Isotonicity and Ocular Irritation of Chloramphenicol in-situ Gel. *International Journal Applied Pharmaceutics, 13*(1), 103-107. https://doi.org/10.22159/ijap.2021v13i1.39925

Kurniawansyah, I. S., Sopyan, I., Wardhana, Y. W., & Gunasekaran, M. (2018). Formulation and Evaluation of Chloramphenicol Hydrogel Ophthalmic Preparation. *Journal of Young Pharmacist, 10*(2S), S73-S78. https://doi.org/10.5530/jyp.2018.2s.14

Miller, D. P., Tan, T., Nakamura, J., Malcolmson, R. J., Tarara, T. E., & Weers, J. G. (2017). Physical Characterization of Tobramycin Inhalation Powder: II. State Diagram of an Amorphous Engineered Particle Formulation. *Molecular Pharmaceutics, 14*(6), 1950-1960. https://doi.org/10.1021/acs.molpharmaceut.7b00036

Mingeot-Leclercq, M.-P., Glupczynski, Y., & Tulkens, P. M. (1999). Aminoglycosides: activity and resistance. *Antimicrobial Agents and Chemotherapy, 43*(4), 727-737. https://doi.org/10.1128/aac.43.4.727

Nikghalb, L. A., Singh, G., Singh, G., & Kahkeshan, K. F. (2012). Solid Dispersion: Methods and Polymers to increase the solubility of poorly soluble drugs. *Journal of Applied Pharmaceutical Science, 2*(10), 170-175.

Patere, S., Newman, B., Wang, Y., Choi, S., Mekjaruskul, C., Jay, M., & Lu, X. (2020). Influence of *in vitro* release methods on assessment of tobramycin ophthalmic ointments. *International Journal of Pharmaceutics, 590*, 119938. https://doi.org/10.1016/j.ijpharm.2020.119938

Patil, M., Waydande, S., & Pravin, P. (2019). Design and evaluation of topical solid dispersion composite of voriconazole for the treatment of ocular keratitis. *Therapeutic Delivery, 10*(8), 481-492. https://doi.org/10.4155/tde-2019-0021

Pooja, Lal, V. K., & Verma, A. (2016). Development and Evaluation of Ophthalmic Drop and In-situ Gel from Roots of Boerhaavia diffusa. *Journal of Pharmaceutical Research International, 11*(1), 1-20. https://doi.org/10.9734/BJPR/2016/24395

Raj, A. L., & Kumar, Y. S. (2018). Preparation and Evaluation of Solid Dispersion of Nebivolol Using Solvent Evaporation Method. *International Journal of Pharmaceutical Sciences and Drug Research, 10*(4), 322-328. https://doi.org/10.25004/IJPSDR.2018.100418

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

Marketed Chloramphenicol Eye Drops. *IOSR Journal of Pharmacy and Biological Sciences, 15*(3), 13-19.

Rosasco, M. A., Bonafede, S. L., Faudone, S. N., & Segall, A. I. (2018). Compatibility study of tobramycin and pharmaceutical excipients using differential scanning calorimetry, FTIR, DRX, and HPLC. *Journal of Thermal Analysis and Calorimetry, 134*, 1929-1941. https://doi.org/10.1007/s10973-018-7282-z

Saha, R. N., Sanjeev, C., Priya, K. P., Sreekhar, C., & Shashikanth, G. (2002). Solubility enhancement of Nimesulide and Ibuprofen by solid dispersion technique. *Indian Journal of Pharmaceutical Sciences*, *64*(6), 529-534.

Schwartz, G. F., Hollander, D. A., & Williams, J. M. (2013). Evaluation of eye drop administration technique in patients with glaucoma or ocular hypertension. *Current Medical Research and Opinion, 29*(11), 1515-1522. https://doi.org/10.1185/03007995.2013.833898

Sebastian-Morello, M., Alambiaga-Caravaca, A. M., Calatayud-Pascual, M. A., Rodilla, V., Balaguer-Fernández, C., Miranda, M., & López-Castellano, A. (2020). Ex-Vivo Trans-Corneal and Trans-Scleral Diffusion Studies with Ocular Formulations of Glutathione as an Antioxidant Treatment for Ocular Diseases. *Pharmaceutics*, *12*(9), 861. https://doi.org/10.3390/pharmaceutics12090861

Shantier S. W., Gadkariem, E. A., Ibrahim, K. E., & Hagga, M. E. (2012). Kinetic Determination of Tobramycin in Drug Formulations. *Research Journal of pharmaceutical, Biological and Chemical Sciences, 3*(1), 566-573.

Sharma, A., & Jain, C. P. (2010). Preparation and characterization of solid dispersions of carvedilol with PVP K30. *Research in Pharmaceutical Sciences, 5*(1), 49-56.

Vyas, M. B., Patel, D., & Shah, S. K. (2015). Formulation & Evaluation of Eye Care Solution of Vasoconstrictor and Antihistaminic Drug for Conjunctivitis. *Global Journal of Medical Research, 15*(2), 19-34.