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Preparation and optimization of voriconazole microemulsion for ocular delivery

Rakesh Kumar, V.R. Sinha *

UIPS, Panjab University, Chandigarh 160014, India

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ABSTRACT

Optimized microemulsions (o/w type) of voriconazole were formulated for efficient ocular delivery. Optimized batches were selected through construction of phase diagrams following stability studies. No significant physiochemical interactions were found between the drug and excipient (oil and surfactant/co-surfactant) as confirmed by H NMR and FTIR studies. Drug content was found between 53 and 72% depending on size and composition. Selected microemulsion batches exhibited shear thinning properties with acceptable viscosities. Globule size analyzed by zetasizer as well as TEM images of selected batches were found within the desired range (<200 nm). In vitro release studies of microemulsions exhibited sustained release property (>70% in 12 h). Ex vivo permeation study also supported the enhanced drug flux through cornea from microemulsions. Based on size, surfactant/co-surfactant concentration, viscosity, drug content and release studies, the microemulsion batch ME-10 was selected for future in vivo studies.

1. Introduction

Topical application is one of the most widely preferred routes of administration for treating ocular infections/diseases as about 90% of the marketed ophthalmic formulations are in the form of eye drops. Features like patient compatibility, tolerability, the easier production method and economical cost make eye drops acceptability wider [1,2]. However, the meager bioavailability (<5%) of conventional eye drops (suspensions, solutions, etc.) is one of the major concern that could be ameliorated through the application of novel pharmaceutical approaches [3].

Application of nanotechnology-based formulations (like nanosuspension, nanoemulsion/microemulsion, solid lipid nanoparticle etc.) in the field of ophthalmology offers several beneficial features like improved solubility (for lipophilic drugs), targeted delivery and controlled release of therapeutic agents [4–7].

Microemulsions are one of the interesting and promising sub-micron carriers for ocular drug delivery. These are transparent, single optically isotropic and thermodynamically stable dispersions of water, oil and amphiphilic compounds (surfactant and co-surfactant) [8]. Microemulsions offer several advantages like improved drug-loading and bioavailability (facilitating transcorneal penetration) with acceptable biocompatibility (due to presence of physiological lipids/oils) over polymeric nanoformulations. Microemulsion of many ocular drugs like ofloxacin, timolol, tacrolimus, everolimus and prednisolone were successfully prepared with sustained effect and better penetrability [9–13].

Voriconazole (VCZ), $C_{16}H_{14}F_3N_5O$, a second generation antifungal agent possesses phenomenal characteristics like broad-spectrum activity, activity against resistant fungal species, and acceptable tolerability [14–16]. Almost 100% in vitro susceptibility was observed against various fungal isolates associated with keratitis and endophthalmitis. Moreover, studies suggested an excellent efficacy of VCZ against several ocular mycoses following topical administration [17–19].

Peng et al. fabricated PLGA [Poly (lactide-co-glycolide)] nanoparticles loaded with VCZ to improve agglomeration and antifungal efficacy in mice (renal tissue) [20]. Similarly, Sinha et al. exhibited excellent pulmonary deposition of VCZ following inhalational delivery of PLGA nanoparticles [21]. El-Hadidy et al. demonstrated significant enhancement in antifungal activity of VCZ microemulsion in comparison to drug supersaturated solution for topical effect (dermal and transdermal) [22].

So far, no topical (ocular) formulation of VCZ has been available in the market, though several researchers supported the need of effective topical delivery. This article proposes the formulation and optimization of o/w microemulsions for ocular delivery of VCZ.

* Corresponding author. Tel.: +911722534101.
E-mail address: Sinha_vr@rediffmail.com (V.R. Sinha).

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2. Experimental

2.1. Materials

VCZ was a gift sample from Lifecare Innovations Pvt. Ltd. (Gurgaon, India). Oleic acid (OA), isopropyl myristate (IPM), tween 80, propylene glycol, cotton seed oil, castor oil liquid paraffin, span 80 were purchased from S.D. Fine Chemicals (Mumbai, India). Plurol oleique was a gift sample from Gattefosse India (Mumbai, India). Dialysis membrane (MW cut-off 12–14 kDa) was purchased from HiMedia Laboratories (Mumbai, India). All other chemicals used in the study were of analytical reagent grade.

2.2. Methods

2.2.1. HPLC analysis of VCZ

A reversed phase HPLC method was developed and validated for analysis of VCZ.

The HPLC (Shimadzu, Kyoto, Japan) instrument was equipped with a model series LC-10 ADVP pump, SCL-10 AVP system controller, DGU-12 A Degasser, Rheodyne 7725i injector with a 20-μL loop and a SPD-10 AVP ultraviolet-visible detector. Separation and quantitation were made on a C18 reverse phase (250 mm × 4.6 mm (internal diameter), 5-μm Inertsil ODS-3) column that was operated at 40 °C. The mobile phase comprises a mixture of methanol and 0.1% acetic acid (pH 4) in the ratio of 70:30 (v/v), which was run at a flow rate of 1 mL/min. The eluents were analyzed spectrophotometrically at 256 nm. The retention time of VCZ was obtained at 5.45 min. The method developed was validated for linearity, precision and accuracy. A linear standard plot was obtained with $R^2 = 0.9985$. For precision (inter- and intra-day), the relative standard deviation was found below 2% while the %recovery (accuracy) was found between 98.84 and 99.65%. Limit of detection (LOD) and limit of quantitation was found as 0.1 and 0.3 μg/mL, respectively.

2.2.2. Equilibrium solubility studies for VCZ

Drug solubility in non-aqueous components is an important parameter for development of effective ophthalmic preparations. Solubility studies of VCZ were performed by mixing an excess amount of drug with various non-aqueous solvents (oils and surfactants) using a water bath shaker at room temperature (37° ± 2 °C) for 72 h to reach equilibrium. The equilibrated samples were centrifuged at 3000 rpm for 15 min. The supernatant was filtered through a 0.45 μ membrane filter and the amount of VCZ solubilized was analyzed using UV–visible spectrophotometer at 256 nm.

2.2.3. Construction of pseudo-ternary diagrams

For construction of each phase diagram, oil and specific S_{mix} ratio (surfactant:co-surfactant) were mixed carefully in diverse weight ratios from 9:1 to 1:9 (% w/w). Each weight ratio mixture was gradually titrated with distilled water and visual appearance was noted down for clear and easily flowable o/w microemulsions.

2.2.4. Stability evaluation of microemulsion and dispersibility test

2.2.4.1. Thermodynamic stability. Various thermodynamic stability tests like heating–cooling cycle, centrifugation and freeze–thaw cycle were performed on selected regions of microemulsions. Formulations that passed these stress tests were further subjected to dispersibility test.

2.2.4.2. Dispersibility test. This test assesses the efficiency of self-emulsification for VCZ microemulsions. 1 mL of each formulation was added to 500 mL of water at 37 ± 0.5 °C and the in vitro emulsification rates as well as appearance of microemulsions was graded visually.

2.2.5. Physiochemical interactions

To determine the presence and extent of interactions of VCZ with different components of microemulsions (oil and surfactant/co-surfactant) NMR and FTIR spectroscopy was used. H NMR measurements were performed on NMR spectrometer (Bruker Avance III 400 MHz, Wageningen, Netherlands) and the chemical shifts were determined using D_2O as internal locking agent. FTIR spectra were obtained using FTIR spectrophotometer (Spectrum Two, PerkinElmer, USA). Transmittance (%T) was recorded in the spectral region of 500–4500 cm^{-1} using a resolution of 4 cm^{-1} and 40 scans.

2.2.6. Globule size, polydispersity index (PDI) and drug content

The average globule size and PDI of microemulsions were determined by photon correlation spectroscopy. Measurements were made using Zetasizer 1000 HS (Malvern Instruments, Worcestershire, UK), wherein light scattering was monitored at 25 °C at a 90º angle. Drug content of microemulsions was analyzed by UV–visible spectrophotometer (Shimadzu 1700, Japan) at 256 nm.

2.2.7. Viscosity, pH and conductivity

The viscosity of undiluted microemulsions was determined using Brookfield DV-II+ viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA 02346, USA) using spindle no. 27 at 25 ± 0.5 °C. The pH and conductivity of nanoformulations were determined using a pH meter (CyberScan pH 510, Eutech Instruments) conductivity meter (ELICO, CM 180) at 25 ± 0.5 °C.

2.2.8. Surface morphology and structure

Transmission electron microscopy (Hitachi, Tokyo, Japan) was used to carry out morphological and structural examination of drug-loaded microemulsions on H7500 machine operating at 100 kV capable of point-to-point resolution. 0.5 mL droplet of the formulation stained with 0.5% aqueous solution of phosphotungstic acid was directly positioned on the copper electron microscopy grids. Combinations of different bright-field imaging at increasing magnification were used to reveal the form and size of the microemulsions.

2.2.9. In vitro drug release

A volume of 2.0 mL of the microemulsion was enclosed in a dialysis bag (cellulose membrane, mw cut-off 12400) and incubated in 40 mL release medium at 37° ± 0.5 °C under mild agitation in a water bath. Simulated phosphate buffer saline (PBS, pH 7.4) was used as the release medium. At predetermined time intervals, 500 μL of the samples were withdrawn from the incubation medium and analyzed spectrophotometrically at 256 nm. After sampling, 500 μL of fresh medium was added in the incubation medium.

2.2.10. Ex vivo permeation study

All animal experiments were carried out after approval of the protocol by the Institutional Animal Ethical Care committee (IAEC), Panjab University, Chandigarh, India, and conducted according to the Indian National Science Academy (INSA) guidelines for the use and care of experimental animals. The ex vivo permeation study was performed on the excised goat eye collected within 30 min after sacrifice from the slaughter house. Fresh excised cornea was tied to one side of the open tube (donor compartment) in such a way that its epithelial surface faced the donor compartment. The tube was submerged carefully in a beaker containing 40 mL of PBS, pH 7.4 (receptor compartment). Phosphate buffer was stirred at 50 rpm and maintained at 37° ± 0.5 °C. About 2.01 cm^2 corneal surface area was exposed to the donor cell and made available for drug permeation. Samples (0.5 mL) were withdrawn from the receptor