Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 1, 2013

Research Article

A BIO-BASED APPROACH IN DESIGNING AN ORAL DRUG DELIVERY SYSTEM FOR FLUCONAZOLE

M. JOYCE NIRMALA[a], AMITAVA MUKHERJEE[a] AND N. CHANDRASEKARAN[a, *]

[a]Centre for Nanobiotechnology, VIT University, Vellore, Tamil Nadu, India. Email: nchandrasekaran@vit.ac.in, nchandra40@hotmail.com

Received: 17 Dec 2012, Revised and Accepted: 25 Jan 2013

ABSTRACT

Fluconazole is a synthetic triazole antifungal agent used in treating many fungal infections. Owing to its poor aqueous solubilization property, we focused at devising a simple microemulsion drug delivery system for fluconazole comprising of clove oil, tween 20 and water (5: 30: 65 wt/wt). Several parameters like conductivity, pH, viscosity and droplet size measurements were done to optimize the formulated microemulsion. The drug incorporated microemulsion measured about 8–20 nm by dynamic light scattering method. The particle size reduction of the drug would prove in improving the efficacy and stability of the drug *in-vivo* as compared to its bulk form. Thus, this formulation acts as a suitable oral drug delivery system with bio-safety.

Keywords: Fluconazole, Clove oil, Microemulsion, Solubility, Tween 20

INTRODUCTION

Fluconazole [2, 4-difluoro-(alpha), (alpha) 1-bis (1H-1, 2, 4-triazol-l-ylmethyl) benzyl alcohol] belongs to the subclass of synthetic triazole antifungal agent used mainly in treating infections due to *Candida albicans* [1]. This drug has prolonged half-life, good bioavailability and easy absorption after oral administration and hence distributed widely in different sites of the body. Yet, poor solubility of fluconazole in water, may pose dissolution related problems [2–4]. Being fungistatic in mechanism, the drug causes incomplete destruction of fungi leading to emergence of certain resistant strains like *C. albicans* [5]. In order to enhance the solubilization of the drug, a simple microemulsion technique was developed to improve its efficacy after oral administration. In recent years, oil-in-water microemulsion system play a vital role to pharmaceutical scientists due to the ease of formation with no high-energy methods, optical clarity, low viscosity, long shelf-life and prolonged stability [6–9].

Hence, in our study, we reported on a simple encapsulation of the drug in herbal oil based microemulsion system. The system comprised of clove oil (*Syzygium aromaticum*), bio-based surfactant and water as the continuous phase. Internal physico-chemical characteristics of the drug-incorporated formulated system were studied using various parameters like conductivity, pH and viscosity measurements. The formulation was subjected to extreme stress conditions to confer on the stability of the system. The size reduction of the bulk drug into tiny droplets that ranged in the nano size was characterized by dynamic light scattering technique. Thus, the system was developed with an aim to enhance its solubility and thereby, the dissolution could be greatly improved *in-vivo*. This could definitely reduce the dosage concentration to a minimum and thereby the patient's compliance could be met with biological safety.

MATERIALS AND METHODS

Materials

Fluconazole was a gift sample from Morepen Laboratories Private Limited (Parwanoo, Himachal Pradesh, India). Tween 20, Bioxtra (Polyoxyethylene sorbitan monolaurate) was obtained from Sigma Aldrich, India. Clove oil and Olive oil was obtained from Hi Media, India. Coconut oil was purchased from local market in Vellore, Tamil Nadu, India. For all experiments, ultrapure water (Cascada™ Biowater System, Pall Corporation, USA) with a resistivity of not less than 18.2 MΩ cm was used. All other reagents used were of analytical grade.

Solubility study

The solubility of drug in different oils was determined by equilibration method, the same method developed from previous literatures [10]. The concentration of the drug was then analyzed using double beam UV–visible spectrophotometer (UV–Vis Systronics-2201) after appropriate dilution with carbinol.

Microemulsion formulation method

Owing to the highest solubilization of fluconazole in clove oil, about 5 mg of the drug was incorporated in clove oil and allowed to stand overnight to ensure complete solubilization. This is followed by addition of 1.4 ml of tween 20 and 3.25 ml of water and mixed thoroughly using vortex. Thus, the drug was encapsulated in the oil phase with no leakage into the water phase. This method of encapsulation was followed from the same protocol followed for the incorporation of bassic acid and diospyrin [11, 12].

Stress tests

The formulated microemulsion was centrifuged at 3500 rpm for 30 min, to ensure physical stability. Followed by, subjecting the formulation to heating cooling cycles between 4 °C and 45 °C for 48 h each time. Then subjecting the same to three freeze-thaw cycles between −21 °C and +25 °C for 48 h each was repeated.

Physico-chemical characterization of the microemulsion

Conductivity measurement

The solubilization of water phase in the selected oily mixture was monitored quantitatively by measuring the electrical conductivity (σ) using a conductivity meter (Conductivity meter, Elico CM 180). Conductivity measurement was done to determine the type of microemulsion formed and the experiments were carried out in triplicates.

pH measurement

The pH value of the thermodynamically stable microemulsion was measured by immersing the electrode directly into the dispersion using a calibrated pH meter (model HI 8417, Hanna Instruments Inc., Woonsocket, USA), at 25 ± 1 °C. The measurement was carried out in triplicates.

Viscosity determination

The viscosity of the stable microemulsion was determined as such without dilution using Brookfield Viscometer (LVF model)-UL-Adapter with spindle set, Spindle # 2 at 25 ± 1 °C. The measurement was carried out in triplicates.

Droplet size measurement

The droplet size of stable microemulsion was determined by dynamic light scattering technique. The instrument used was 90Plus Particle Size Analyzer (Brookhaven Instruments Corp., Holtsville,

New York, USA). The measurement was carried out in triplicates. A sample volume of 3 ml was used for the study without any dilution.

RESULTS AND DISCUSSION

Solubility study

An oil-in-water microemulsion system play a better role in incorporating hydrophobic drug molecules as they show improved solubility and stability. Hence, different oils including clove oil, coconut oil and olive oil were chosen for our study. The oil system helps in maintaining the drug in its solubilized form. Based on the highest solubility of fluconazole in clove oil (146.25 mg/ml), clove oil was selected as the suitable oil system for the development of microemulsion system as shown in Table 1.

Table 1: The solubility of the drug (mean ± S. D., n=3) in different oils

Oils	Solubility (mg/ml)
Olive oil	1.57 ± 0.05
Coconut oil	0.08 ± 0.02
Clove oil	146.25 ± 2.09

Stress tests

The formulated microemulsion was subjected to different stress tests such as centrifugation, heating cooling cycle and freeze thaw cycle. From the study, it was observed that, the formulated system passed through all stress tests and was found stable for a period of 8 months. Hence the formulation was further subjected to characterization.

Physico-chemical characterization

Conductivity measurements

Conductivity measurement is done to understand whether the formulated system is oil-continuous, bi-continuous or water-continuous type. The conductivity measurement is done on the basis of percolation theory [13]. In Fig. 1, a comparison between both drug-unloaded and drug-loaded microemulsion is shown, which interprets that the formulated system has no significant change concerning the microstructures formed. The electrical conductivity of the system, expressed in terms of µS/cm, was very low as long as the water concentration was titrated to 15% (w/w), which is the percolation threshold. On further titration of water to 55% (w/w), there was a linear and a sharp increase in the conductivity range beyond the percolation threshold. The peak occurred at around 55% water content and this is the transition point to form oil-in-water microemulsion region. After this transition point, the conductivity decreased slowly on addition of water phase to the system due to dilution of oil-in-water microemulsion. Thus, the conductivity measurement demonstrates three different structural regions in the system: water-in-oil, bi-continuous and oil-in-water region.

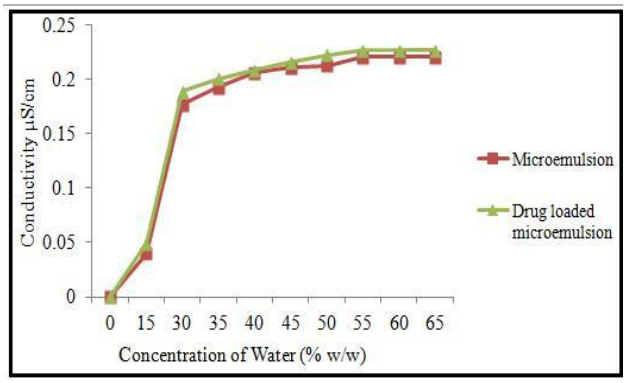

Fig. 1: Comparative analysis of electrical conductivity of drug-loaded and drug-unloaded microemulsion

Measurement of pH range

The pH of the thermodynamically stable formulation was found to be 4.2. With further increase in the surfactant concentration, the pH value also increased.

Viscosity

The viscosity of the stable formulation measured 40 cPs as determined by viscometer. The viscosity increased with further increase in the surfactant concentration. The increase may be likely due to the water molecules that get trapped into the cross-linking portions of the surfactant molecule [14].

Droplet size analysis

The droplet size of the system is measured using dynamic light scattering technique. The size distribution yields useful information regarding the stability of the microemulsion formed [15]. The formulated system showed uniform size distribution of 8–20 nm. The uniform distribution of droplets confers good stability.

CONCLUSION

The present work describes the incorporation of fluconazole using simple herbal oil based microemulsion system. The system involves no high energy methods and also yields small droplets in the nanometer range that confers good stability at extreme conditions. The minimal viscosity due to low surfactant concentration is an added advantage as it may avoid gastric irritation. Moreover, the enhanced solubility of the drug would greatly improve the efficacy of the drug at a minimum concentration.

ACKNOWLEDGEMENT

The authors thank the management of VIT University for the provision of funding to carry out our research work.

REFERENCES

1. Park HJ, Kim MS, Lee S, Kim JS, Woo JS, Park JS, Hwang SJ. Recrystallization of fluconazole using the supercritical antisolvent (SAS) process. Int J Pharm. 2007; 328, 152–160.
2. Debruyne D, Ryckelynck JP. Clinical pharmacokinetics of fluconazole. Clin Pharmacokinet. 1993; 24, 10–27.
3. Kauffman CA. Role of azoles in antifungal therapy. Clin Infect Dis. 1996; 22, S148–S153.
4. Sun S, Lou H, Gao Y, Fan P, Ma B, Ge W, Wang X. Liquid chromatography–tandem mass spectrometric method for the analysis of fluconazole and evaluation of the impact of phenolic compounds on the concentration of fluconazole in *Candida albicans*. J Pharm Biomed Anal. 2004; 34, 1117–1124.
5. Rex JH, Rinaldi MG, Pfaller MA. Resistance of *Candida* species to fluconazole. Antimicrob Agents Chemother. 1995; 39, 1–8.
6. Bagwe RP, Kanicky JR, Palla BJ, Patanjali PK, Shah DO. Improved drug delivery using microemulsions: rationale, recent progress and new horizons. Crit Rev Ther Drug. 2001; 18, 77–140.
7. Talegaonkar S, Azeem A, Ahmad JF, Khar RK, Pathan SA, Khan ZI. Microemulsions: a novel approach to enhanced drug delivery. Recent Pat Drug Deliv Formul. 2008; 2, 238–257.
8. He CX, He ZJ, Gao JQ. Microemulsions as drug delivery systems to improve the solubility and the bioavailability of poorly-water soluble drugs. Expert Opin Drug Deliv. 2010; 7, 445–60.
9. Anjali CH, Dash M, Chandrasekaran N, Mukherjee A. Antibacterial activity of sunflower oil microemulsion. Int J Pharm Pharm Sci. 2010; 2, 123–128.
10. Nirmala MJ, Mukherjee A, Chandrasekaran. N. Enhanced solubilization of aqueous insoluble anti-hypertensive drug. Int J Pharm Pharm Sci. 2012; 4, 366–368.
11. Lala S, Gupta S, Sahu NP, Mandal D, Mondal NB, Moulik SP, Basu MK. Critical evaluation of the therapeutic potential of bassic acid incorporated in oil-in-water microemulsions and poly-D, L-lactide nanoparticles against experimental leishmaniasis. J Drug Target. 2006; 14, 171–179.
12. Gupta S, Moulik SP, Hazra B, Ghosh R, Sanyal SK, Datta S. New pharmaceutical microemulsion system for encapsulation and

delivery of diospyrin, a plant-derived bioactive quinonoid compound. Drug Deliv. 2006; 13, 193–199.

13. Rao J, McClements DJ. Formation of flavor oil microemulsions, nanoemulsions and emulsions: influence of composition and preparation method. J Agric Food Chem. 2011; 59, 5026.
14. Eini DIDE, Barry BW, Rhodes CT. Micellar size, shape and hydration of long-chain polyoxyethylene nonionic surfactants. J Colloid Interface Sci I. 1976; 54, 348.
15. Constantinides PP, Yiv SH. Particle size determination of phase inverted water-in-oil microemulsions under different dilution and storage conditions. Int J Pharm. 1995; 115, 225.