Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 2, 2013

Research Article

DEVELOPMENT OF A SUITABLE DRUG DELIVERY SYSTEM FOR AZITHROMYCIN: FORMULATION AND CHARACTERIZATION

M. JOYCE NIRMALA[a], MURUGESH SHIVASHANKAR[b], AMITAVA MUKHERJEE[a], N. CHANDRASEKARAN[a, *]

[a]Center for Nanobiotechnology, VIT University, Vellore, India, [b]Pharmaceutical Chemistry Division, School of Advanced Sciences, VIT University, Vellore, India. Email: nchandrasekaran@vit.ac.in, nchandra40@hotmail.com

Received: 07 Feb 2013, Revised and Accepted: 19 Mar 2013

ABSTRACT

Azithromycin, an important member of the azalides subclass is found effective in the treatment of both Gram-positive and Gram-negative organisms. But it has relatively low bioavailability of 37% due to its water insoluble property. We aimed at designing a thermodynamically stable microemulsion formulation of azithromycin to improve the solubility of azithromycin to a greater extent and therefore, the efficacy of the drug could be greatly enhanced. A typical wt/wt% composition of clove oil (5%), tween 20 (30%) and water (65%) when incorporated with drug in the oil core produced droplets in the diameter size range of 8–20 nm as confirmed through dynamic light scattering method. The small size would aid in easy permeation through capillaries and thereby, the efficacy could be greatly attained. The system was further characterized using conductivity, pH, and viscosity measurements to understand the physico-chemical nature. Thus, the system stands out to be the best system for the oral drug delivery of azithromycin.

Keywords: Azithromycin, Microemulsion, Solubility, Dynamic light scattering, Viscosity

INTRODUCTION

Azithromycin is a commonly used antibiotic used in the treatment of infections caused by both Gram-positive and Gram-negative [1] organisms. The mechanism of action is that it works by binding to 50S subunit of the 70S bacterial ribosome and thereby inhibits the protein synthesis of micro-organisms [2]. Amidst the wide usage of azithromycin, it has certain physico-chemical properties to be modified due to its poor water solubility and relatively low oral bioavailability of about 37% after administration [3]. Hence, we aimed at designing a suitable drug delivery system to enhance its solubilization with improved stability, and thereby, the efficacy of the drug could be improved.

Our study is the first time to report on microemulsion based system with minimum number of components in them, for the incorporation of azithromycin. Eventhough, the same system has been used for the encapsulation of many other drugs. The microemulsion drug delivery system was chosen as it has compartmentalized hydrophilic and hydrophobic domains in which both non-polar and polar compounds could be incorporated. Also, oil-in-water microemulsions are greatly known for improving the bioavailability of highly lipophilic and aqueous insoluble drugs [4–7]. The ease of preparation, higher solubilization of organic compounds, small droplet size, high surface area, enhanced shelf life and good thermodynamic stability are the added advantages of the system [8–12].

Owing to the highest solubilization, clove oil based microemulsion system was chosen as the best system for azithromycin incorporation. Few literatures on clove oil microencapsulation of natural bioactive products like Bassic acid, Quercetin and Diospyrin have reported the system to be more efficient, non-toxic and biocompatible [13–15]. Also, clove oil exhibits good anti-bacterial activity against certain micro-organisms, hence, this clove oil microemulsion system has additional importance in enhancing the efficacy of an anti-bacterial drug, azithromycin.

MATERIALS AND METHODS

Chemicals

Azithromycin was a gift sample from Aurobindo Pharma Limited, Hyderabad, India. Tween 20, Bioxtra was obtained from Sigma Aldrich, India. Clove oil was purchased from S. D. Fine Chemicals (Mumbai, India). Peppermint oil and olive oil were obtained from Hi Media, 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 reagent grade.

Solubility studies

The solubility of azithromycin in different oils was determined by conventional equilibration method, the method developed from previous literatures [16]. The concentration of drug was analyzed using double beam UV–visible spectrophotometer (UV–Vis Systronics-2201) after appropriate dilution with methanol at 215 nm.

Preparation of microemulsion system

Based on the highest solubilization of azithromycin in clove oil, a minimum amount (5 mg) of drug that is completely soluble in clove oil was incorporated in oil core and allowed to stand overnight for solubilization to occur. Followed by, addition of 1.4 ml of tween 20 and 3.25 ml of water to it. Mix thoroughly using a vortex mixer. The drug was completely encapsulated in the oil phase with no leakage into the water phase. This method of clove oil microemulsion was developed from the same literature studies of bassic acid, quercetin and diospyrin incorporation.

Stress tests

(1) Centrifugation: The formulation was centrifuged at 3500 rpm for 30 min, to ensure physical stability.

(2) Heating cooling cycle: Six cycles between refrigerator temperature of 4 °C and 45 °C for 48 h was examined.

(3) Freeze thaw cycle: Three freeze-thaw cycles between −21 °C and +25 °C was also checked.

Physico-chemical characterization

Conductivity measurement

The conductivity measurement (Conductivity meter, Elco CM 180) helps in determining whether the microemulsion system formed is oil-continuous, bi-continuous or water-continuous. The solubilization of water phase in the selected oily mixture was monitored quantitatively by measuring the electrical conductivity (σ). The conductivity (σ) of formulated samples was measured using a conductivity meter. The measurements were performed in triplicates.

pH measurement

The pH value of the formulated 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 measurements were done in triplicates.

Viscosity measurement

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

Size analysis

Scanning electron microscopy

The surface morphology of the bulk drug azithromycin pure was observed by a scanning electron microscope (FEI, Quanta 200F). The bulk drug in its white crystalline form was mounted on a 10 mm metal stubs using carbon tape. The sample was then sputtered with gold under vacuum in nitrogen atmosphere. The analysis was made using a potential difference of 20 kV for the field emission gun.

Droplet size measurement

The droplet size of the microemulsion formulation was determined by dynamic light scattering technique. The instrument used was 90Plus Particle Size Analyzer (Brookhaven Instruments Corp., Holtsville, New York, USA). The size measurement was carried out in triplicates, and the average results were reported in this paper. A sample volume of 3 ml was used in this measurement.

RESULTS

Solubility study

As determined from the solubility studies, azithromycin showed the highest solubilization in clove oil. The oil system plays an important role in maintaining the drug in its solubilized state. The results are shown in Table 1.

Table 1: Solubility of azithromycin (mean ± S. D., n=3) in different oils

Oils	Solubility (mg/ml)
Olive oil	0.02 ± 0.01
Coconut oil	3.01 ± 0.06
Clove oil	140.26 ± 1.10

Drug loaded microemulsion formulation

The drug was initially incorporated in the oil core. Optically clear, transparent and easily flowable microemulsion system was formed within few seconds on addition of oil (drug-loaded), tween 20 and water (5:30:65 wt/wt) without the use of any high-energy methods. The system was then characterized and checked for its internal stability.

Physico-chemical characterization

Stability study

The formulated drug-loaded microemulsion system was found physically stable for a period of one year with no phase separation. No phase separation, flocculation or coalescence was observed during storage at different extreme conditions and also during centrifugation. Thus, the formulated system of azithromycin is said to exhibit good thermodynamic stability.

Conductivity and pH measurement

The conductivity of the microemulsion system as determined by conductivity meter was 0.228 µS/cm. The pH was measured to be 4.2. The pH showed no significant change upon storage due to the presence of non-ionic surfactant in the system.

Viscosity determination

The viscosity of the microemulsion was 38 cPs as determined by viscometer. The viscosity of a particular microemulsion system increases with an increase in surfactant concentration due to the presence of water molecules that get trapped into the cross-linking portions of the surfactant molecule [17].

Size analysis

Scanning electron microscopy

The morphology of the bulk drug, azithromycin pure was examined by scanning electron microscopy. The surface topography of the white, crystalline powder form of azithromycin was seen as cuboidal images that are sharp edged as observed through SEM. As evidenced from electron microscopic images as shown in Fig. 1, the size of the bulk drug ranged around 100 µ (microns) in length. The original size and morphology of the bulk drug was visualized in order to study the morphological difference and size reduction of the drug when designed to a microemulsion formulation.

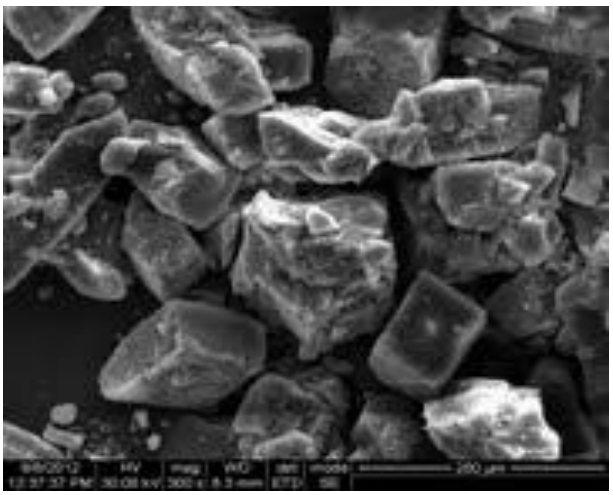

Fig. 1: It shows scanning electron microscopic image of azithromycin pure

Droplet size measurement

The droplet size diameter of the formulation was found to be in the range of 8–20 nm. This size distribution correlated well with our previous report of clove oil microemulsion encapsulation of fluconazole [18]. Also, the measurements were taken as such without any dilution as it may alter the morphology of the microstructure formed.

CONCLUSION

Our study presents a suitable drug delivery system for azithromycin utilizing simple clove oil based microemulsion concept. The particle size reduction may enhance the efficacy of the drug and also leads to good stability and increased solubilizing capacity of the drug incorporated system. Also, the use of clove oil, that by itself, possess anti-microbial property, may add to superior anti-bacterial activity of the drug against the action of microbes. Henceforth, the dosage could be reduced by the usage of this system.

ACKNOWLEDGEMENTS

The authors thank the management of VIT University, Chancellor, for providing facility to carry out our research. We acknowledge Sophisticated Analytical Instrumentation Facility (SAIF), Department of Science & Technology (DST) at Indian Institute of Technology (IIT), Madras, for Scanning Electron Microscopy facility used in our research.

REFERENCES

1. Dinos GP, Michelinaki M, Kalpaxis DL. Insights into the mechanism of azithromycin interaction with an *Escherichia coli* functional ribosomal complex. Mol Pharmacol. 2001; 59, 1441–1445.
2. Champney WS, Burdine R. Azithromycin and clarithromycin inhibition of 50S ribosomal subunit formation in *Staphylococcus aureus* cells. Curr Microbiol. 1998;36:119–23.

3. Luke DR, Foulds G. Disposition of oral azithromycin in humans. Clin Pharmacol Ther. 1997; 61, 641–648.
4. Chen H, Chang X, Du D, Li J, Xu H, Yang X. Microemulsion-based hydrogel formulation of ibuprofen for topical delivery. Int J Pharm. 2006; 315, 52–58.
5. Yuan Y, Li S, Mo F, Zhong D. Investigation of microemulsion system for transdermal delivery of meloxicam. Int J Pharm. 2006; 321, 117–123.
6. Damasceno BP, Dominici VA, Urbano IA, Silva JA, Araujo IB, Santos-Magalhaes NS, Silva AK, Medeiros AC, Oliveira AG, Egito ES. Amphotericin B microemulsion reduces toxicity and maintains the efficacy as an antifungal product. J Biomed Nanotechnol. 2012; 8, 290–300.
7. Shah KA, Joshi MD, Patravale VB. Biocompatible microemulsions for fabrication of glyceryl monostearate solid lipid nanoparticles (SLN) of tretinoin. J Biomed Nanotechnol. 2009; 5, 396–400.
8. He CX, He ZJ, Gao JQ. Microemulsions as drug delivery systems to improve the solubility and bioavailability of poorly-water soluble drugs. Expert Opin Drug Deliv. 2010; 445–60.
9. Fatouros DG, Karpf DM, Nielsen FS, Mullertz A. Clinical studies with oral lipid based formulations of poorly soluble compounds. Ther Clin Risk Manag. 2007; 3, 591–604.
10. 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.
11. Nagarajan R. Molecular theory of microemulsions. Langmuir. 2000; 16, 6400–6415.
12. 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.
13. Gupta S, Moulik SP, Lala S, Basu M, Sanyal SK, Datta S. Designing and testing of an effective oil-in-water microemulsion drug delivery system for *in vivo* application. Drug Del. 2005; 12, 267–274.
14. Gupta S, Moulik SP, Hazra B, Ghosh R, Sanyal SK, Datta S. New pharmaceutical microemulsion system for encapsulation and delivery of a plant-derived bioactive quinonoid compound, diospyrin. Drug Del. 2006; 12, 193–199.
15. Lala S, Gupta S, Basu MK, Moulik SP. Critical evaluation of the therapeutic potential of basic acid entrapped in oil-in-water microemulsions and classical polylactide nanoparticles against Leishmaniasis. J Drug Target. 2006; 14, 171–179.
16. Nirmala MJ, Mukherjee A, Chandrasekaran. N. Enhanced solubilization of aqueous insoluble anti-hypertensive drug. Int J Pharm Pharm Sci. 2012; 4, 366–368.
17. 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.
18. Nirmala MJ, Mukherjee A, Chandrasekaran. N. A bio-based approach in designing an oral drug delivery system for fluconazole. Int J Pharm Pharm Sci. 2013; 5, 273–275.