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Research Article

IMPROVED PHYSICO-CHEMICAL ASPECTS OF AZITHROMYCIN THROUGH NOVEL MICROEMULSION SYSTEM

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ABSTRACT

Azithromycin, a semi-synthetically important macrolide is well known for its wide applications as anti-microbials. This drug could act as both bacteriostatic and bactericidal depending on the dosage administered. Apart from its good activity in both Gram positive and Gram-negative micro-organisms, azithromycin was found to be sparingly soluble in water as it is highly lipophilic. Also, the oral bioavailability was found to be as low as 37% due to incomplete absorption. Hence, we worked on the concept of microemulsion technique to increase the solubilization of the drug in a lipophilic environment and also to reduce the particle size to a greater extent that may, on the other hand, enhance the permeation. Our formulation included gentle mixing of cinnamon oil, tween 20 and water to obtain clear and transparent oil-in-water microemulsion system. This system showed enhanced solubility rate, reduced droplet size diameter of 10-45 nm and excellent stability. In addition, the inclusion of cinnamon oil in the system may have added benefit in improving the anti-bacterial activity in certain pathogens.

Keywords: Cinnamon oil, Azithromycin, Bio-based surfactant, Solubility, Conductivity.

INTRODUCTION

Azithromycin, a semi-synthetic derivative and structural analogue of erythromycin is an important member of the macrolides [1]. It is one of the world's best selling antibiotic because of its wide array of pH dependent anti-microbial activity and numerous desired qualities such as long half-life, high tissue penetration, high acid stability and gastrointestinal tolerability and the substantially increased potency against Gram-negative bacteria is due to presence of methyl substituted nitrogen atom in the macrolide ring [2–4]. This drug which could be bacteriostatic and bactericidal depending upon the dosage administered, interferes with the protein synthesis by binding to the 23s rRna of 50s ribosomal unit [5]. This action leads to the blocking of 50s ribosomal subunit assembly and thereby nullifies the production of important proteins required for the growth of pathogen. Due to the differences in the way proteins are made in bacteria and humans, the macrolyte antibiotics do not interfere with the humans' ability to synthesize proteins [6]. This drug which has 3 routes of administration is rapidly taken up by phagocytes and delivered to the sites of infection [7]. Higher tissue concentration ensures prolonged half-life and slows down drug distribution that helps in the maintenance of similar bacteriostatic levels for several days [8]. Amidst the wide usage, azithromycin may lead to undesirable effects. This azane substituent of erythromycin has objectionable taste and makes the person photosensitive. This drug also kills some important species of gut flora that are essential for human digestion. Common side effects include diarrheoa, gastro-intestinal irritation, prolonged QTc intervals, skin rash and the effects are dependent on the route of administration [9]. Antagonistic effects of antacids containing Mg^{2+} decrease the peak serum concentration and efficacy of this drug. Azithromycin may bind to food and hence should be administered on empty stomach. Since hepatic metabolism plays a key role in the mechanism of action of azithromycin, this drug should not be administered if the patient has impaired hepatic function. Because of the lipophilic nature, this drug has low water solubility which stands out as one of the reason for low oral availability and decreased in vivo efficacy of the drug. Hence there is a need for the design of novel drug delivery system to improve the solubilization of this drug.

In the recent years the concept of micro-emulsions has drawn attention as drug delivery agents due to compartmentalized hydrophobic and hydrophilic domains, ease of preparation, optical clarity, low viscosity, long shell life and prolonged stability [10–12]. Hence, we aimed at designing a micro-emulsion based novel drug delivery system to enhance the solubilization of this drug with improved stability and to achieve particle size reduction of the drug to reduce the dosage. Our study regarding the microemulsion drug delivery system for azithromycin is detailed in Fig. 1.

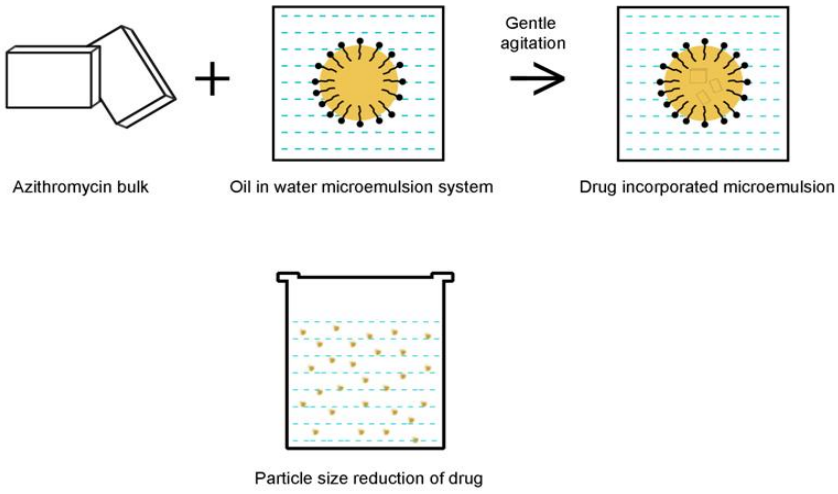

Fig. 1: Schematic representation of azithromycin drug delivery system

MATERIALS AND METHODS

Chemicals

Azithromycin was obtained from Aurobindo Pharma Limited, Hyderabad, India. Tween 20 (Bioxtra) and cinnamon oil was obtained from Sigma Aldrich, India. Peppermint oil, castor 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. Other reagents used were of analytical reagent grade.

Solubility

The solubility of azithromycin in various lipophilic environments was determined by conventional equilibration method. The concentration of drug was analyzed using double beam UV–Visible spectrophotometer (UV–Vis Systronics-2201) after appropriate dilution with ethanol at 215 nm.

Microemulsion technique

Based on the highest solubilization of azithromycin in cinnamon oil, a minimum fixed concentration of the drug was loaded into the oil core (5%) and allowed to stand overnight for solubilization to occur. Followed by, addition of bio-based surfactant (tween 20) and water. After thorough mixing, the drug was fully incorporated in the oil phase with no leakage into the water phase.

Stability

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

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

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

Conductivity measurement

The solubilization of water phase in the selected oily mixture was monitored quantitatively by measuring the electrical conductivity (σ) using conductivity meter (Elco CM 180). The measurements were performed in triplicates.

Viscosity

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 ℃. Viscosity measurement was carried out in triplicates.

Droplet size

The droplet size of our formulation was determined by dynamic light scattering (DLS) - 90Plus Particle Size Analyzer (Brookhaven Instruments Corp., Holtsville, New York, USA). The hydrodynamic diameter of the system was carried out in triplicates, and the average results were reported in this paper.

RESULTS

Solubility study

The lipophilic system plays an important role in maintaining the drug in its solubilized state. The solubilization potential of azithromycin in various oils are shown in Table 1.

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

Oils	Solubility (mg/ml)
Peppermint oil	12.57 ± 0.46
Olive oil	0.02 ± 0.01
Castor	4.18 ± 0.04
Cinnamon oil	64.41 ± 1.34

Microemulsion formation

The drug was solubilized in cinnamon oil initially. Optically clear, transparent and easily flowable microemulsion system was formed within few seconds, followed by, the addition of tween 20 and water respectively (5:25:70 v/v). This was done by gentle mixing with hand to bring the components together. The system was then subjected to characterization.

Stability

The drug-loaded cinnamon oil based microemulsion system was found physically stable for a period of one year with no phase separation, flocculation or coalescence. The formulation passed through all three stress tests and therefore said to demonstrate good thermodynamic stability.

Conductivity

The conductivity of the microemulsion system as determined by conductivity meter was 0.279 µS/cm. The conductivity study was based on percolation theory and this study clearly explains that our drug-loaded system was of oil-in-continuous type.

Viscosity

The viscosity of the drug-loaded system was 21 cPs as determined by viscometer. With increase in the surfactant concentration, the water molecules get trapped into the cross-linking portions of surfactant molecule. Therefore, the surfactant concentration has a positive correlation with the viscosity readings.

Droplet size

The droplet size diameter of the formulation was found to be in the range of 10–45 nm as measured by dynamic light scattering technique. Our result more or less coincides with our previous report of clove oil microemulsion encapsulation of the same drug. The size distribution analysis was taken without dilution as the microstructure may get altered.

CONCLUSION

Cinnamon oil microemulsion drug delivery system for azithromycin is a novel and potential system due to its high solubility, good stability, small droplet size and improved efficacy. Also, the use of this essential oil based system may reduce the toxicity rate to a very lower level.

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REFERENCES

1. Petropoulos AD, Kouvela EC, Starosta AL, Wilson DN, Dinos GP and Kalpaxis DL. Time-resolved binding of azithromycin to *Escherichia coli* ribosomes. J Mol Biol. 2009; 385, 1179–1192.
2. Zuckerman JM. Macrolides and ketolides: azithromycin, clarithromycin, telithromycin. Infect Dis Clin N Am 2004; 18, 621–649.
3. Farmer S, Li Z and Hancock REW. Influence of outer membrane mutations on susceptibility of Escherichia coli to the dibasic macrolide azithromycin. J Antimicrob Chemother. 1992; 29, 27–33.
4. Yousef AA and Jaffe A. The role of azithromycin in patients with cystic fibrosis. Paediatr Respir Rev. 2010; 11, 108–114.
5. Gaynor M and Mankin AS. Macrolide antibiotics: binding site, mechanism of action, resistance. Front Med Chem. 2005; 2, 21–35.
6. Dewan I, Amin T, Hossain MF, Hasan M, Chowdhury SF, Gazi M and Islam SMA. Development and validation of a new HPLC method for the estimation of azithromycin in bulk and tablet dosage form. Int J Pharm Sci Res. 2013; 4(1), 282–286.
7. Lode H, Borner K, Koeppe P and Schaberg T. Azithromycin-review of key chemical, pharmacokinetics and microbiological features. J Antimicrob Chemother. 1996; 37, Suppl.C, 1–8.

8. Hunter RP, Koch DE, Coke RL, Goatley MA and Isaza R. Azithromycin metabolite identification in plasma, bile and tissues of ball python (Python regius). J vet Pharmacol Therap. 2003; 26, 117–121.
9. Xin-Li L, Jian-Jun L, Da-Chang W, Cui-Li Z and Yi X. Detection of intestinal microflorafom mice affected by macrolide antibiotics. Afr J Microbiol Res. 2013; 7(16), 1475–1479.
10. Bagwe RP, Kanicky JR, Palla BJ, Patanjali PK and Shah DO. Improved drug delivery using microemulsions: rationale, recent progress, and new horizons. Crit Rev Ther Drug Carrier Syst. 2001; 18(1), 77–140.
11. Nirmala MJ, Shivashankar M, Mukherjee A and Chandrasekaran N. Development of a suitable drug delivery system for azithromycin: formulation and characterization. Int J Pharm Pharm Sci. 2013; 5(2), 598–600.
12. Nirmala MJ, Mukherjee A and Chandrasekaran N. A bio based approach in designing an oral drug delivery system for fluconazole. Int J Pharm Pharm Sci. 2013; 5, 273–275.