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Formulation and characterization of nanomicelles loaded with azithromycin for improved solubility

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ABSTRACT

The objective of the present investigation was to prepare nano-micelles loaded with azithromycin for improving its solubility and bioavailability. Nanomicelles were prepared using Soluplus as the surfactant and thin film method. The CMC was determined to be 1.0mM. The particle size of the formulations below and above the CMC was more than 100 nm with a PDI of more than 0.2. At the CMC, the PDI of the formulation was close to less than 0.2. The drug loaded formulation at just above the CMC presented particle size of 98.3nm and PDI of 0.128. The zeta potential of the AZI loaded nano-micelles ranged from -2.45 to -3.54. The encapsulation efficiency was found to be 94.8 to 97.2% slightly increasing with an increase in concentration of the surfactant. The *in vitro* release of AZI from suspension and the optimized nanomicelle (NM4) was determined by shake flask method. The AZI suspension release 51% drug in 2 hours and the concentration decreased to 48% in 5 hours. No azithromycin was found in the release medium at the 8th hour till 24h. On the other hand the nanomicelle released 29% AZI in 2h and 74% at the 24th hour of the study.

Introduction

The major problem being faced by modern pharmaceutical research is the hydrophobic characteristic of drugs, which is associated with ever-increasing solubility problems for the past ten years [1, 2]. Currently, approximately 70% of new drug candidates are insoluble in water and organic media. Besides, 40% of marketed oral drugs are considered hydrophobic drugs [3, 4]. The enhancement of oral bioavailability of poorly water-soluble drugs remains one of the most challenging aspects of drug development. The most commonly used techniques to increase dissolution rate are particle size reduction, salt formation and lyophilization, but all these methods have practical limitations like improper enhancement of solubility and all the drugs are not suitable for these techniques.

Polymeric micelles are comparatively more stable than surfactant micelles. It can solubilize a substantial quantity of hydrophobic molecules in their central core. Because of their structural aspects like size and hydrophilic shell they possess prolonged circulation times in vivo and can accumulate in tumor tissues [5].

In the pharmaceutical industry, micellar solubilization finds an important application for the enhancement of solubility and bioavailability of drugs [6-8]. The poorly water-soluble drugs may be entrapped within the hydrophobic core or linked covalently to the surface of polymeric micelles to improve their aqueous solubility. Solubilization is controlled by the characteristics of the drug as well as those of the micellar systems.

Azithromycin (AZI) is a macrolide antibiotic that binds to the 23S rRNA of the bacterial 50S ribosomal subunit. It stops bacterial protein synthesis by inhibiting the transpeptidation/translocation step of protein synthesis and by inhibiting the assembly of the 50S ribosomal subunit [9-11]. The low solubility of azithromycin (AZI) makes it a favourable candidate for micellar solubilization. The nano sizing of the particles improves the bioavailability of the formulation further. It was therefore envisioned to prepare nano-micelles loaded with azithromycin for improving its solubility and bioavailability.

Material and Methods

All the material used in the study were procured from various sources either as gift sample or purchased and used without any processing or purification.

Preformulation characterization

The procured AZI (Ind Swift, Baddi) was subjected to solubility, melting point, partition coefficient determination [12]. The calibration curve of AZI was prepared in phosphate buffer pH 6.8 by UV spectrophotometry at 285 nm.

Preparation of blank nano-micelles

In a round-bottomed flask, the required amount of azithromycin (50 mg) was dissolved in 10 mL of acetone. Soluplus® was added (Table 1), and the mixture was stirred until a limpid solution was obtained. The solvent was evaporated off by a rotating evaporator at 60 °C for 2–3 h, until a thin film was produced. The flask was kept under vacuum overnight and the rehydrated with water (10 mL) under magnetic stirring at 650 rpm at room temperature to achieve a final drug concentration of 0.5% (w/v) and a various concentrations of Soluplus® micellar suspension [13,14]. Blank Soluplus® solution was prepared by dissolving the

polymer in distilled water, under constant magnetic stirring at room temperature for 48 h [13,14].

Table 1. Batch formula for nano-micelle preparation

Formulation code	Azithromycin (mg)	Soluplus® (g)	Soluplus® micellar concentration achieved
NM1	50	0.0575	0.5 mM
NM2	50	0.0862	0.75 mM
NM3	50	1.150	1.0 mM
MN4	50	1.725	1.5 mM
NM5	50	2.30	2.0 mM
NM6	50	2.875	2.5 mM
Blank NM	-	1.725	1.5 mM

Critical Micellar Concentration

Dilute the formulation with water in the ratios (formulation: Water) 10:0, 7:3, 5:5, 3:7, and 0:10. The above ratios are further analyzed using conductivity method. The conductivity of each dilution was measured using conductivity meter and the CMC for each formulation was determined [15].

Shape, size and zeta potential determination

The size distribution, mean size, and polydispersity index (PDI) of both nanosystem were determined by dynamic light scattering (DLS) using the equipment Zetasizer Nano ZS (Malvern Instruments, UK) [16].

Entrapment Efficiency

The encapsulation efficiency (EE%) was determined by membrane filtration method [17]. The filtrate (20 µL) was disrupted with 980 µL of MeOH by vortexing for 15 min. The amount of AZI encapsulated and loaded in the nanomicelles was quantified by measuring the absorbance by UV Spectrophotometry.

***In vitro* release**

A suitable medium for the *in vitro* release of AZI was selected using the shake-flask method. The release of AZI from nanomicelle was evaluated using dialysis bags (MWCO of 8000–12000 kDa) [18].

Briefly, 1875 mg of nanomicelle (containing 1.5 mg of AZI) or 1.5 mg of free AZI (control sample) was added to 15 mL of DDW and dialyzed in 150 mL of PBS containing Tween 80 (2.75 mg/mL) under stirring at 100 rpm and 37°C in an incubator shaker. At 5 min, 15 min, 30 min, 1 h, 2 h, 5 h, 8 h and 24 h, 1 mL of dialysis medium was collected, and the volume was replaced with fresh medium. AZI in the eluate was quantified by UV.

Anti-bacterial activity of the formulation

The microorganisms used for the antimicrobial study were procured from Institute of Microbial Technology, Chandigarh (MTCC). *Staphylococcus aureus* (MTCC 3160) was used for the present investigation.

Agar plates were prepared by pouring the sterilized medium into sterilized petridishes suitably marked and labeled. The plates were allowed to solidify in the laminar flow bench and stored packed for culturing with microbes and antimicrobial screening. The agar plates were inoculated with a few drops of the bacterial suspension by swabbing on the surface of agar. The antimicrobial action was screened using disc diffusion method [19]. Wells were bored into the agar plate at equal distances using cork borer (10mm) and 200µL of the nanomicelle (50, 75, 100 & 150 µg/mL) were placed in each hole. The plates were incubated for 24h at 37 ± 0.1°C to allow for microbial growth. The zone of inhibition in each plate was measured in millimeters.

Results and Discussion

Preformulation Studies

The procured AZI was white, odorless powder that melted at 117-120°C and was insoluble in water and slightly soluble in phosphate buffer. The sample displayed partition coefficient of 4.05 and loss on drying of 0.15%. The linear regression analysis for the calibration curve was Abs = 0.006(conc) + 0.030 with a regression coefficient of 0.993 (Figure 1).

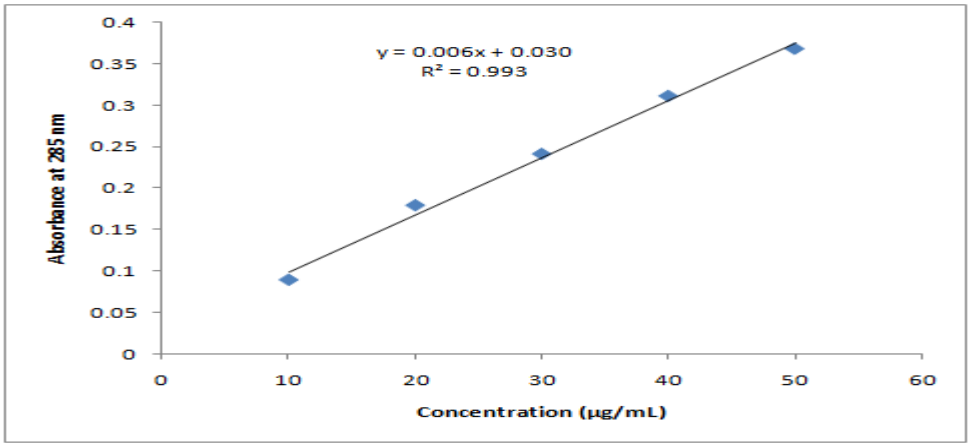

Figure 1. Calibration curve of Azithromycin in phosphate buffer

Nanomicelle preparation

In this work, the thin film method was used to prepare nanomicelles formulations [39]. Since a low CMC value indicates a high resistance of nanomicelles against dilution by body fluids [20], the total polymer concentration was selected above the CMC value of the surfactant as the best for NM formulation. Soluplus consists of polyvinyl caprolactam (57%), polyvinyl acetate (30%) and polyethylene glycol (13%). It has a very low CMC value (7.6 mg/L) that confers stability to micellar formulations upon dilution in vivo, and it is considered a safe excipient since no adverse effects were observed in animals at a dose of 1000 mg/Kg [21-23].

Critical micellar concentration

The literature reports a CMC of 7.6mg/L for soluplus. Herein for the sake of formulating AZI loaded nano-micelles, the critical micellar concentration was determined for the drug containing micelles formulation by dilution and measurement of conductivity. The conductivity of solution usually increases with increasing the concentration of surfactant. Once the CMC is achieved, the addition of surfactant either has no effect on conductivity or the conductivity even decreases occasionally. The CMC was determined to be 1.0mM (Table 2).

Table 2. Conductivity measurement of various formulations at fixed dilution ratios

Formulation code	Conductivity (µS/m)				
Formulation:Water	10:00	7:03	5:05	3:07	0:10

NM1	$1.8x10^{-4}$	$1.7x10^{-4}$	$1.7x10^{-4}$	$1.9x10^{-4}$	$1.8x10^{-4}$
NM2	$2.1x10^{-4}$	$1.9x10^{-4}$	$2.0x10^{-4}$	$2.1x10^{-4}$	$2.0x10^{-4}$
NM3	$2.6x10^{-4}$	$2.5x10^{-4}$	$2.4x10^{-4}$	$2.5x10^{-4}$	$2.3x10^{-4}$
NM4	$2.4 x 10^{-4}$	$2.4x10^{-4}$	$2.3x10^{-4}$	$2.3x10^{-4}$	$2.1x10^{-4}$
NM5	$2.1 x10^{-4}$	$2.0x10^{-4}$	$2.1x10^{-4}$	$2.1x10^{-4}$	$2.0x10^{-4}$
NM6	$1.9x10^{-4}$	$2.1x10^{-4}$	$1.9x10^{-4}$	$1.8x10^{-4}$	$2.0x10^{-4}$

Particle size, PDI and zeta potential

The particle size of the formulations was determined by DLS and it was found that the particle size of the blank nanomicelle at concentration above the CMC was less than 100 nm with PDI almost 0.1. The particle size of the formulations below and above the CMC was more than 100 nm with a PDI of more than 0.2. At the CMC, the PDI of the formulation was close to less than 0.2. The drug loaded formulation at just above the CMC presented particle size of 98.3nm and PDI of 0.128 (Table 3).

Table 3. Properties of the nanomicelles

Formulation code	Particle size	PDI	Zeta potential	Encapsulation efficiency
NM1	208.4	0.217	-3.47	94.8
NM2	183.5	0.211	-3.54	95.1
NM3	157.1	0.189	-3.18	95.8
NM4	98.3	0.128	-2.45	96.4
NM5	124.3	0.233	-3.18	96.5
NM6	143.5	0.248	-3.11	97.2
Blank NM	87	0.103	-0.87	-

The zeta potential of soluplus blank nanomicelles was -0.87. In the AZI loaded nanomicelles, the zeta potential slightly increased suggesting the presence of some drug on the micellar surface. The zeta potential of the AZI loaded nano-micelles ranged from -2.45 to -3.54. The encapsulation efficiency was determined by disruption of the nanomicelles to release out the drug in methanol and measuring the absorbance to calculate the concentration of AZI present in inside the micelles. The encapsulation efficiency was found to be 94.8 to 97.2% slightly increasing with an increase in concentration of the surfactant.

In vitro release

The *in vitro* release of AZI from suspension and the optimized nanomicelle (NM4) was determined by shake flask method. The AZI suspension release 51% drug in 2 hours and the concentration decreased to 48% in 5 hours. No azithromycin was found in the release medium at the 8th hour till 24h. On the other hand the nanomicelle released 29% AZI in 2h and 74% at the 24th hour of the study (Figure 2).

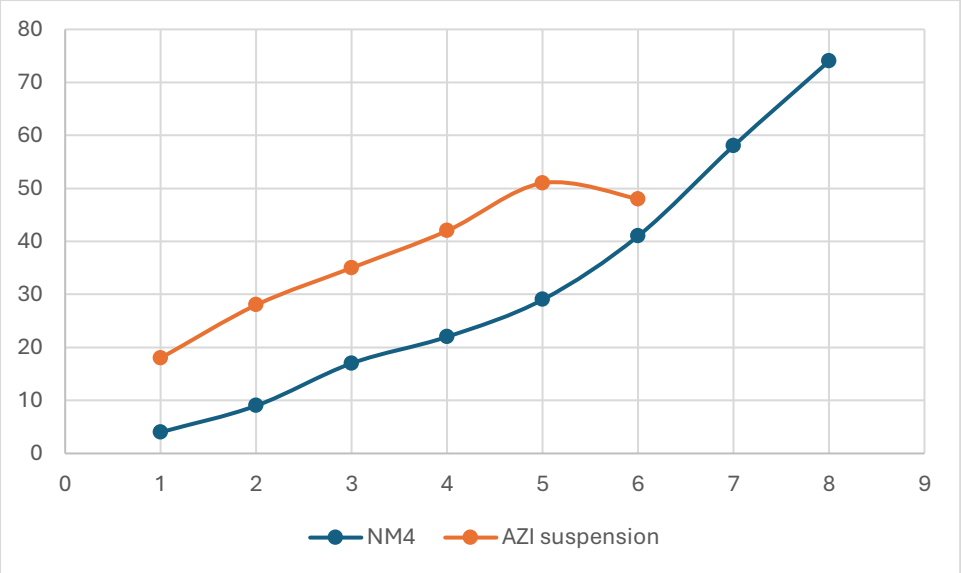

Figure 2. _In vitro_ release of AZI from nanomicelles and suspension

Antibacterial Activity of Azithromycin loaded nanomicelle

The antibacterial action of the liposomal formulation was compared to that of the pure drug solution and it was found that the nanomicelle formulation loaded with Azithromycin was able to exhibit comparable antibacterial activity against *Staphylococcus aureus* in the disc diffusion

assay, as measured using the zone of inhibition.

Conclusion

In this study, Soluplus based nanomicelles loaded with azithromycin were prepared. The low CMC values of soluplus showed that it forms stable nanomicelle and keeps it structure intact upon dilutions with body liquids thus they would protect the drug until the target site. The release study revealed that azithromycin release could be controlled to 24 h suggesting an improvement in the bioavailability of azithromycin in the nano-micelle formulation.

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