The Impact of Lithocholic Acid as A Surfactant on the Characteristics and Cytocompatibility of Azithromycin Loaded Self-Emulsifying Drug Delivery System

Reem Abou Assi[1,2], Ibrahim M.Abdulbaqi [1,2], Toh Seok Ming[1], Chan Siok Yee[1]*, Habibah A. Wahab[1]*, Yusrida Darwis [1]*

[1] The Discipline of Pharmaceutical Technology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800, Penang, Malaysia.
[2] The Discipline of Pharmaceutical Technology, College of Pharmacy, Al-Kitab University, Altun kupri, Kirkuk, Iraq.
Corresponding authors: Chan Siok Yee, sychan@usm.my ; Habibah A. Wahab, habibahw@usm.my ; Yusrida Darwis, yusrida@usm.my

INTRODUCTION

The self-emulsifying drug delivery system (SEDDS) has emerged as an effective pharmaceutical strategy for addressing the issue of poorly soluble drug bioavailability, specifically candidates belonging to BCS classes II and IV. Aside from oil, the main component in a SEDDS is the surfactant, which is present in high concentrations. Due to the complexity of the surfactant molecule's properties, complex reactions or physiological interactions are frequently difficult to monitor. This, in turn, raises the possibility of toxicity due to changes in protein structure or malfunctioning enzymes and phospholipids in the membrane. Surfactants derived from bile acid have transpired as an excellent choice of bio-compatible pharmaceutical excipient [1]. Unconjugated lithocholic acid (LA) has recently been reported to improve formulations' drug release and stability [2,3]. Lipophilic drugs in BCS class IV have poor gut absorption after an oral dose, requiring high doses and causing subsequent side effects, this includes azithromycin (AZM, log p = 4) [4]. Therefore, the objective of this study is to investigate LA as a safe and effective surfactant in liquid SEDDS (L-SEDDS) and compare it with LA-free L-SEDDS and solid SEDDS (S-SEDDS) states for AZM. The desirable properties of SEDDS, such as reduced particle size (PS), dispersity (Đ), self-emulsification efficiency (T%), zeta potential charge, and cellular viability, were studied in particular.

METHODOLOGY

LA-Free SEDDS: The initial L-SEDDS was formulated with Capryol 90® oil (22.22%), Tween 20® as surfactant and Transcutol HP® (2:1 ratio) as co surfactant. After which AZM was loaded. S-SEDDS was produced by adsorbing the L-SEDDS(s) to Aerosil 200 ® as a solid carrier (at 2:1 ratio of L-SEDDs to Aerosil 200®).

LA-SEDDS: As per L-SEDDS, where additionally LA was incorporated at high (B-L-SEDDS3), medium (B-L-SEDDS2), and low (B-L-SEDDS1) concentrations of 7.75, 3.6, and 1.03 mg/ml respectively, then AZM was added. The PS, Đ, T% and ZP measurements were performed in distilled water (DW), 0.1 mM HCl, and simulated intestinal fluids (SIF). Cell viability was done by MTT assay as illustrated in figure 1.

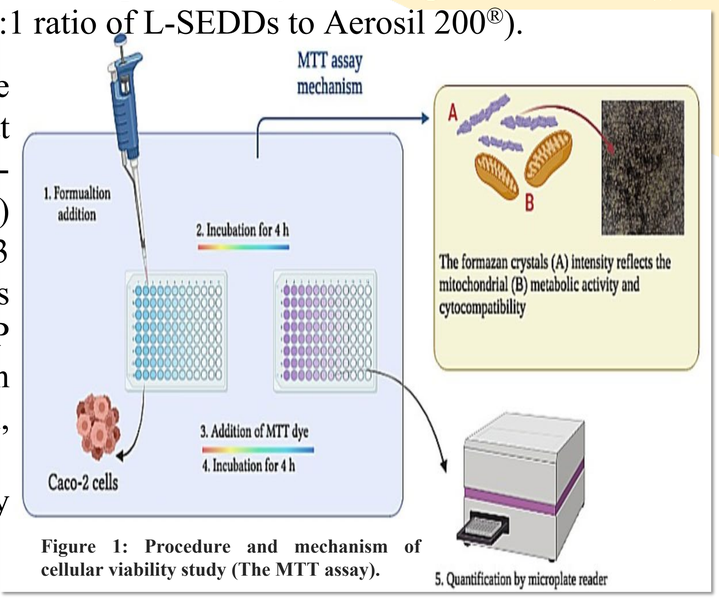

Figure 1: Procedure and mechanism of cellular viability study (The MTT assay).

RESULTS & DISUCSSION

1. Characterization:

Particle Size | Dispersity | Transmittance | Zeta potential

Particle size (PS) of all the tested formulation were < 200 nm which is the recommended size for oral route delivery (Figure 2A and 2E). Further significant reduction in PS, and Đ values was observed upon the addition of LA ($p<0.05$) in both blank and loaded B-L-SEDDS and compared to LA-free AZM-loaded liquid and solid SEDDSs, respectively (Figure 2A and 2E). Besides, the size reduction is LA concentration dependent. The PS reduction is an advantage for drug absorption due to increase surface area of the dispersed phase. In addition, the smaller size of the B-SEDDS could promote higher proportion of lymphatic uptake which occurs preferentially for particle in the range of 10-100nm. On the other hand, the Đ reduction represents SEDDS improved homogeneity. Lowest PS and Đ values were reported in B-L-SEDDS3 (12.7 nm ±0.5, and 0.29 ± 0.01), while B-L-SEDDS1 and B-L-SEDDS2 have the highest T% (100.2% and 100.05%).

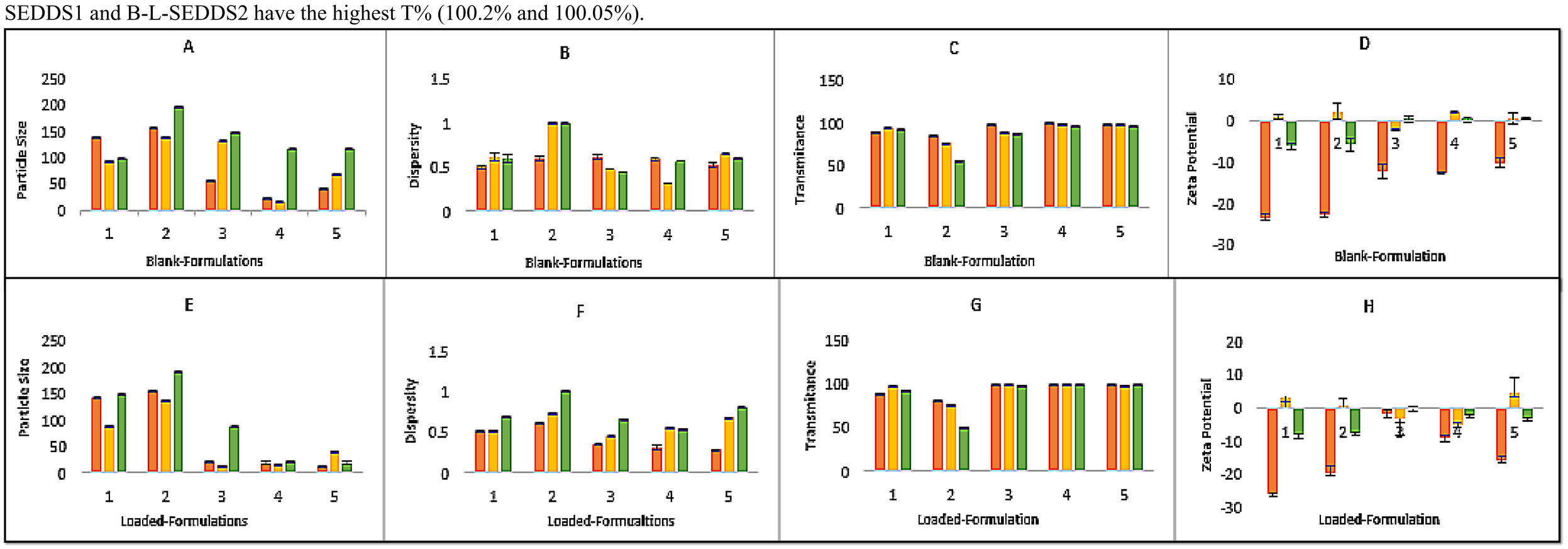

Figure 2: PS, Đ, T%, and ZP of blank (A, B, C, D) and Azithromycin loaded (E, F, G, H) L-SEDDs (1), S-SEDDs (2), B-L-SEDDs 1 (3), B-LSEDDs 2 (4), and B-L-SEDDs (5), in DW (red), 0.1mM HCl (orange), and SIF (green) respectively. (n = 3 ± SD)

Based on transmittance result, L-SEDDS shows high transmittance, while S-SEDDS were cloudy in blank or loaded forms (Figure 2C and 2G). However, after the addition of LA, the transmittance of light increase up to ≈ 100%. This indicates the formation of a very small internal phase of emulsion , i.e. <200nm, size that is referred to colloidal system. The formation of colloidal system at biological condition indicates its supreme stability which allow absorption along the gastrointestinal tract. ZP charges were negative in DW and SIF, with charge shift to positive in HCl diluent. This could be due to the neutralization of fatty acids and their negatively charged hydroxyl groups (OH−) by the available positively charged hydrogens (H+) in such acidic medium. Such positive charge might also be linked to enhanced drug solubility and uptake upon consumption [5].

2. Cellular viability

In this study MTT assay (figure 1) was performed to ensure safety of the prepared formula. This assay assessing cell metabolic activity in term of viability. As expressed in figure 3, L-SEDDS had the highest viability of ~90% at all studied formulation's concentrations, and S-SEDDS viability ranged from 85% to 90% in concertation dependent manner, while the B-SEDDS viability was ~85%. The addition of LA significantly ($p<0.05$) reduced viability in comparison to LA-free L-SEDDS and S-SEDDS. However, nearly all SEDDSs formulations demonstrated good cytocompatibility (> ~85%).

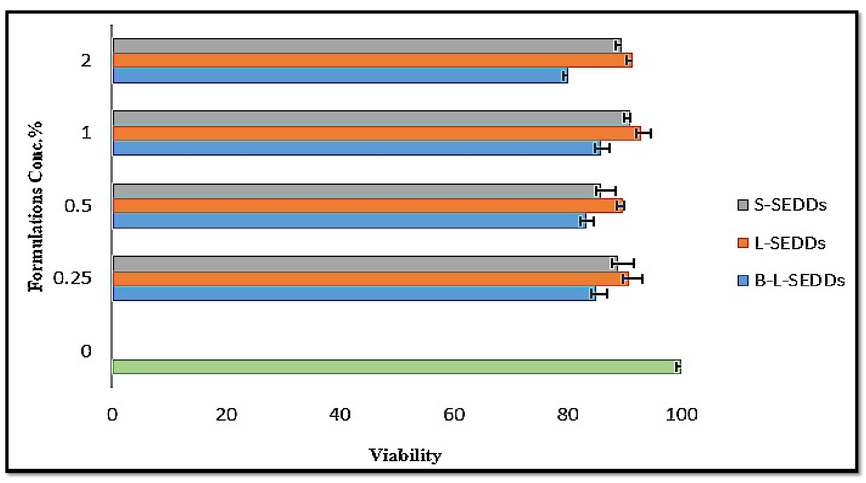

Figure 3: Viability percentage of blank L-SEDDs, S-SEDDs and B-SEDDs2 at 0.25, 0.5, 1, and 2 v/v% respectively, compared to untreated cells (in green). (n=3±standard error of the mean (SEM)).

CONCLUSION

This study revealed the significant role of using bile acids LA as surfactant in SEDDS formulation for particle size reduction, and enhanced homogeneity. The high transmittance reveal successful formation of colloid system upon emulsification. Furthermore, the developed system showed cytocompatibility which indicate its safety. Therefore, lithocholic acid is a potential surfactant for desired feature of SEDDS with a good safety profile.

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