P-031	**Antimicrobial analysis of microemulsions containing Lipoid® S100, Tween® 80 and Miglyol® 812N**	

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INTRODUCTION AND OBJECTIVES

The development of novel pharmaceutical preparations with acceptable levels of product preservation has rapidly grown. The interest in the use of microemulsions (ME) as drug delivery systems is due to their relatively high oil content, which improves the bioavailability of hydrophobic drugs (Al-Adham 2000). It is suggested that MEs can be used to control the rate of drug release (Trotta 1989). Therefore, this system is considered a promising tool in chemical, pharmaceutical and cosmetic industry.

The aim of this work was to analyze the self-preserving antimicrobial properties of a ME and its pure components against standard strains of bacteria and yeasts of medical interest.

MATERIALS AND METHODS

The ME was obtained from the mixture of Lipoid® S100 (Lipoid® GMBH, Germany) and Tween® 80 (Vetec Química Fina Ltda, Brazil) in a ratio of 3:7, hydrophilic phase of 0.2M pH 7.4 phosphate buffer solution (PBS) (dibasic sodium phosphate and monobasic sodium phosphate – Vetec Quimica Fina Ltda, Brazil) and lipophilic phase of Miglyol 812N (Sasol, Germany). This formulation contained 11% of Miglyol® 812N, 6.3% of Lipoid® S100, 14.7% of Tween® 80 and 68% of 0.2M PBS.

The antimicrobial assay was adapted from the American Pharmacopoeia (USP 27 2007). Solutions were prepared with the following microrganisms: *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739 and *Salmonela* sp, separately, in tubes containing Tryptic Soy Agar (TSA) (Difco, USA) followed by the biased incubation at 35 ± 2.5°C for 24 hours. A dilution was then performed and the micro-organisms presented in the suspension were counted comparing to the 0.5 McFarland scale. A subsequent dilution was adjusted to a concentration of 10^3 CFU/mL.

The samples were placed on plates at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 9, 12, 24, 48 hours containing TSA and incubated at 35 ± 2.5°C for 48 hours (USP 27 2007).

The same methodology was used for yeasts (*Candida albicans* ATCC 90028, *Candida tropicallis* ATCC 0750) using Sabouraud Agar (Difco, USA) and incubation time in accordance to the growth of the yeast. In the second dilution, the concentration of the inoculum was adjusted to 2.5x 10^4 CFU/mL. The yeast cultures in fresh medium were added at $10\%_{(v/v)}$ to the ME and incubated at 25 ± 2.5°C for 24 hours. Subsequently, the plates were removed from the incubator and growth potential was observed (USP 27 2007).

The minimum inhibitory concentration (MIC) test was also performed for the pure components of ME.

RESULTS AND DISCUSSION

An experiment was designed to observe the behavior of strains of bacterial on ME preparations. The bacterial growth was observed after 48 hours (Figure 1).

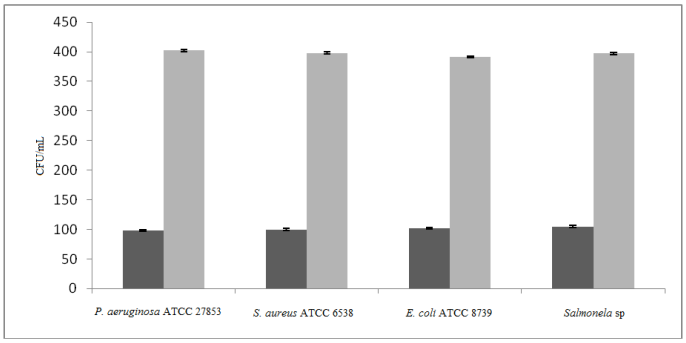

Figure 1- Bacterial cells viability before (■) and after the addition of microemulsion (■) at 35 ± 2.5°C for 48 hours.

Some preparations are particularly susceptible to microbial growth because of the nature of their ingredients. Such preparations are protected by the addition of preservatives that prevent alteration and degradation of the product formulation (Boukarim 2009). The ME prepared without antimicrobial compounds are capable of inhibiting microbial growth and viability (Al-Adham 2000). These systems are indeed self-preserving systems (Zhang 2010). However, the results here presented showed a different antimicrobial behavior with the ME containing Lipoid S100. Concerning both, the number and the size of colonies incubated for 48h at 35 ± 2.5ºC, the ME was not effective in inhibiting the bacteria growth, when compared to the control.

The antimicrobial activity of the pure components of ME was carried out and the results were analyzed by the MIC

test. These results indicate an inherent antimicrobial activity of Miglyol 812N and Tween® 80 (Figure 2). The intense bacteria growth was observed in the experiments with Lipoid S100 and 0.2 M pH 7.4 PBS.

These findings suggest that the antimicrobial behavior of the ME studied and the absence of antimicrobial activity can be associated with the high concentrations of Lipoid S100 and PBS 0.2 pH 7.4.

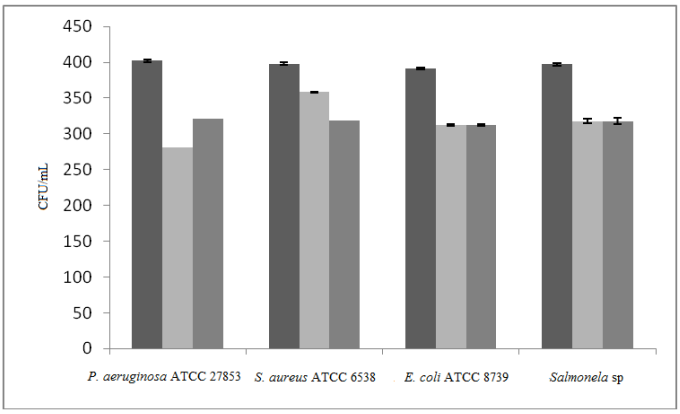

Figure 2- Bacterial cells viability before (■) and after addition of 12.5% (v/v) Miglyol® 812N (■) and Tween® 80 (■) at 35 ± 2.5°C for 48 hours.

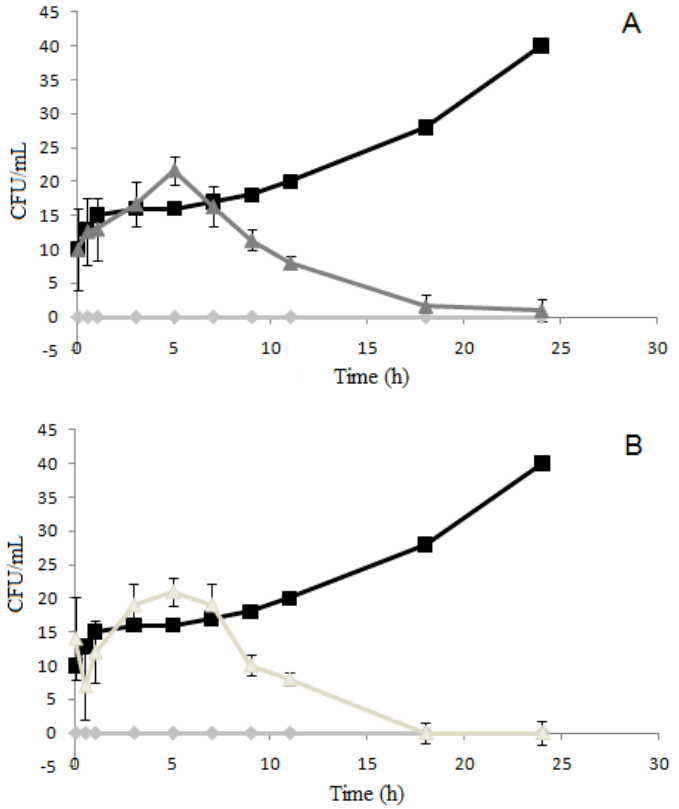

Figure 3 – Time exposure viability curve for cultures of *Candida albicans* ATCC 90028 (A) and *Candida tropicallis* ATCC 0750 (B) after addition of the microemulsion, the negative control (◆) and the positive control (■) at 25°C ± 2.5 for 24 hours.

In the experiment with yeast cells after a short exposure time to ME, the changes in viability of the culture of yeast cells were observed. The Figure 3 shows the rate of the killing observed for these cultures and gives clear evidence of a true biocidal dynamic. The isolated components do not have an antimicrobial activity, but when they are mixed to form ME, interestingly, yeast cells do not show growth. This fact reinforces the hypothesis that ME can span lipid layer due to their unique features that enhance the intrinsic permeation of membranes.

CONCLUSION

The antimicrobial activity of the ME was observed to be dependent on both the chemical and the inherent antimicrobial nature of their compounds. It may be proposed that antibiotics encapsulated into ME will probably present enhanced antimicrobial activity due to the inherent antimicrobial activity of the drug delivery system itself against the fungal cytoplasmic membrane.

Further analysis to study this phenomenon regarding the use of ME is desirable.

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