



## Extrinsic contamination of propofol non-lipid nanoemulsion

### Contaminação extrínica de propofol nanoemulsão não-lipídica

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#### RESUMO

Existem vários casos reportados de contaminação extrínica de propofol por bactérias associada a complicações infecciosas, que resultaram em inúmeros estudos relativos ao efeito conservante de diferentes substâncias em propofol. Este trabalho avaliou a contaminação extrínica de emulsões de propofol (com e sem edetato dissódico) e nanoemulsão não lipídica de propofol. Alíquotas de propofol foram inoculadas com suspensões de 10 cepas de micro-organismos. Contagens microbianas foram realizadas após 0, 3, 6, 12, 18 e 24 horas. A nanoemulsão não-lipídica de propofol demonstrou ser mais efetiva contra todos os micro-organismos. Portanto, a nanoemulsão não-lipídica de propofol apresenta uma segurança adicional às boas práticas assépticas, uma vez que está menos sujeita a crescimento microbiano que a emulsão lipídica de propofol convencional.

**Palavras-chave:** propofol, contaminação extrínica, nanoemulsão

#### ABSTRACT

There are several cases reported of bacteria extrinsically contaminating propofol, what has been associated with infective complications and led to numerous studies concerning the preventive effect of different substances on bacterial growth in propofol. This work evaluated extrinsically contamination of propofol lipid emulsion (with and without disodium edetate) and propofol non-lipid nanoemulsion. Amounts of propofol were inoculated with suspensions of 10 strains of microorganisms. Plate counts were performed after 0, 3, 6, 12, 18 and 24 hours. The addition of disodium edetate to propofol lipid emulsion retards microbial growth. Propofol non-lipid nanoemulsion proved to be more effective against all microorganisms. Therefore propofol non-lipid nanoemulsion is an additional safety precaution to good aseptic practice, as it provides less microbial growth than conventional propofol lipid emulsion.

**Keywords:** propofol, extrinsic contamination, nanoemulsion

#### INTRODUÇÃO

Propofol is a short-acting, intravenously administered hypnotic agent used for induction and maintenance of general anesthesia, sedation for mechanically ventilated adults, and procedural sedation. Chemically, propofol (Figure 1) is unrelated to barbiturates, has largely replaced sodium thiopental for induction of anesthesia, and the recovery from propofol is faster and "clear" if compared to thiopental. As Propofol is not considered an analgesic, opioids such as fentanyl may be combined with propofol to alleviate pain (Miner & Burton, 2007). Due to its amnesic effects and its appearance, as a white liquid, propofol has been humorously dubbed "milk of amnesia" by medical professionals (Gravenstein, 2004).

When formulated in a lipid vehicle propofol supports the growth of microorganisms (Fukada & Ozaki, 2007, Jansson *et al.*, 2006). Several reports describe it as prone to be extrinsically contaminated by bacteria, been these cases usually related to infusions or delays in administration after the ampoule has been opened (Jansson *et al.*, 2006; Soong, 1999; Aydin *et al.*, 2002). The number of contaminated opened ampoules increased with time: 20-26% after 12 hours (Aydin *et al.*, 2002). Contamination of propofol has been associated to infective complications (Ozer *et al.*, 2002).

Therefore it is essential for medical professionals to follow strict aseptic precautions when handling

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propofol, as recommended by manufacturers, the Center for Disease Control and Prevention (USA) and the Sanitary Vigilance (Brazil). Non-adherence to these recommendations increases the risk of nosocomial postoperative infections, which impose a heavy burden of morbidity and mortality besides serious economic consequences (Fukada & Ozaki, 2007; Jansson *et al.*, 2006; Bennett *et al.*, 1995; Bach *et al.*, 1997).

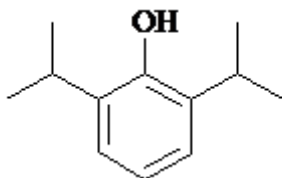


Figure 1. Propofol chemical structure

Many studies have been carried out concerning the preventive effect of different substances on bacterial growth in propofol, which, in association with 0.1% lidocaine (Aydin *et al.*, 2002; Güzelant *et al.*, 2008) or 0.05-0.1% lignocaine (Ozer *et al.*, 2002) did not exhibit adequate antibacterial activity to prevent bacterial growth. Diphenhydramine inhibited bacterial growth in propofol solutions in a dose-dependent manner (Güzelant *et al.*, 2008). Alfaxalone supports growth of some microorganisms but less readily than propofol (Strachan *et al.*, 2008). Midazolam reduces the growth of *S. aureus* and also completely inhibits the growth of *E. coli*, *P. aeruginosa* and *A. baumannii*. Like propofol, dexmedetomidine and etomidate-lipuro do not inhibit bacterial growth (Kele *et al.*, 2006).

*In vitro* studies confirmed that disodium edetate retards microbial growth when added to propofol (Fukada & Ozaki, 2007; Jansson *et al.*, 2006; Noriega *et al.*, 1999). While the latter, with disodium edetate, inhibits bacterial growth more efficiently than without it (Noriega *et al.*, 1999).

The aim of this work is to evaluate extrinsically contaminated bacterial growth in propofol lipid emulsion formula, propofol lipid emulsion with disodium edetate formula and a propofol non-lipid nanoemulsion formula.

## MATERIALS AND METHODS

Ten microorganisms, recognized as the most frequent in postoperative infection, were used in this study: *Acinetobacter calcoaceticus* (ATCC 19606), *Candida albicans* (ATCC 10231), *Enterobacter aerogenes* (CDC 5039), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 8739), *Klebsiella pneumoniae* (ATCC 23357), *Pseudomonas aeruginosa* (ATCC 9027), *Serratia marcescens* (CDC 6572), *Staphylococcus aureus* (ATCC 6538) and *Staphylococcus epidermidis* (ATCC 12228).

Each microorganism was cultivated in tryptic-soy agar (TSA) and incubated at 30-35°C, for 24 hours, except *Candida albicans* (ATCC 10231), which was cultivated in Sauboraud-dextrose agar (SDA) and incubated at 20-25°C for 48 hours. After incubation, each microorganism was diluted in 0.9% sodium chloride sterile solution to a

suspension of  $10^5$ - $10^6$  counting forming units per milliliter (CFU.mL<sup>-1</sup>).

Amounts of 10-mL of propofol 1.0% lipid emulsion (Propovan, Cristália), propofol 1.0% lipid emulsion with disodium edetate (Cristália), propofol 2.0% lipid emulsion with disodium edetate (Cristália), propofol 1.0% non-lipid nanoemulsion (Cristália) and a control group (0.9% sodium chloride solution) were inoculated with 100-µL suspensions of each microorganism.

Immediately after inoculation (T0), decimal dilutions of each propofol formula were performed and amounts of 1-mL were transferred to Petri dishes (2 replicas per dilution). Amounts of 10-20-mL of TSA were transferred to each plate, except for *Candida albicans* (ATCC 10231), where SDA was used. All plates were incubated at 30-35°C for 24-48 hours, except *Candida albicans* (ATCC 10231), whose plates were incubated at 20-25°C for 48-72 hours. This procedure was repeated after 3 (T3), 6 (T6), 12 (T12), 18 (T18) and 24 (T24) hours.

## RESULTS AND DISCUSSION

The results of microbial growth were not the same in all preparations, due to different levels of resistance or behavior of microorganism. In general, propofol 1.0% lipid emulsion with disodium edetate, propofol 2.0% lipid emulsion with disodium edetate and propofol 1.0% non-lipid nanoemulsion support the growth of microorganisms, but less readily than propofol 1.0% lipid emulsion (without disodium edetate). The behavior of each microorganism towards each challenge can be observed in figures 2-11, and is described below.

*Acinetobacter calcoaceticus*. In the first 12 hours, all propofol formulas and the control group presented a little decrease concerning the CFU results. After 12 hours, the CFU of propofol 1.0% lipid emulsion, propofol 1.0% lipid emulsion with disodium edetate, propofol 2.0% lipid emulsion with disodium edetate and control group increased. The results of propofol 1.0% non-lipid nanoemulsion CFU had no significant change.

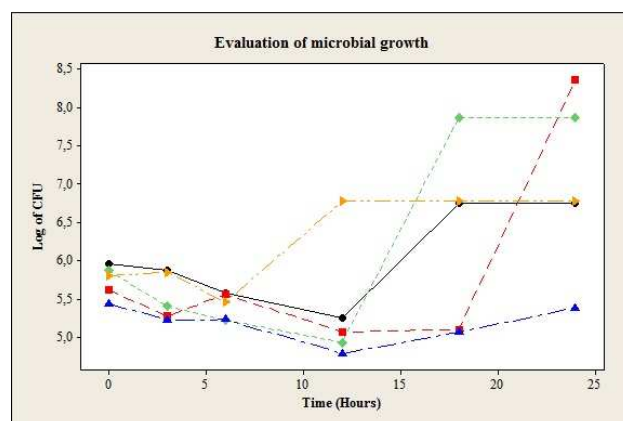
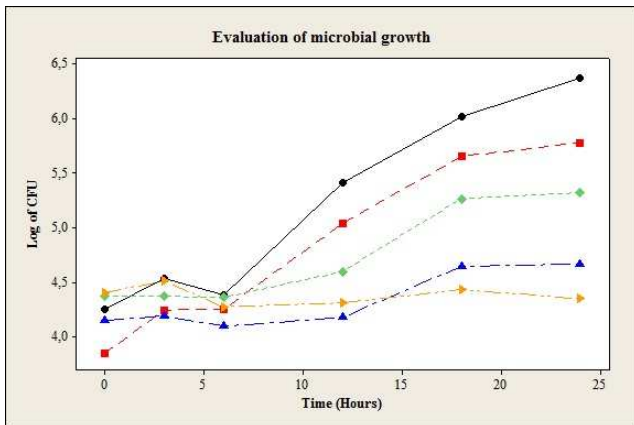


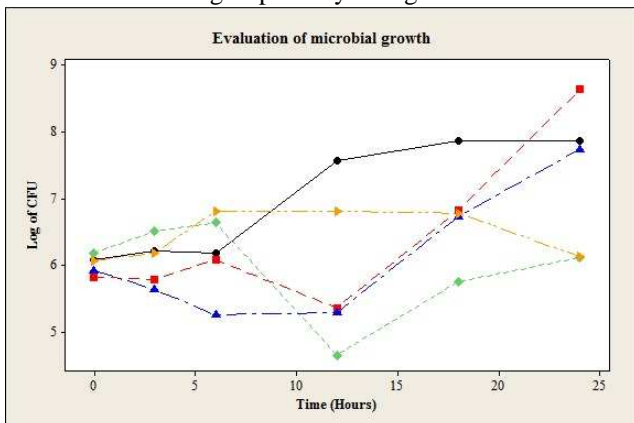
Figure 2. Evaluation of microbial growth of *Acinetobacter calcoaceticus* (ATCC 19606) in propofol 1.0% lipid emulsion (●), propofol 1.0% lipid emulsion with disodium edetate (■), propofol 2.0% lipid emulsion with disodium edetate (◆), propofol 1.0% non-lipid nanoemulsion (▲) and control (▶)

*Candida albicans*. The results of CFU relatively to propofol 1.0% lipid emulsion, propofol 1.0% lipid emulsion with disodium edetate, propofol 2.0% lipid emulsion with edetate and propofol 1.0% non-lipid nanoemulsion showed an increase. However, propofol 1.0% non-lipid nanoemulsion revealed microbial growth less readily than propofol 1.0% lipid emulsion. The CFU of the control group had no significant change.



**Figure 3.** Evaluation of microbial growth of *Candida albicans* (ATCC 10231) in propofol 1.0% lipid emulsion (●), propofol 1.0% lipid emulsion with disodium edetate (■), propofol 2.0% lipid emulsion with disodium edetate (◆), propofol 1.0% non-lipid nanoemulsion (▲) and control (►)

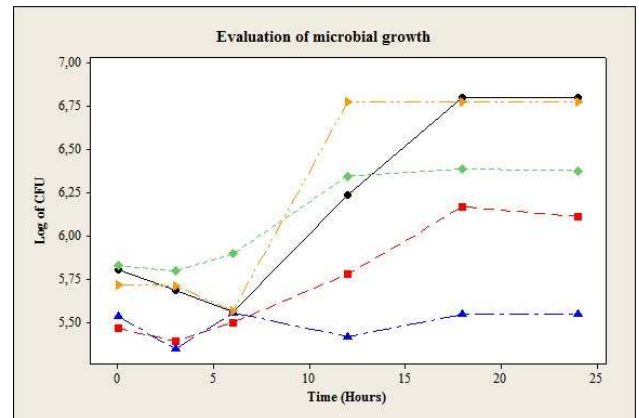
*Enterobacter aerogenes*. Propofol 1.0% lipid emulsion showed rapid microbial growth, after 6 hours. Propofol 1.0% lipid emulsion with disodium edetate, propofol 2.0% lipid emulsion with disodium edetate and propofol 1.0% non-lipid nanoemulsion also presented microbial growth but less readily than propofol 1.0% lipid emulsion. The CFU of the control group hardly changed.



**Figure 4.** Evaluation of microbial growth of *Enterobacter aerogenes* (CDC 5039) in propofol 1.0% lipid emulsion (●), propofol 1.0% lipid emulsion with disodium edetate (■), propofol 2.0% lipid emulsion with disodium edetate (◆), propofol 1.0% non-lipid nanoemulsion (▲) and control (►)

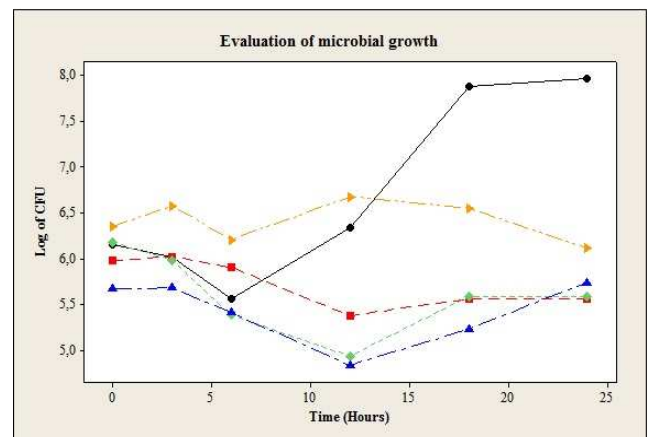
*Enterococcus faecalis*. The results of the CFU relatively to propofol 1.0% lipid emulsion, propofol 1.0% lipid emulsion with disodium edetate and propofol 2.0% lipid

emulsion with edetate showed an increase. These hardly changed relatively to propofol 1.0% non-lipid nanoemulsion as well as control group.



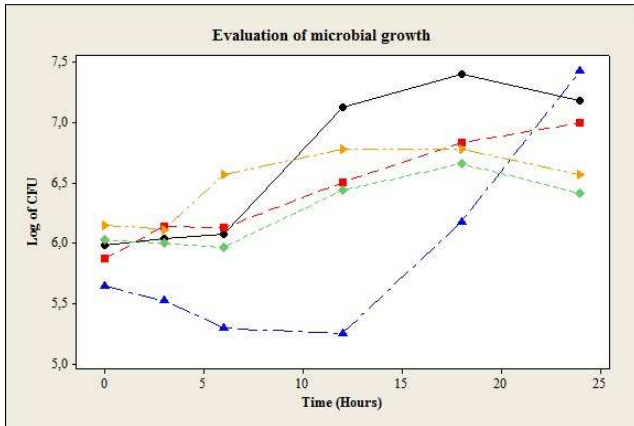
**Figure 5.** Evaluation of microbial growth of *Enterococcus faecalis* (ATCC 29212) in propofol 1.0% lipid emulsion (●), propofol 1.0% lipid emulsion with disodium edetate (■), propofol 2.0% lipid emulsion with disodium edetate (◆), propofol 1.0% non-lipid nanoemulsion (▲) and control (►)

*Escherichia coli*. Propofol 1.0% lipid emulsion had a rapid increase in the results of CFU results after 6 hours, whereas the results concerning propofol 1.0% lipid emulsion with disodium edetate, propofol 2.0% lipid emulsion with disodium edetate and propofol 1.0% non-lipid nanoemulsion had no significant change.



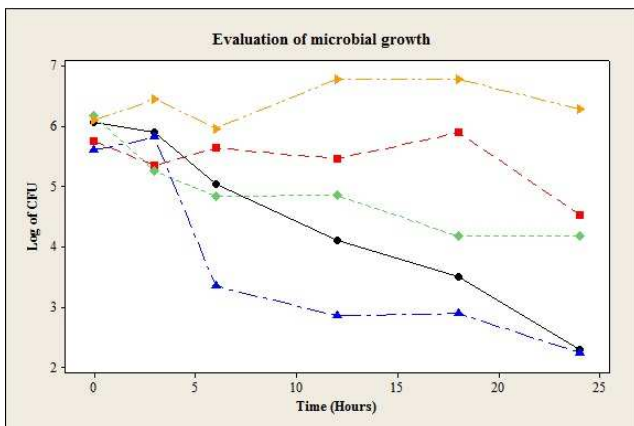
**Figure 6.** Evaluation of microbial growth of *Escherichia coli* (ATCC 8739) in propofol 1.0% lipid emulsion (●), propofol 1.0% lipid emulsion with disodium edetate (■), propofol 2.0% lipid emulsion with disodium edetate (◆), propofol 1.0% non-lipid nanoemulsion (▲) and control (►)

*Klebsiella pneumoniae*. The results of the CFU relatively to propofol 1.0% lipid emulsion, propofol 1.0% lipid emulsion with disodium edetate, propofol 2.0% lipid emulsion with edetate and propofol 1.0% non-lipid nanoemulsion showed an increase. However, propofol 1.0% non-lipid nanoemulsion presented microbial growth less readily than propofol 1.0% lipid emulsion.



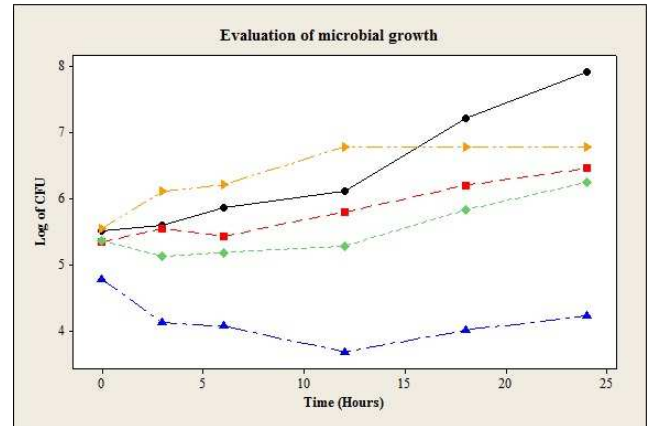
**Figure 7.** Evaluation of microbial growth of *Klebsiella pneumoniae* (ATCC 23357) in propofol 1.0% lipid emulsion (●), propofol 1.0% lipid emulsion with disodium edetate (■), propofol 2.0% lipid emulsion with disodium edetate (◆), propofol 1.0% non-lipid nanoemulsion (▲) and control (►)

*Pseudomonas aeruginosa*. The results of CFU relatively to propofol 1.0% lipid emulsion, propofol 1.0% lipid emulsion with disodium edetate, propofol 2.0% lipid emulsion with edetate and propofol 1.0% non-lipid nanoemulsion showed a decrease. Propofol 1.0% non-lipid nanoemulsion had the most rapid decrease in microbial growth. The results concerning the control group had no significant change.



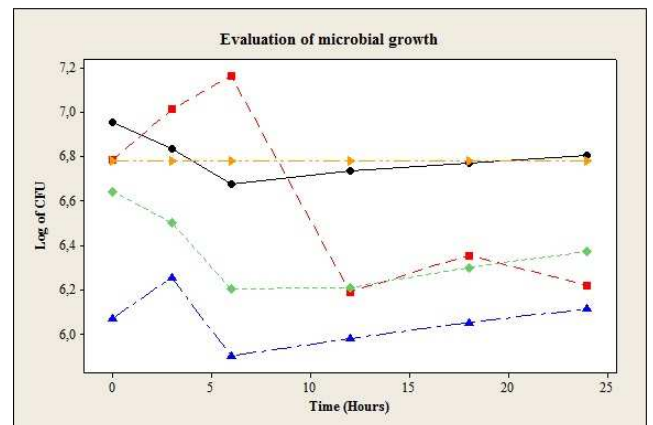
**Figure 8.** Evaluation of microbial growth of *Pseudomonas aeruginosa* (ATCC 9027) in propofol 1.0% lipid emulsion (●), propofol 1.0% lipid emulsion with disodium edetate (■), propofol 2.0% lipid emulsion with disodium edetate (◆), propofol 1.0% non-lipid nanoemulsion (▲) and control (►)

*Serratia marcescens*. The results of the CFU relatively to propofol 1.0% lipid emulsion, propofol 1.0% lipid emulsion with disodium edetate and propofol 2.0% lipid emulsion with edetate showed an increase, whereas the results concerning propofol 1.0% non-lipid nanoemulsion and control group had no significant change.



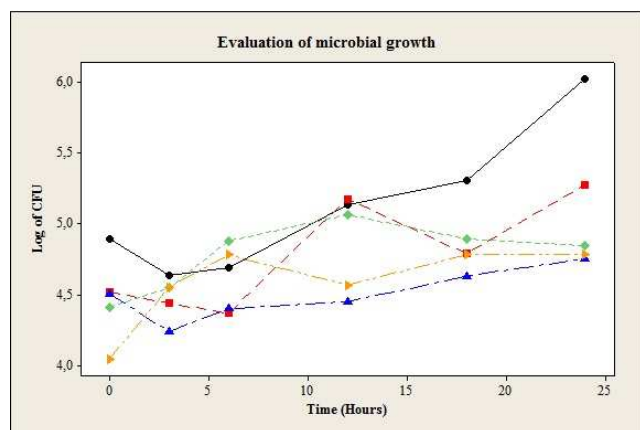
**Figure 9.** Evaluation of microbial growth of *Serratia marcescens* (CDC 6572) in propofol 1.0% lipid emulsion (●), propofol 1.0% lipid emulsion with disodium edetate (■), propofol 2.0% lipid emulsion with disodium edetate (◆), propofol 1.0% non-lipid nanoemulsion (▲) and control (►)

*Staphylococcus aureus*. The results of the CFU relatively to propofol 1.0% lipid emulsion, propofol 1.0% lipid emulsion with disodium edetate, propofol 2.0% lipid emulsion with disodium edetate and propofol 1.0% non-lipid nanoemulsion had no significant change.



**Figure 10.** Evaluation of microbial growth of *Staphylococcus aureus* (ATCC 6538) in propofol 1.0% lipid emulsion (●), propofol 1.0% lipid emulsion with disodium edetate (■), propofol 2.0% lipid emulsion with disodium edetate (◆), propofol 1.0% non-lipid nanoemulsion (▲) and control (►)

*Staphylococcus epidermidis*. The results of the CFU relatively to propofol 1.0% lipid emulsion, propofol 1.0% lipid emulsion with disodium edetate, propofol 2.0% lipid emulsion with edetate and propofol 1.0% non-lipid nanoemulsion showed an increase. However, propofol 1.0% lipid emulsion with disodium edetate, propofol 2.0% lipid emulsion with edetate and propofol 1.0% non-lipid nanoemulsion presented microbial growth less readily than propofol 1.0% lipid emulsion.



**Figure 11.** Evaluation of microbial growth of *Staphylococcus epidermidis* (ATCC 12228) in propofol 1.0% lipid emulsion (●), propofol 1.0% lipid emulsion with disodium edetate (■), propofol 2.0% lipid emulsion with disodium edetate (◆), propofol 1.0% non-lipid nanoemulsion (▲) and control (►)

With the increasing use of lipid-based medications, which support rapid bacterial growth at room temperature, strict aseptic techniques are essential while handling these agents to prevent extrinsic contamination and dangerous infectious complications (Bennett *et al.*, 1995). The addition of disodium edetate to propofol lipid emulsion retards microbial growth.

Propofol non-lipid nanoemulsion shows better results for all microorganisms. These results could be explained by the lack of nutrients for microbial growth in propofol non-lipid nanoemulsion, in comparison to propofol lipid emulsion (with or without disodium edetate). The variation of results among the different microorganisms could be explained by metabolism differences of each microorganism and also by differences of membrane permeability resistance.

The results of this study showed that non-lipid nanoemulsion inhibits bacterial growth until about 12 hours after inoculation and incubation. It is known that propofol emulsion is an excellent supporting vehicle when the growth of several microorganisms is concerned; likewise, the addition of disodium edetate in lipidic formulations delayed the microbial growth due its bacteriostatic action, what can be explained by the fact that although edetates have been used as preservatives in several formulations, their role as antimicrobial agents is limited.

In both cases, of lipidic and non-lipidic emulsions, the addition of surfactants or emulsifiers is necessary to stabilize formulations by modifying the surface tension and improving cell membrane permeation. In the case of non-lipid emulsion, the presence of surfactants not only decreased the surface tension of the emulsion, generating nanoemulsion, which allowed a better contact with microorganisms, but also increased the adsorption of surfactants to the surfaces and increased cell killing rate. These results follow Kurup *et al.* (1991), who studied the effect of surfactants on the antibacterial activity of preservatives.

It is clear that both, edetates and surfactants can not be considered exactly an antimicrobial agent, since microorganisms recover their growth capacity after their adaptation to environment. This could explain the inhibition of growth until about 12 hours after incubation of non-lipid nanoemulsion and the higher rate of growth from the initial time concerning other lipidic samples, containing or not edetate.

In comparison to other studies (Güzelant *et al.*, 2008; Noriega *et al.*, 1999), in this work decimal dilutions of samples inoculated with microorganisms and plated with 1 mL of each dilution were prepared, what resulted in more accurate values than if we applied 1 or 10  $\mu$ L onto Agar to detect colony forming units.

## CONCLUSIONS

Propofol non-lipid nanoemulsion is therefore an additional safety precaution to attain good aseptic practice, as it provides less microbial growth than propofol lipid emulsion with disodium edetate and much less than propofol lipid emulsion without disodium edetate.

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