Analyses of amphotericin B superagreggates stability by oxidant agent and size behavior

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INTRODUCTION AND OBJECTIVE

The amphotericin B on its micellar system (M-AmB) is a formulation widely used to treat systemic fungal infections. Unfortunately, its use is limited because of the toxicity, mainly nephotoxicity (Uehara 2005).

In aqueous solutions, M-AmB is present as monomers and soluble and insoluble aggregates. The later one is responsible for its side effects (Gaboriau 1997).

It has been demonstrated that the controlled heating of M-AmB leads to a micellar rearrangement, commonly known as "superaggregates" (H-AmB). This form has demonstrated to be less toxic while keeping its activity (Silva-Filho 2012). It is believed that this lower toxicity could be consequent of its higher stability under autoxidation process and also due to its higher size, which could make the superaggregates a reservoir of monomeric forms.

The aim of this work was to investigate the superaggregates size behavior under the dilution procedure by means of diffratometry light scattering. The H-AmB stability under the oxidant agent potassium permanganate by spectrophotometry was also evaluated.

MATERIALS AND METHODS

Preparation of the H-AmB and M-AmB samples
Stock solution of M-AmB was prepared at $5x10^{-3}$ M concentration. The H-AmB was prepared under controlled heat of the M-AmB stock solution at 70ºC for 20 min. The samples were subsequently diluted to $5x10^{-5}$ M, $5x10^{-6}$ M, $5x10^{-7}$ M and $5x10^{-8}$ M in order to proceed with the assay.

Dynamic light scattering analysis
Dynamic light scattering (DLS) was carried out in a ZetaPlus particle sizer at the previously described concentrations.

Spectrophotometry analysis
The optical path of the quartz cuvettes was 0.1 cm for $5x10^{-5}$ M, 1 cm path for $5x10^{-6}$ M and $5x10^{-7}$ M and 10 cm for the concentration of $5x10^{-8}$ M. These paths were chosen to obtain spectra of aggregate and monomer states of the micellar systems at a good signal range. Their molar extinction coefficients (ε) were calculated using the Beer–Lambert equation.

Chemical oxidation
Both AmB formulations at the concentration of 5 $mg.L^{-1}$ were incubated in a solution containing increasing amounts of potassium permanganate (0-2000 µM) for 24h at 25ºC. Subsequently, the remaining AmB content was measured by spectrophotometry. A previous analytical curve was built with an r^2 of 0.9996 (Tufteland 2009).

RESULTS AND DISCUSSION

After heating, the micellar solution presented an important turbidity, which was consequence of its higher size as it can be seen in Figure 1. This higher size was observed at the higher concentrations (5 and 50 $mg.L^{-1}$). After successive dilutions, those sizes have decreased. For M-AmB, the sizes values were significantly (lower) compared to H-AmB (Figure 1).

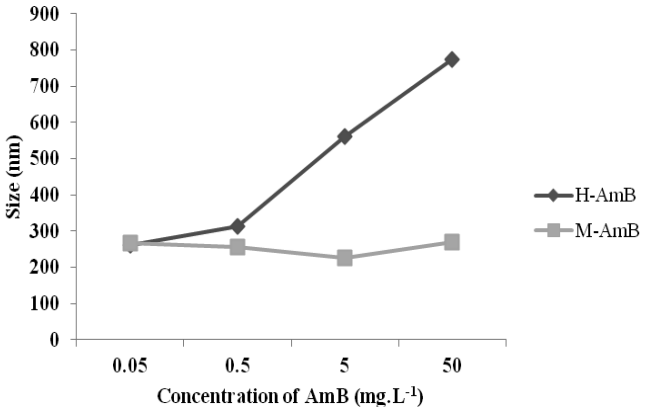

Figure 1: Sizes of M-AmB and H-AmB

Concerning the spectroscopic study, H-AmB presented a blue-shift from 327 nm to 323 nm, characteristic of superaggregates. Additionally, from Figure 2 and 3 it is possible to observe that the spectra of both M-AmB and H-AmB were concentration dependent. At higher concentrations, the aggregated form of M-AmB and H-AmB predominates and following dilution, a bathochromic effect from 327 nm (for M-AmB) and 323 nm (for H-AmB) was observed and the band at 408 nm, which is characteristic of monomeric forms, increase.

The study of the stability of H-AmB under oxidation was important once a major environment that can limit the use of polyene antibiotics is the oxidation. The double conjugated bonds, characteristic of this family of molecules, represent a target for oxidation and consequent loss of activity (Tufteland 2009). In order to observe if H-AmB is more resistant to oxidation, it was incubated in buffer containing increasing concentrations of potassium permanganate for 24h, followed by determination of its remaining amount by spectroscopy. It was observed that the main significant difference between M-AmB and H-AmB was obtained at the higher concentrations of the oxidant agent (0.002 and 0.0002 M) ($p < 5$) (Figure 4). Then, at least at this two analyzed concentrations, H-AmB was more resistant to this process. Actually, by means of a different method, Gaboriau has already shown the higher stability of H-AmB under that process (Gaboriau 1997).

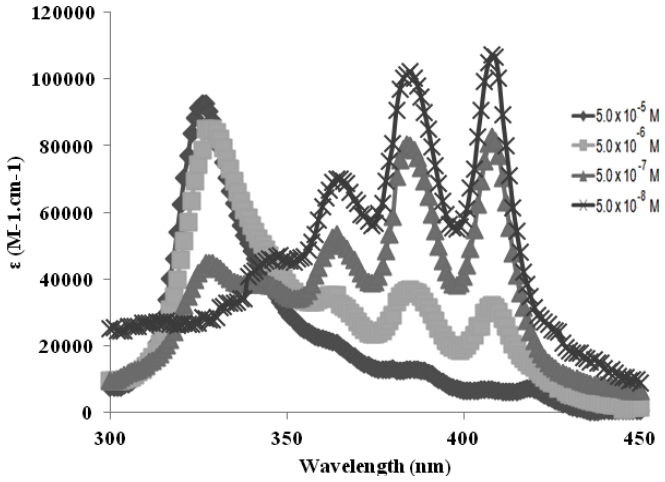

Figure 2: Spectra of M-AmB.

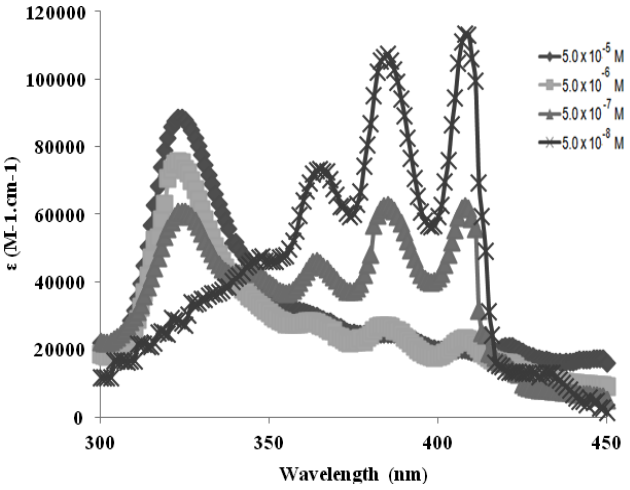

Figure 3: Spectra of H-AmB.

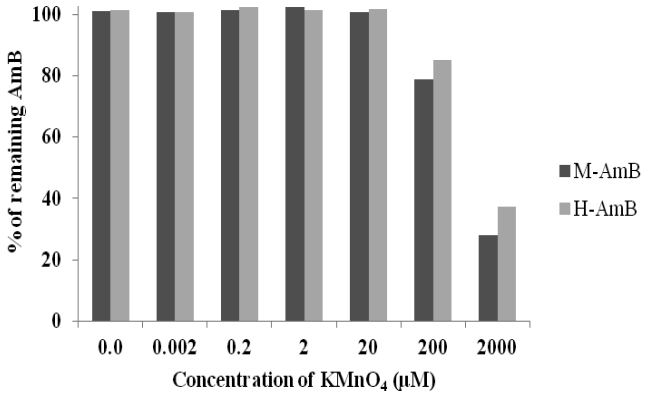

Figure 4: Percentage of remaining H-AmB and M-AmB under different concentrations of potassium permanganate.

CONCLUSION

This study confirmed that H-AmB presented a higher stability under the potassium permanganate agent and that it really had a higher size compared to M-AmB. Additionally, it also presented a different behavior by spectrophotometric analysis. Taking together these differences could be correlated to the H-AmB lower toxicity found in previous works.

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ACKNOWLEDGMENTS

We thank to Cristália by provided us with the micellar amphotericin B.