Development of the Liposomal Amphotericin B Release Assay

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Introduction

The goal of this study is to develop a USP4 apparatus flow-through assay for measuring Amphotericin B release from liposomal formulations capable of discriminating liposome manufacturing and product compositional differences.

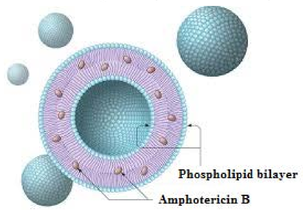
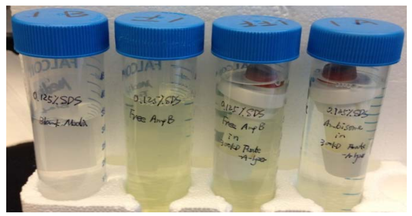


Figure1. Schematic picture of AmBisome®

Methods

The basic release media used in the assay contained 5% sucrose, 10 mM HEPES and 0.01% NaN_3 (pH=7.4). In the single-unit vial-based assay, AmBisome® or free Amphotericin B (Amp B) were placed in Float-A-Lyzer® dialysis tubes (Spectrum Labs, molecular weight cut-off 300 kDa) and inserted in release media, then incubated in a shaking water bath. To increase the in vitro release of AmBisome®, sodium dodecyl sulfate (SDS), isopropanol (IPA) and γ-cyclodextrin (γ-CD) were tested. The assay was adapted to USP 4 apparatus flow-through apparatus Sotax® CE7 Smart. Each cell contained 50 mL of the release media re-circulated at 16 mL/min flow rate. The effects of temperature and drug concentration on the Amp B release were investigated.

A



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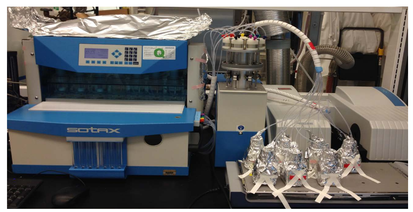
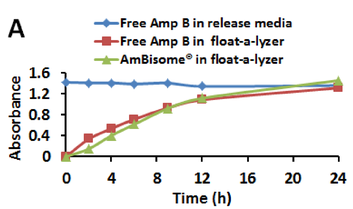
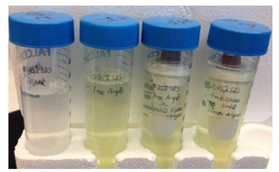


Figure 2. Picture of single-unit vial-based assay system **(A)** and USP 4 apparatus CE7-smart (SOTAX®) dissolution system **(B)**

Results





Picture after 24 h release (45ºC)

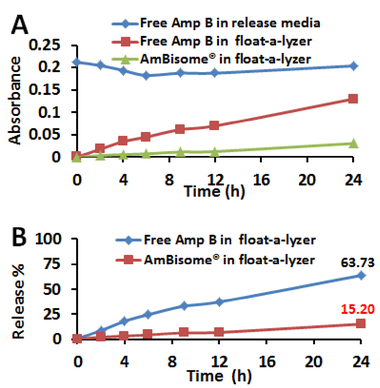
D

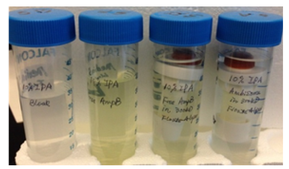
Size distribution

Sample Group	Size(nm)	PDI
AmBisome® after release	20.63	0.539
AmBisome®before release	96.67	0.113

Figure 3. The absorbance **(A)** and cumulative release **(B)** of Amp B in the release media containing 0.25% (w/v) SDS. **(C)** The pictures of test tubes after release. **(D)** The size distribution of AmBisome® before and after release.

As shown in figure 3, addition of SDS resulted in faster release of Amp B, however AmBisome® liposomes decreased from 100 nm to 20 nm, indicating liposome structural degradation.





Picture after 24 h release (45ºC)

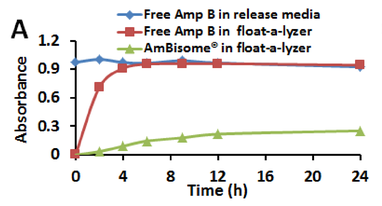
D

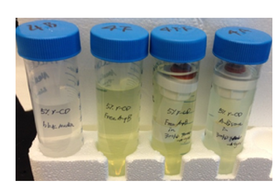
Size distribution

Sample Group	Size(nm)	PDI
AmBisome® after release	141.9	0.138
AmBisome® before release	96.67	0.113

Figure 4. The absorbance **(A)** and cumulative release **(B)** of Amp B in the release media containing 10% IPA. **(C)** The pictures of test tubes after release. **(D)** The size distribution of AmBisome® before and after release.

The above results showed that both free Amp B release and AmBisome® release were slow and limited with addition of 10% IPA to the release media.





Picture after 24 h release (45ºC)

D

Size distribution

Sample Group	Size(nm)	PDI
AmBisome® after release	204.4	0.766
AmBisome® before release	96.67	1

Figure 5. The absorbance **(A)** and cumulative release **(B)** of Amp B in the release media containing 5% γ-CD. **(C)** The pictures of test tubes after release. **(D)** The size distribution AmBisome® before and after release.

When 5% γ-CD was added to the release media, AmBisome® release was only 27.24% in 24 hours at 45° C, however, the free drug release was quick and thorough.

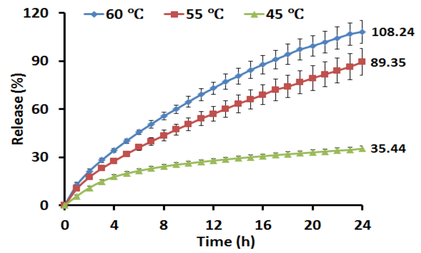


Figure 6. The cumulative release of Amp B in the release media containing 5% γ-CD at 45, 55 and 60 ℃, respectively.

Increase in temperature had significant effect on the rate of Amp B release with 35%, 89% and 108% of cumulative release in 5% γ-CD median within 24 hours at 45, 55 and 60 ℃, respectively.

Conclusion

Amp B could be released completely from AmBisome® in the media containing 5% sucrose, 10 mM HEPES, 0.01% NaN_3 and 5% γ-CD (pH = 7.4) within 24 hours at 60 ℃. This release assay could be used in the future to potentially discriminate possible differences between generic and innovator liposomal Amp B products and provide a more accurate prediction of *in vivo* drug release kinetics. This would be a major advance in the quality evaluation of complex generic products.

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