

LC-UV as tool for nanovectorized anticancer peptide quality control: evaluation of encapsulation efficiency.

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1. Introduction

Many peptides have today a recognized and growing therapeutic interest. Unlike conventional small molecules, peptides present a wide range of challenges related to physicochemical stability during the formulation or analytical development. Furthermore, many compounds of this emerging class of therapeutics have their targets at the intracellular level hence, they must be incorporated into suitable carriers to achieve their therapeutic success. This general concept raises the need to develop analytical tools enabling the determination of the encapsulation efficiency of LB19, a peptide inhibitor of LDHB proposed as a new therapeutic approach for cancer treatment, into pH sensitive liposomes and quantitatively demonstrate the ability of these nanocarriers to achieve intracellular delivery of their payload *in-vitro*.

2. Objectives

- ❖ To generate stability data of LB19 in order to support its formulation development.
- ❖ To evaluate the impact of adsorption of LB19 on the estimation of EE%
- ❖ To develop a LC method to quantify LB19 both in clean and complex medium.

3. Material and Methods

3.1. Stability testing

The stress factors were selected based on the handling conditions during the liposomes preparation (thin film hydration method). Solutions of LB19 (500 µM) in water and HEPES buffer pH 7.4 as two media of preparation of liposomes were kept at 45°C and 1500 RPM for 4 days using Thermomixer (Eppendorf ThermoMixer™). Samples were withdrawn at regular time intervals (0, 24, 48, 72 and 96 hrs) and analysed for LB19 content and detection of potential degradation products.

3.2. LB19 Loaded liposomes preparation

Liposomes made up of DOPE:CHEMS:CHOL:PEG750-DSPE (43:21:30:6 molar % ratio) were prepared, then characterized for size and surface charge by Zetasizer Nano (Malvern).

3.3. Determination of encapsulation efficiency

Evaluation of LB19 adsorption

LB19 samples of concentration between 10-50 µg/mL were used to evaluate the adsorption to solid surfaces of vials, pipette tips and Eppendorf. Then recovery tests were carried out on centrifugal filters attended to be used for the purification of LB19 loaded liposomes and on Ostro™ 96-well Plate as well (lipid removal system used in sample preparation).

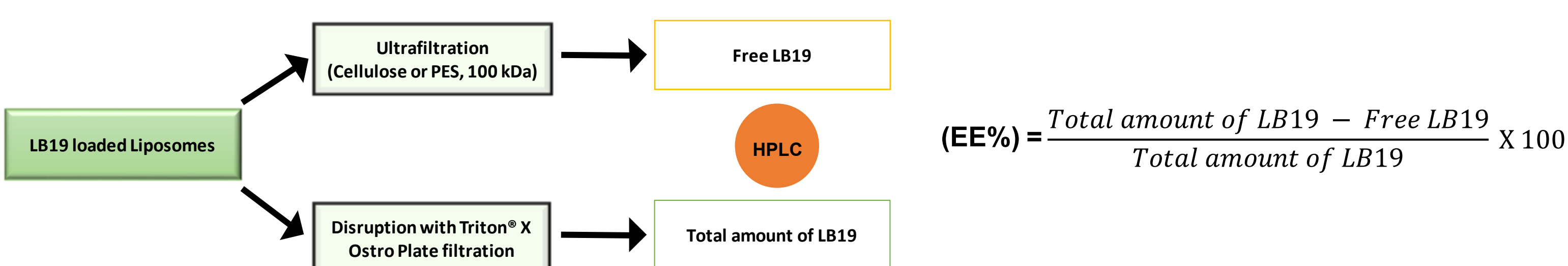


Fig 1. Processing of LB19 Loaded liposomes for the determination of Encapsulation Efficiency (EE)

4. Results and discussion

4.1. Stability testing

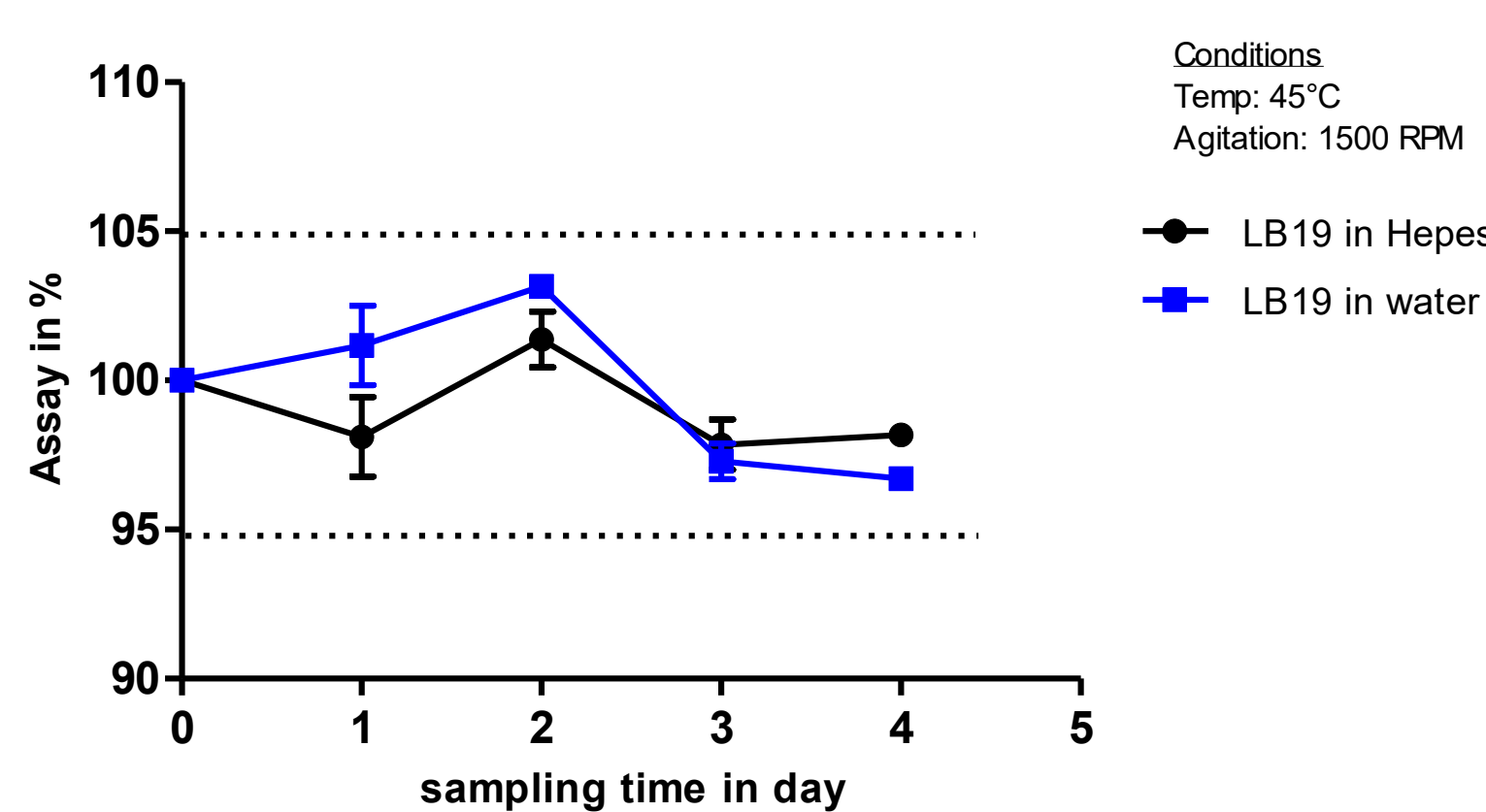


Fig 2. Result of forced degradation study of LB19; Assay by HPLC-UV; n=3

LB19 remains stable under the studied stress conditions (45°C with 1500 RPM agitation) (less than 5% variation after 4 days). However, the selectivity must be also checked with LC-MS to confirm these results.

4.2. Liposomes characterization

Table 1. Liposomes size distribution and charge (samples diluted 100 times in MilliQ water, n=3)

	Size (nm)	PDI	Zeta (mV)
Empty	167.60 ± 8.85	0.17 ± 0.23	(-53.83 ± 4.7)
LB19	163.60 ± 7.85	0.16 ± 0.59	(-58.74 ± 6.8)

The presence of LB19 does not affect the physicochemical properties of liposomes.

4.3. Determination of encapsulation efficiency

Evaluation of adsorption & recovery of LB19

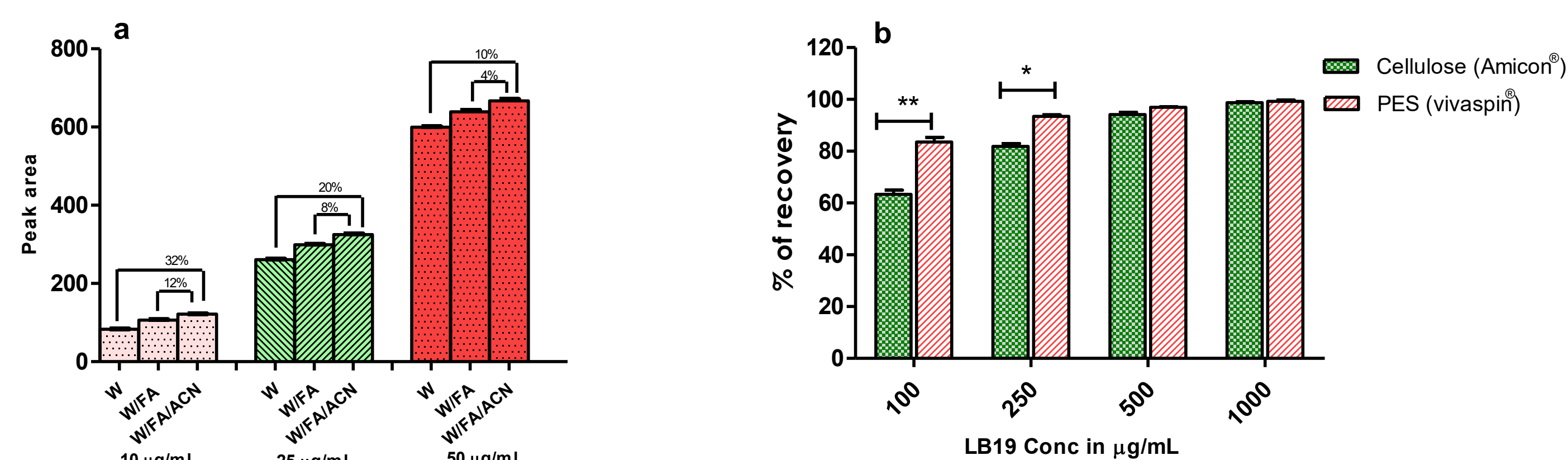


Fig 3. (a) adsorption of LB19 to solid surface of commonly used Lab materials, the value in % represent the lost of LB19. (b) Recovery test of LB19 HEPES buffer solution after filtration through centrifugal filters (* p<0.05, ** p<0.01) (mean ± SEM, n=3).

LB19 adsorbs to solid surfaces (Eppendorf, Vials, pipette tips etc.). The rate of adsorption depends on the composition of dissolution medium and concentration of the LB19 in solution. In the same way, LB19 adsorbs to filters membrane. However, at concentrations below 500 µg/mL, PES provides higher LB19 recovery compared to cellulose membrane.

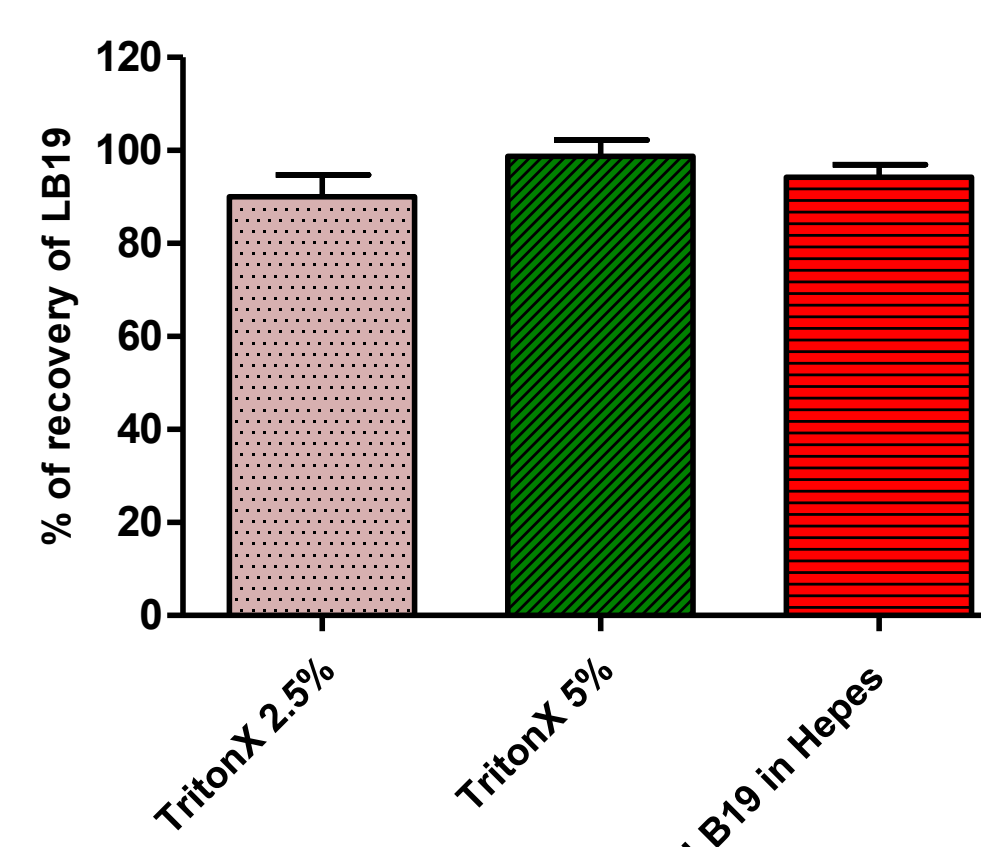


Fig 4. Comparison of LB19 recovery between liposomes disrupted with Triton® X-100 and LB19 HEPES solution. The concentration of LB19 was 500 µM. All samples were filtrated through Ostro™ 96-well Plate (n=3).

Triton® X-100 at 5 % improves slightly the LB19 recovery (98%), but the difference is not significant (T-test).

Encapsulation efficiency of LB19

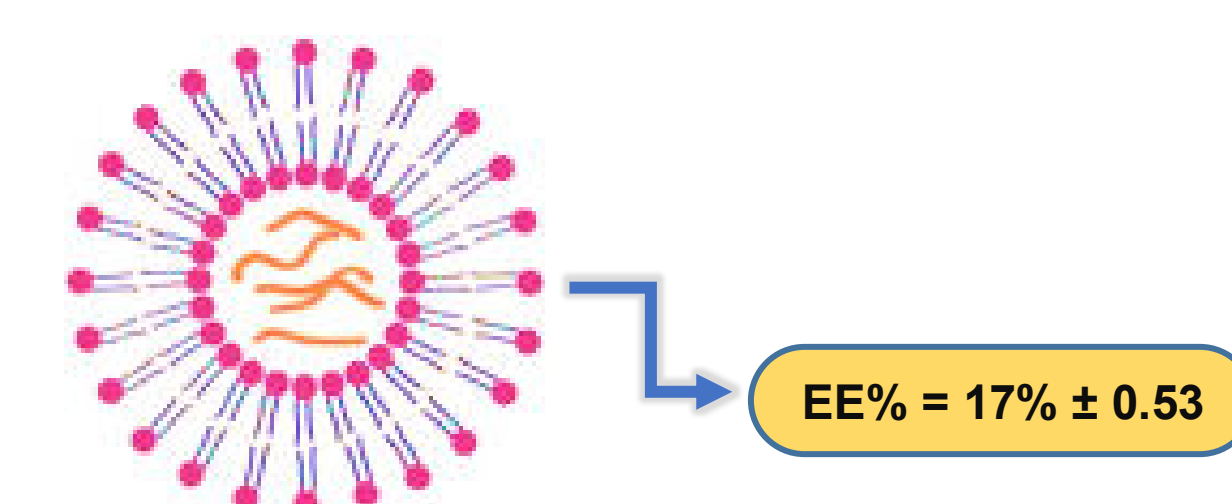


Fig 5. Encapsulation efficiency of LB19 Loaded Liposomes, n=2.

Early formulation development leads to 17% of encapsulation efficiency. Several approaches will be used to optimize the EE% in the near future.

5. Conclusion & Perspective

- The results of stability testing suggest that LB19 can withstand the processing conditions of the chosen preparation method (Thin film hydration method)
- In the framework of determination of encapsulation efficiency for LB19 loaded liposomes, selection of devices with low binding characteristic along with recovery tests could provide a way to ensure reliable measurement of this important quality control parameter.
- Centrifugal filters with PES are more suitable for LB19 since provide better recovery rate.
- The use of Triton® X-100 could be useful but further investigations need to be done.
- In prospect, validation of LC-UV method and development of LC-MS/MS method will be carried out for the *in-cellulo* assay of LB19.

References

- [1] S. Warwood, A. Byron, J. M. Humphries, D. Knight, The effect of peptide adsorption on signal linearity and a simple approach to improve reliability of quantification, J. of Proteomics 85 (2013) 160 – 164.
- [2] K. Kristensen, J.R. Henriksen, L.T. Andresen, Adsorption of Cationic Peptides to Solid Surfaces of Glass and Plastic, PLoS One (2015) 10.
- [3] A. Penwell, K Sharp, M. Mansour, L. Sammatour, Development and validation of an HPLC/UV assay for separation and quantification of peptide antigens from a liposomal vaccine delivery platform, J. Pharm & Biomed Analysis 66 (2012) 176-182.