Measuring Mass of Liposome Cargo Using Centrifugal Field-Flow Fractionation

General Information ID0067

Application	Nanomedicine, Drug Delivery, Biopharmaceutical
Technology	CF3-UV
Info	Postnova CF2000, PN3211 UV/Vis
Keywords	Centrifugal Field-Flow Fractionation, Nanomedicine, Drug Delivery, Porphysome

Introduction

Lipid-based nanoparticles (liposomes) have been developed for a number of applications in both medical and non-medical fields. In the field of biomedical therapy, imaging and chemotherapy, drug-loaded liposomes have shown great potential for treating diseases including cancers as the biocompatible nature of liposomes helps to avoid an undesired immune response. However, the liposomes themselves do not act as therapeutic or imaging agents.

Porphyrins are a group of organic molecules which have been used in clinical settings for a variety of imaging applications, and also find application as a photosensitizer for photodynamic therapy. However, porphyrins are lipophilic, making them prone to aggregation in aqueous solution, so incorporating them into liposomes results in a more stable delivery vehicle which retains the imaging and photosensitive properties of the porphyrins [1]. These hybrid materials are referred to as porphysomes and were once named a top 10 cancer breakthrough. [2].

Measuring the amount of drug 'cargo' loaded into these drug delivery particles is one of the main analytical challenges in nanomedicine. Separating by size will not help as the filled and unfilled particles are often the same size. Therefore, in order to achieve mass-based separation, centrifugal field-flow fractionation (CF3) was used (Figure 1) where the particles are separated based on their masses.

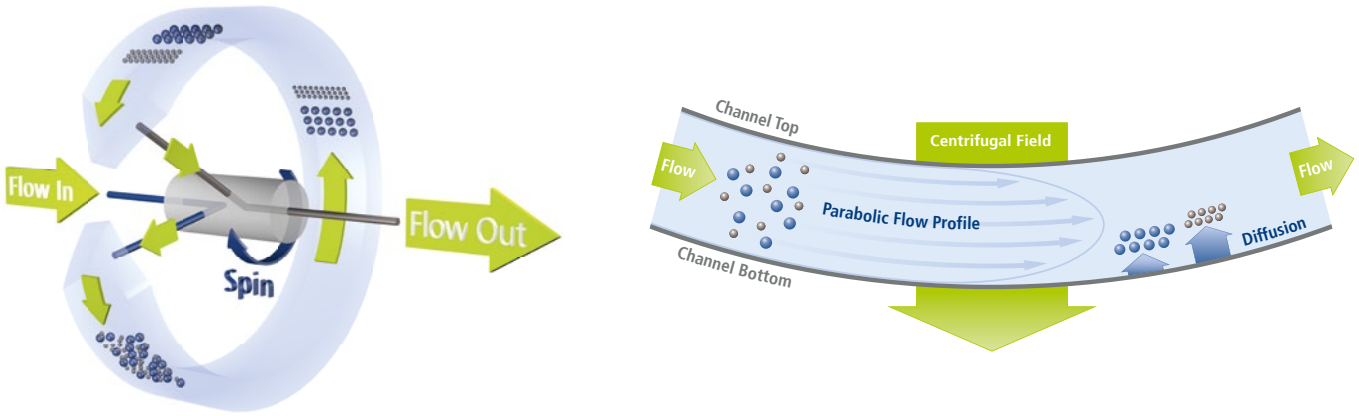

Figure 1: Schematics of the CF3 channel.

Experimental Details and Results

Sample solutions of porphysomes, both empty and filled with human serum albumin (HSA), were analyzed by CF3 (Postnova CF2000) coupled with a UV/Vis detector (PN 3211). The UV/Vis signal was used as a concentration detector to generate particle mass distributions, and the difference in measured mass between the empty and drug-filled porphysomes was calculated to be the cargo mass.

POSTNOVA

info@postnova.com
www.postnova.com

Postnova Analytics GmbH
86899 Landsberg, GERMANY
T: +49 8191 985 688 0

Postnova Analytics UK Ltd.
Worcestershire, WR14 3SZ, UK
T: +44 1684 585167

Postnova Analytics Inc.
Salt Lake City, UT 84102, USA
T: +1 801 521 2004

Postnova Northern Europe
01630 Vantaa, FINLAND
T: +358 9 8545 510

The UV response for the empty and filled porphysome samples is shown in Figure 2. The empty porphysome has less mass and elutes first, with the peak maximum at about 12 minutes. The filled porphysome has more mass due to the cargo contained in it and elutes later, with a peak maximum around 21 minutes. This shift in retention time is proportional to the average mass of HSA per porphysome.

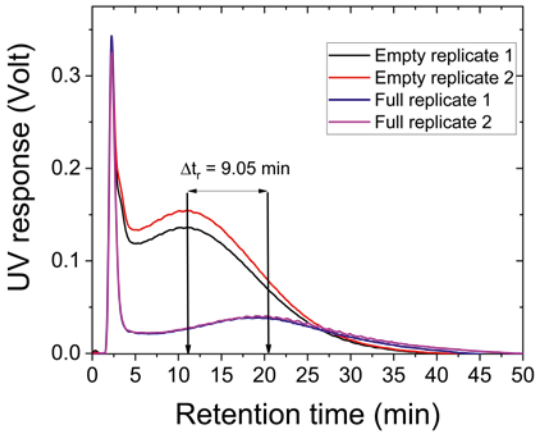

Figure 2: UV/Vis-fractograms of empty and full porphysomes.

The UV-based fractograms in Figure 2 were converted into particle mass distributions (Figure 3) using CF3 theory [3]. By subtracting the mass of the empty porphysome from the mass of the loaded porphysome, the mass of the cargo (HSA) can be calculated. In this example, the mass of HSA was calculated to be 15.6 ± 1.8 attograms (15.6 ± 1.8 x 10^{-18} grams) or ~143 ± 17 HSA per porphysome. This information can be very valuable for determining how much drug payload each particle can potentially deliver to a target such as a tumor.

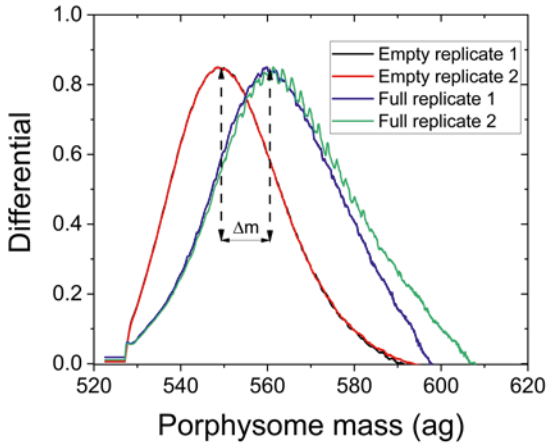

Figure 3: Mass distributions of empty and full porphysomes.

Conclusion

We observed that centrifugal field-flow fractionation can discern differences in retention time related to the drug cargo mass in filled porphysomes versus empty porphysomes. This should be possible for a variety of similar drug delivery particles, given that the loaded mass is appreciable enough to cause a significant mass difference between the loaded and empty particles.

References

[1] E. Huyn and G. Zheng, NanoToday, 2014, 9(2), 212-222.
[2] https://technainstitute.com/porphysomes/
[3] S. Tadjiki, M.D. Montaño, S. Assemi, A. Barber, J. Ranville, R. Beckett, Analytical Chemistry, 2017, 89(11), 6056-6064.

POSTNOVA
info@postnova.com
www.postnova.com
Postnova Analytics GmbH
86899 Landsberg, GERMANY
T: +49 8191 985 688 0
Postnova Analytics UK Ltd.
Worcestershire, WR14 3SZ, UK
T: +44 1684 585167
Postnova Analytics Inc.
Salt Lake City, UT 84102, USA
T: +1 801 521 2004
Postnova Northern Europe
01630 Vantaa, FINLAND
T: +358 9 8545 510