

# Quantification of Liposome-Encapsulated Drug Using Centrifugal Field-Flow Fractionation

## General Information

ID0050

<b>Application</b>	Pharmaceutical, Drug Delivery, Nanomedicine
<b>Technology</b>	CF3 - Diode Array PN3241 UV-Vis, Offline Optical Microscopy
<b>Info</b>	Postnova CF2000, Postnova Diode Array UV-Vis, Optical Microscope
<b>Keywords</b>	Centrifugal Field-Flow Fractionation, Liposomes, Drug Delivery, Fraction Collection

## Introduction

Nanoparticles including liposomes are increasingly used for delivery of drug molecules. During formulation of a liposome around a drug particle, a relatively large amount of free drug may remain unencapsulated and therefore not available for drug delivery via the liposomal carrier. It is important to quantify the amount of free vs encapsulated drug in a product in order to accurately know the delivered dose of drug to a patient [1]. In this study, Centrifugal Field-Flow Fractionation (CF3) was used to separate free drug from liposomes and drug-containing liposomes in order to quantify the free drug.

## Principle of CF3

The CF3 fractionation channel is mounted on a centrifuge, which spins to generate a centrifugal field perpendicular to the channel flow. Less massive particles can diffuse against the centrifugal field and elute sooner than more massive particles, resulting in a mass separation.

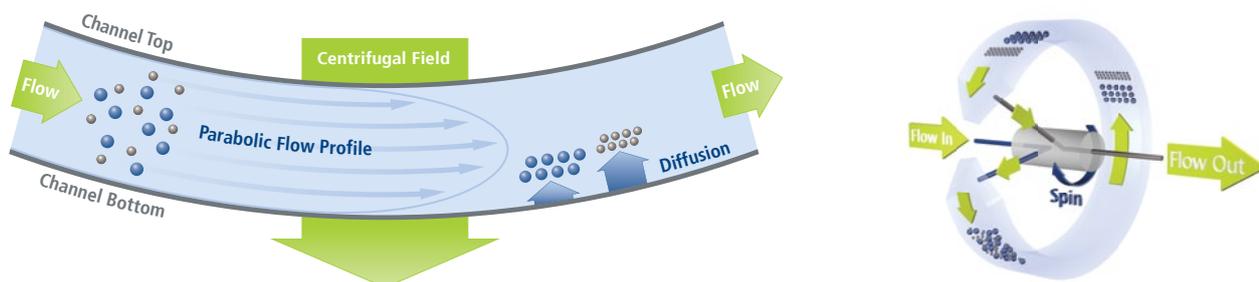


Figure 1: Schematics of the CF3 channel: left, side-on view; right, 3D view.

## Experimental Details and Results

Optical microscopy image of the liposome-drug product (Figure 2) shows a mix of three species: free drug (black specks), empty liposomes (bubble-like structures), and liposome-encapsulated drug. The goal of the study was to quantify the amount of free drug using CF3 coupled with diode array UV-Vis detection and verify the separation with optical microscopy.

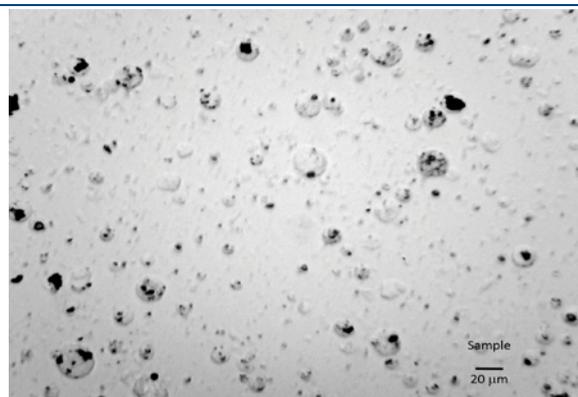


Figure 2: Optical microscopy image of liposome-drug product.

The CF3-UV fractogram (Figure 3) shows clear separation of two populations in the sample. As CF3 separates by mass, the very light drug particles are likely to elute at the beginning of the separation, and the heavier liposome-encapsulated drugs will elute later. Fractions were collected every 15 seconds (0.25 minutes) and imaged using optical microscopy. Representative microscopy images of the fractions are shown in Figure 4 and Figure 5. Figure 4 shows a fraction collected in the initial peak from Figure 3, at 0.75 to 1 minutes, and only free drug particles are observed in this fraction. Figure 5 shows a fraction collected in the second peak from Figure 3, at 2.25 to 2.5 minutes, and both empty liposomes and liposome-encapsulated drug are observed in this fraction. Integration of the peak areas allowed calculation of the amount of free drug, at  $29.0 \pm 1.6$  % of peak area.

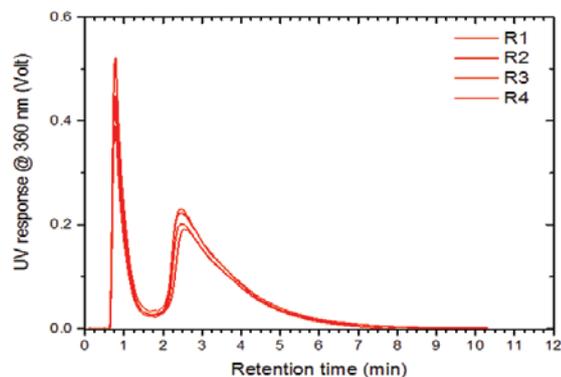


Figure 3: CF3-UV fractogram of the liposome-drug sample. Multiple replicates were analyzed to test for repeatability.



Figure 4: Optical microscopy image of a fraction collected from CF3 at 0.75 – 1 minutes (Figure 3).

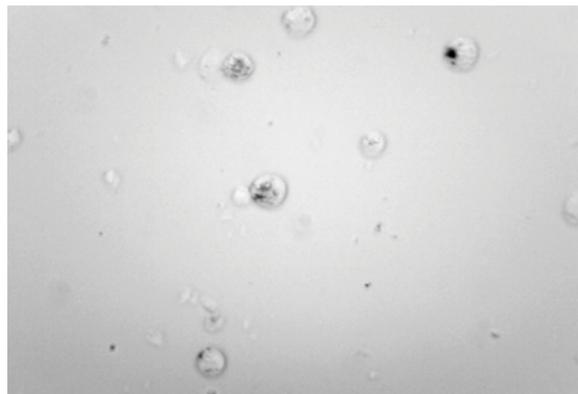


Figure 5: Optical microscopy image of a fraction collected from CF3 at 2.25 – 2.5 minutes (Figure 3).

## Conclusion

Centrifugal Field-Flow Fractionation coupled with UV-Vis detection was able to separate and quantify the amount of free drug in a liposome-drug product. The separation was verified using optical microscopy. This is a promising application using FFF for separation and quantification of drug particles, as the field of nanomedicine and liposomal drug delivery grows.

## References

- [1] Mayer L.D., St. -Onge G., Analytical Biochemistry, 1995, 232(2), 149-157.