Application Note

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Dissolution of a Propofol Liposomal Formulation Using the Agilent NanoDis System

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Abstract

Liposome formulations have numerous advantages over conventional unencapsulated drug formulations. Drug targeting, decreasing toxicity, promoting intracellular uptake, improving safety profiles, and reducing side effects are the most important improvements of a drug formulation that can be achieved by a liposomal formulation. Controlling the release of the active ingredient from the liposomal formulations ensures that drug concentrations remain within the therapeutic window at the site of action.

A predictive *in vitro* release method is required during the development and manufacturing of liposome formulations to provide evidence on the *in vivo* fate of the liposome formulation. The method must consider changes in liposome characteristics based on the process parameters or during stability storage or as a quality control method to ensure the reproducibility of the drug release in different batches.

There are several *in vitro* release methods to determine the drug release from liposome formulations. The first group of methods uses sample-and-separate techniques such as filtration, size exclusion, or centrifugation. These methods lack the efficiency of separation of the liposomes from the dissolution medium and potentially alter the sample properties due to the physical stress applied to samples. This application note develops a novel sample-and-separate technique using the Agilent NanoDis system to investigate the effects of dissolution medium on drug release rate.

Introduction

Liposome formulations have numerous advantages over conventional unencapsulated drug formulations. Drug targeting, decreasing toxicity, promoting intracellular uptake, improving safety profiles, and reducing side effects are the most important improvements of a drug formulation that can be achieved by a liposomal formulation. Controlling the release of the active ingredient from the liposomal formulations ensures that drug concentrations remain within the therapeutic window at the site of action. Ensuring that drug concentrations are maintained can ensure efficacy and safety of the formulation.

There are many factors affecting the release rate of the active ingredient from the liposomal formulation. These factors include composition and proportion of the lipids, particle size and distribution, structure of the liposomes, and the physicochemical properties and physical state of the drug.

A definitive *in vitro* release method is required during development and manufacturing of liposome formulations to provide evidence on the *in vivo* fate of the liposome formulation. The method must consider a change in liposome characteristics based on the process parameters, during stability storage, or as quality control method to ensure the reproducibility of the drug release in different batches.

There are several *in vitro* release methods to determine the drug release from liposome formulations. The first group of methods use sample-and-separate techniques such as filtration, size exclusion, or centrifugation. These methods lack efficiency of separation of the liposomes from the dissolution medium and potentially alter the sample properties due to physical stress applied on samples. Another group of methods uses dialysis membranes to physically separate the dissolved drugs from the liposomes. Underestimating the drug release rate can result if the drug release rate is higher than the permeation rate of the active ingredient through the dialysis membrane. Therefore, dialysis membrane separation is not an appropriate method for the separation of dissolved drugs. Drug release from liposomes can be measured in real time using UV absorbance of fluorescence of the active ingredient. There can be carrier-related interferences when using UV absorbance, so this technique is limited to drug molecules with fluorescence emission.

This application note develops a novel sample and separation technique using the Agilent NanoDis system to investigate the effects of dissolution medium on drug release rate. See reference 1 for more information on the overall functionality of the NanoDis system.

Experimental

HPLC method for the quantification of dissolution samples

An HPLC-UV method was used for the quantification of the samples (Table 1).

Table 1. Method parameters of HPLC analysis.

Column	Intersil ODS-3 C18, 4.6 × 250 mm, 5 µm
Mobile Phase	A: HPLC water B: Acetonitrile
Flow	1.2 mL/min
Mode	Isocratic Mobile phase A: 35% Mobile phase B: 65%
Run Time	15 minutes
Temperature	Column oven: 25 °C Autosampler: 25 °C
Injection Volume	10 µL
UV Detection	220 nm

Particle size measurement

Particle size measurements were conducted with a Malvern ZS90 Zetasizer. Liposome formulations were diluted 1:20 with distilled water before measuring the particle size.

Dissolution method

Selection of membrane chemistry: A propofol solution of 10 µg/mL in PBS buffer containing 1% Tween 80 was filtered through different filters and assayed for recovery after filtration.

Selection of membrane pore size: 100, 300, and 500 kDa cross-flow filtration membranes were used for selection of the membrane pore size. Propofol liposomes were pumped through the membranes and concentrated five-fold. The filtrate was then assayed for propofol content.

Release response time: The quantification of the release response time was realized by introducing dissolved propofol into the vessels shortly before the sampling point. The release response time of the NanoDis was qualified using propofol dissolved in DMSO. The dissolved propofol was added to the vessels 5 seconds before each sampling time point.

Dissolution: Dissolution experiments were conducted with an Agilent 708-DS dissolution apparatus coupled with a NanoDis module and an Agilent 850-DS sampling station (Table 2).

Table 2. Parameters of dissolution experiments.

Dissolution Apparatus	USP II
Volume	900 mL
Rotation Speed	50 rpm
Sampling Time Points	5, 15, 30, 45, 60, 75, and 90 min
Buffer	Phosphate buffer saline with 0.1 to 1% Tween 80

At predetermined time points, the samples were pulled from the vessel, filtered by the NanoDis module, and collected into vials by the 850-DS sampling station (Table 3). The workflow was controlled by Agilent Dissolution Workstation software.

Table 3. Parameters for the Agilent NanoDis system.

Operation	Setting
Pretest	
Peristaltic Flow Through Duration	240 s
Syringe Purge Volume	4 mL
Peristaltic Air Purge Duration	60 s
Pre Time Point Filter Conditioning	
Peristaltic Flow Through Duration	60 s
Syringe Purge Volume	2 mL
Time Point Sampling Properties	
Sample Volume	5 mL
Filter Outer Cylinder Rinse Volume	4 mL
Peristaltic Pump Sample Duration	45 s
Peristaltic Syringe Overlap	0 s

Results and discussion

Particle size characterization of propofol liposomes

Particle size characterization for propofol liposomes revealed an average particle size of 135 nm and a PDI of 0.166 (Figure 1).

Selection of membrane chemistry

Propofol solution with a concentration of 10 µg/mL in PBS buffer containing 0.25% Tween 80 was filtered through 0.45 µm mixed cellulose ester (ME), polyethersulfone (PES), and polysulfone syringe filters. The solutions were assayed for recovery after filtration (Table 4).

Table 4. Recovery results of propofol solution after filtration.

Filter Material	Recovery %
ME	93.4
PES	99.8
PS	99.5

The PES membrane showed the highest recovery for propofol and was therefore selected for further dissolution experiments.

Selection of membrane pore size

Propofol liposomal formulations with a concentration of 1 mg/mL were concentrated using 100, 300, and 500 kDa PES filters five-fold in a closed-loop system. Concentration was done using a peristaltic pump and a process pressure of 1.5 bar at a flow rate of 60 mL/min. The filtrate was assayed for propofol content (Table 5).

The 300 kDa PES membrane was efficient enough to retain nanoparticles in the retained solution and was therefore selected for further experiments.

Release response time

Release response time studies revealed an immediate response for propofol. The amount added into the dissolution vessel was recovered in the sample at the respective time points (Table 6).

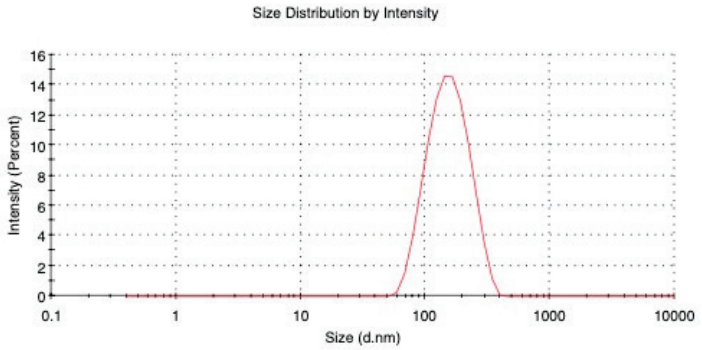

Figure 1. Particle size distribution of propofol liposomes.

Table 5. Propofol assay determination in the filtrate.

Molecular Cut-Off Membrane	Propofol Assay (µg/mL)
100 kDa	<5 µg/mL
300 kDa	<5 µg/mL
500 kDa	12 µg/mL

Table 6. Results of release response time measurements.

Time Points (min)	Theoretical Concentration (%)	Experimental Concentration (%)
5	16.7	16.3
10	33.3	32.9
15	50.0	49.8
30	66.7	66.6
45	83.3	83.5
60	100.0	99.8

Dissolution

Dissolution studies were conducted in PBS buffer with different concentrations (0.1 to 1.0 %) of Tween 80 to investigate the effect of Tween 80 concentration on the drug release from the propofol liposomes (Figure 2).

The drug release of propofol from the liposome formulation was highly dependent on the Tween 80 concentration in the medium. An immediate release was observed for the Tween 80 concentrations of 0.25 to 1%, where 100% drug release was achieved within 15 minutes. In contrast, once 0.1% Tween 80 concentration was used, a burst release was observed for the first 15 minutes, followed by a constant slow release for the next 65 minutes.

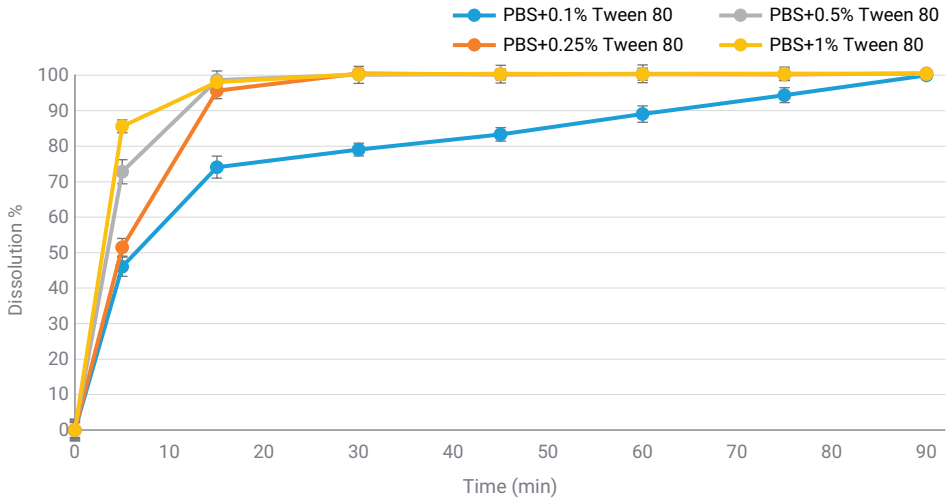

Figure 2. Drug release of propofol from liposome formulations in the presence of Tween 80.

Conclusion

A dissolution method for propofol liposome formulation was successfully developed using the NanoDis system. An immediate separation of nanoparticles from dissolution medium was possible due to the use of the cross-flow filtration technique. The effect of Tween concentration on drug release was investigated using four different Tween 80 concentrations. At the lowest Tween 80 concentration of 0.1%, a two-stage dissolution kinetic was observed for the liposome formulation. In the first 15 minutes a burst release was observed followed by a slow release of drug content for up to 75 minutes. For the higher Tween 80 concentrations, an immediate release over 15 minutes could be observed. The big advantage of using cross-flow filtration with the NanoDis system could be demonstrated with the determination of different release profiles for the various Tween 80 concentrations. A burst release is often not detectable with a dialysis system[2] as the release profile is diffusion-controlled and time-delayed, rather than reflecting the actual release profiles from the liposomes, as shown in this study.

References

1. Agilent NanoDis System Method Development Guide. *Agilent Technologies white paper*, publication number 5994-2347EN, **2020**.
2. Lombardo, S. M. *et al.* Reliable Release Testing for Nanoparticles with the NanoDis System, an Innovative Sample and Separate Technique. *Int. J. Pharm.* **2021**, *609*, 121215. ISSN 0378-5173, https://doi.org/10.1016/j.ijpharm.2021.121215.

Appendix

Dissolution data of propofol liposomes using NanoDis

Formulation	Dissolution %						
	5 min	15 min	30 min	45 min	60 min	75 min	90 min
PBS + 0.1% Tween 80	46.0	74.1	79.0	83.3	89.1	94.4	100.0
PBS + 0.25% Tween 80	51.5	95.6	100.5	100.2	100.3	100.2	100.5
PBS + 0.5% Tween 80	72.8	98.6	100.1	100.3	100.4	100.4	100.4
PBS + 1% Tween 80	85.6	98.1	100.3	100.4	100.3	100.4	100.4
SD							
PBS + 0.1% Tween 80	2.4	2.7	3.1	1.8	1.9	2.3	2.1
PBS + 0.25% Tween 80	2.8	2.5	2.2	1.2	1.5	1.7	1.6
PBS + 0.5% Tween 80	3.1	3.4	2.6	2.4	2.5	2.5	1.9
PBS + 1% Tween 80	2.1	1.8	1.3	0.9	1.2	1.1	0.8
RSD %							
PBS + 0.1% Tween 80	5.2	3.6	3.9	2.2	2.1	2.4	2.1
PBS + 0.25% Tween 80	5.4	2.6	2.2	1.2	1.5	1.7	1.6
PBS + 0.5% Tween 80	4.3	3.4	2.6	2.4	2.5	2.5	1.9
PBS + 1% Tween 80	2.5	1.8	1.3	0.9	1.2	1.1	0.8

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