White Paper

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Agilent NanoDis System Method Development Guide

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Introduction

One of the major challenges in modern drug development is the poor aqueous solubility of many drug candidates. Nanoparticle formulations are an attractive solution to increase the bioavailability of drugs with low aqueous solubility. Dissolution studies are one of the most important methods used to characterize the expected *in vivo* bioavailability under *in vitro* conditions. Thus, an efficient dissolution method is necessary during the nanoparticle development process to facilitate the selection of lead formulations with an expected higher bioavailability. It is equally important for quality control purposes during later stages of the product life cycle. Therefore, the separation of dissolved and undissolved drug is one of the most important factors of all dissolution studies.

The nanoparticle is considered an undissolved substance and needs to be separated from the dissolution media. Current dissolution methodologies suffer from the inefficient separation of nanoparticles from the dissolution medium, independent of the equipment used for the dissolution studies. What's more, standard filtration methods used for conventional formulations are insufficient for the separation of nanoparticles from the dissolution medium during the sampling process. Filtration through membrane-type filters is a very common method used for the separation of undissolved API. Syringe filters with a pore size of 0.22 or 0.45 µm are applied, but this also presents challenges. The disadvantages are that any particles smaller than 500 nm, in average particle size, either block and rupture the filter or pass straight through, resulting in too high dissolution values. There are filters with smaller pore sizes available, such as 0.02 µm. However, these tend to block quickly during the sampling process particularly with high nanoparticle concentration in the medium. Inefficient separation of nanoparticles will result in nanoparticles remaining in the dissolution medium after the separation process, which in turn leads to incorrectly high readings for the dissolution profile analysis. In summary, current dissolution processes used in the development labs are not sufficient to predict the *in vivo* performance of the nanoformulations, because of improper filtration means.

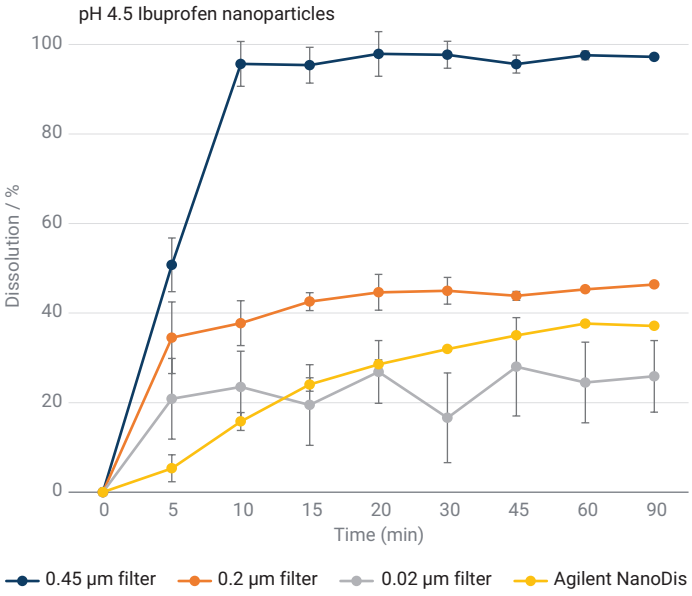

Figure 1. Dissolution profiles of ibuprofen nanoparticles 200 mg/900 mL in acetate buffer pH 4.5 using different syringe filters (0.45 µm, 0.2 µm, and 0.02 µm) and the Agilent NanoDis System.

Agilent NanoDis System for dissolution

The filtration principle of the Agilent NanoDis System is based on cross flow filtration (CFF) combined with conventional dissolution apparatus and with the aim to automate the filtration process. The NanoDis System uses the advantages of CFF to separate the nanoparticles from the dissolution medium. Since CFF is an open filtration system, the formation of a filter cake is prevented, and the nanoparticles are not forced into the filter, which often results in the blockage of filters in a dead-end filtration. Furthermore, the membrane size of the CFF filters can be chosen according to the particle size of the nanoparticles in the formulation. In the NanoDis System, the dissolution medium containing nanoparticles is pumped through CFF filters where the filtrate, free from particles, is collected with the Agilent 850-DS Dissolution Sampling Station. Filtration efficiency and the related time required to collect the filtrate is dependent on the formulation and the concentration of nanoparticles in the medium. However, even the lowest filtration rate for challenging formulations is higher than 10 mL/min with the NanoDis System, which generates enough sample volume for quantitative analysis of the dissolution samples and allows for very short time point intervals.

Selection of the filter membrane size and type

The filter types and the pore sizes of cross flow filters used in dissolution studies are selected according to the nanoparticle size and type, and to ensure complete separation. These specifications enable the determination of dissolved drug without any interference caused by undissolved nanoparticles.

There are many CFF membranes available with different chemistries. A filter validation test is required to select the right membrane chemistry to prevent the adsorption of the nanoparticles and the API to the filter. Filter validation can be conducted with the standard syringe filters, these having the same chemistry as the CFF membranes. By contrast, for an API filter validation, API at a known concentration is dissolved in dissolution medium and assayed after filtration through syringe filters with a pore size of 0.45 µm, for instance. Note

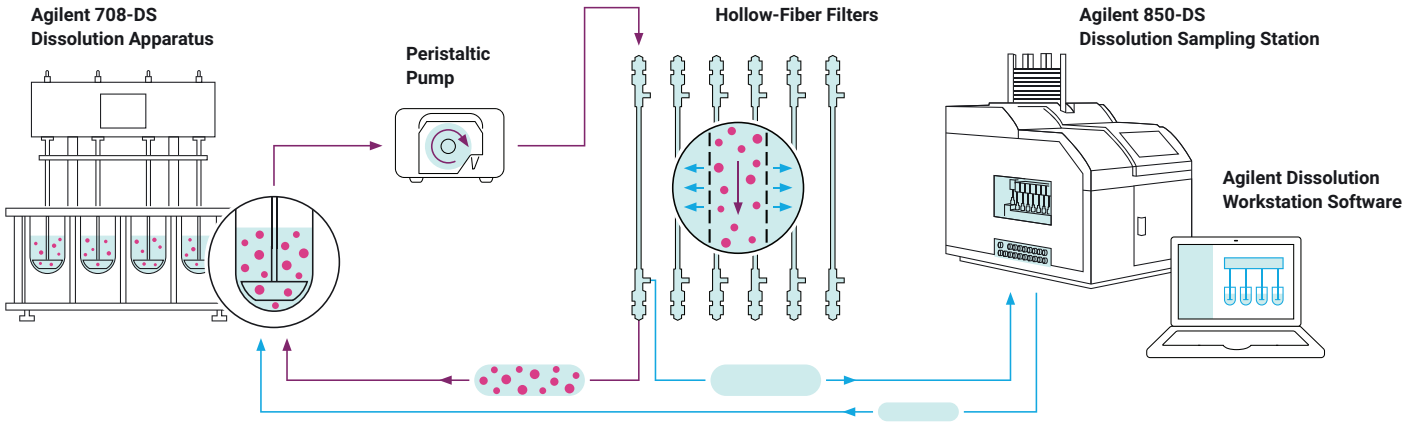

Figure 2. Diagrammatic representation of the NanoDis System showing the directional flow and separation of dissolved API and dispersed nanoparticles using the USP-compliant Agilent 708-DS Dissolution Apparatus and 850-DS Dissolution Sampling Station, operated and electronically documented using the Agilent Dissolution Workstation Software (DWS).

that a higher pore size of the syringe filters might be required for the filter validation of nanoparticle formulations.

The nanoparticle formulations are dispersed in dissolution medium at a known concentration and passed through a syringe filter with an appropriate pore size to ensure the passage of the nanoparticles through the filter. API before and after filtration is qualified to calculate the possible adsorption of the nanoparticles on the filter.

While the average particle size of the drug formulation is the primary consideration, particle size distribution and particle size distribution in the dissolution medium are also important parameters to consider. In nonhomogeneous particle formulations, the particle size of the smallest particles that are present in the sample can be far below the average particle size, or vice versa (i.e., the highest particle size of the biggest particles present in the sample can be significantly higher than the average particle size). Furthermore, especially once the particles are dispersed in *in vivo* relevant media such as FeSSIF and FaSSIF, a corona can be formed around the particle surface, increasing the average particle size.

In general, Table 1 can be considered for the selection of the right membrane pore size:

Table 1. Selection of the membrane pore size.

Average Particle Size (nm)	Membrane Pore Size (kDa)
<10	10
10 to 25	50
25 to 50	100
50 to 150	300
>150	500

If the particle size distribution is wide or if there is a bimodal distribution with smaller particles present in the sample, the CFF filter membrane size needs to be adjusted accordingly, also taking the particle fraction with the smallest particle size into consideration. Another important parameter is the particle size of the micelles, which are formed in cases where surfactants are used in the dissolution medium. The micelles of dissolved API can also be retained by the CFF membranes. Thus, the membrane size needs to be selected in accordance to the micelle size. Further information on filter validation can also be found in the reference section[1] and on the Repligen website.[2]

Figure 3. Agilent NanoDis system with crossflow filters.

Equipment and software parameters

Optimization of equipment and software parameters are part of the dissolution method development process to ensure the efficient separation of the nanoparticles from the dissolution medium.

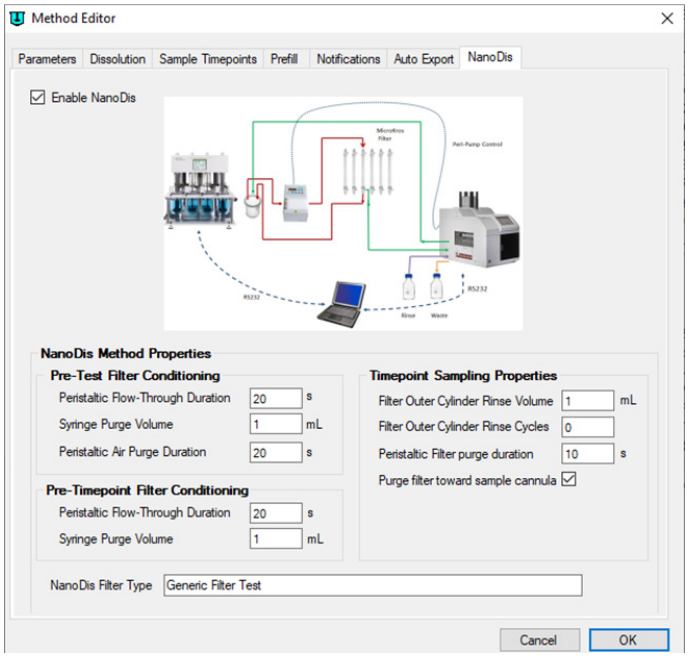

Figure 4. Screenshot of Agilent Dissolution Workstation Software, NanoDis Tab.

The NanoDis System is controlled by the Agilent Dissolution Workstation Software (DWS). Below is an outline of the important parameter settings to guide you through the method development process.

The NanoDis System's method is run in two phases: the filter preconditioning phase prior to the start of the dissolution run, followed by the actual dissolution run. Each time point during the dissolution run is preceded by an additional filter preconditioning step. A typical sequence would therefore look as follows:

Phase 1: Filter preconditioning

Pump rate: Pump rate is adjusted manually on the peristaltic pump. Transmembrane pressure of the CFF membranes increases with the increased flow rate, which results in the increased permeation of the filtrate. In summary, the amount of the filtrate sample collected can be increased by increasing the flow rate.

Pretest filter conditioning: Pretest filter conditioning is used to wet the filters with the current dissolution medium. It is begun before the start of the dissolution and before the introduction of the product into the dissolution vessel. Dissolution medium that is filtered through the CFF membranes is directed back to the dissolution vessel, thus there is no loss of dissolution medium during the pretest filter conditioning. At the end of the pretest filter conditioning step, air is purged through the CFF membranes so that the complete dissolution medium is directed back into the vessel.

Pretest filter conditioning is important in terms of preparing the CFF membranes for the first time point. If the pretest filter conditioning is not sufficiently applied, the sample amount is reduced for the first time points. The correct sample volume is important for accurate calculation of your %dissolved results.

The following starting parameters can be used for the pretest filter conditioning:

Operation	Recommended Setting
Peristaltic flowthrough duration Time in seconds to pump medium through the filter	240 s
Syringe purge volume Volume in mL pumped out of the outer cylinder of the tangential flow filter back to the vessel	4 mL
Peristaltic air purge duration Peristaltic pump is reversed to empty the central core of the filter back into the vessel	50 s

Phase 2: Dissolution

The dissolution runs starts by introducing the dosage form into the vessels. When using the NanoDis System, the dissolution test sequence is slightly modified. Before each time point, it is necessary to condition the filter again, which completes just before the time point is due.

Pre-timepoint filter conditioning variables

Operation	Recommended Setting
Peristaltic flowthrough duration Time in seconds to pump medium through the filter	100 s
Syringe purge volume Volume in mL pumped out of the outer cylinder of the tangential flow filter back to the vessel	2 mL

Time point sampling properties

Operation	Recommended Setting
Prime loss volume Volume of tubing from tip of sampling cannula to needle (850-DS) to allow for time-correct sampling	3.5 mL
Sample volume Volume dispensed into sample tray	1.0 to 5 mL (depending on your analysis)
Filter outer cylinder rinse volume Volume of rinse medium that will be pumped into the outer cylinder of the filter; Total purge volume = purge volume + filter outer cylinder rinse volume	4 mL
Peristaltic filter purge duration After sampling, sample medium within the central core of the filter is purged back to the vessel. Parameter determines how long the peristaltic pump will run to purge the central core. Direction is controlled by the purge filter toward sample cannula check box.	30 s
Syringe purge Syringe purge cycle empties the outer cylinder of the filter and all the tubing between the filter and the vessel in the syringe flow path. Syringe purge volume = filter outer cylinder rinse volume + purge volume	4 mL
Filter outer cylinder rinse cycles At the conclusion of a time point, the outer core of the filter can be rinsed using rinse medium. The value can be set to zero to avoid the rinse cycle.	1 to 3 times with 4 mL

Tips and tricks

Membrane first use, cleaning, and storage

CFF membranes are shipped in glycerol to maintain the pore structure. It is recommended to wash the membranes with at least 500 mL of purified water before the first use; alternatively, dissolution medium can be used for washing the membranes. Purified water, alcoholic solution (20% ethanol or 20% isopropanol), or 0.1 N NaOH can be used for the cleaning of the membranes in between dissolution runs depending on your nanoparticle formulation. It is best to store the membranes at room temperature and in a suitable storage medium—for instance, 0.1 N NaOH. This prevents microbial growth and keeps the fibers from drying out.

Membrane test for integrity

The integrity testing of the CFF membranes is controlled with standard nanoparticles with desired average particle size and adjusted to the CFF-desirable membrane pore size. PLGA-Lumogen nanoparticles allow for the visualization of membrane integrity with the absence or presence of the color in the samples.

Membrane test for blockage

Blockage in the CFF membrane is determined by conducting a dissolution experiment with water and quantification of amount of sample filtered.

References

1. Filter Validation Protocol: Agilent 850-DS Dissolution Sampling Station. *Agilent Technologies technical overview*, publication number 5991-3341EN, **2013**.
2. Repligen 'Find a Spectrum Hollow Fiber Filter' selection tool (https://www.repligen.com/resources/configurators/selection-tools/find-hollow-fiber-filter).

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DE.1479976852

Printed in the USA, October 26, 2020
5994-2347EN

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