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Evaluation of NanoDis as an Automated Sampling Technology for In Vitro Release Testing of Nanomedicines

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ABSTRACT

Nanoparticles can be used in pharmaceuticals to provide a targeted and prolonged release of active pharmaceutical ingredient (API). Nanoparticles are growing in application in the field of oncology due to developments in the field, but still there are issues faced with studying the in vitro release of long-acting injectables. A method using the sample and separate approach via ultracentrifugation was used for a polymeric nanoparticle product with an in vitro release over 10 days. This method is laborious, with many areas of manual intervention, which reduces robustness and provides limited temporal resolution of the in vitro release profile due to sampling timepoints. NanoDis is a recently developed automated sampling system that uses tangential flow filtration (TFF) to separate released and encapsulated API over the in vitro release profile, with minimal analyst input and enhanced temporal resolution compared to other methods. This article highlights the success of implementing NanoDis for automated sampling of polymeric nanoparticles, with release profiles comparable to the ultracentrifugation method, showing potential for a more robust and quality control-friendly method.

KEYWORDS: In vitro release, nanoparticles, nanomedicine, NanoDis, dissolution

INTRODUCTION

The *United States Pharmacopeia* (*USP*) defines nanomaterials as materials with features or structures that exist on the 1–100 nm scale in any of the three spatial dimensions (*1*). The knowledge around nanomaterials is growing rapidly due to advances in research, with one area of growing application being within pharmaceuticals. The first publications were in the 1990s, building on the developments of nanotechnology made in the earlier 20th century (*2*, *3*). Recently, the use of nanoparticles in drug delivery has been seen through the mRNA vaccines for COVID-19, packing the mRNA strands in a lipid nanoparticle as a drug delivery vehicle (*4*, *5*). Their use is also growing within oncology, due to the ability of nanoparticles to distinguish between the healthy and tumorous cells owing to increased blood pressure within tumorous tissues because of waste and toxin build up within the cells (*3*, *6*). As of May 2021, there were 16 nanomedicines approved for cancer treatment, with the first approved in 1994 and the most recent in 2018 (*7*).

There is a wide breadth of formulations available for nanomaterials as delivery vehicles, encompassing both organic and inorganic nanoparticles, so the methods available for in vitro release testing (IVRT) of these parenterals does not provide a universal solution for their analysis (*8–10*). Some common techniques used in IVRT include sample and separation with ultracentrifugation, dynamic dialysis, and continuous flow (*10–12*).

The sample and separate with ultracentrifugation method is popular but presents challenges, such as the stress applied to samples through manually intensive and laborious processes. The identification of these challenges and similar limitations of other available methods (e.g., membrane kinetics being a rate limiting factor for dynamic dialysis, filter clogging preventing accurate data from continuous flow methods) have facilitated

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research into the use of tangential flow filtration (TFF) (*10*, *11*, *13*, *14*). TFF involves a parallel stream of sample to the filter membrane, allowing the API to pass through the membrane, whereas the nanomaterial is unable to pass through (*15*). This technique can reduce potential filter clogging seen in dead end filtration, preventing a decrease in the flux rate (*15*) (Fig. 1).

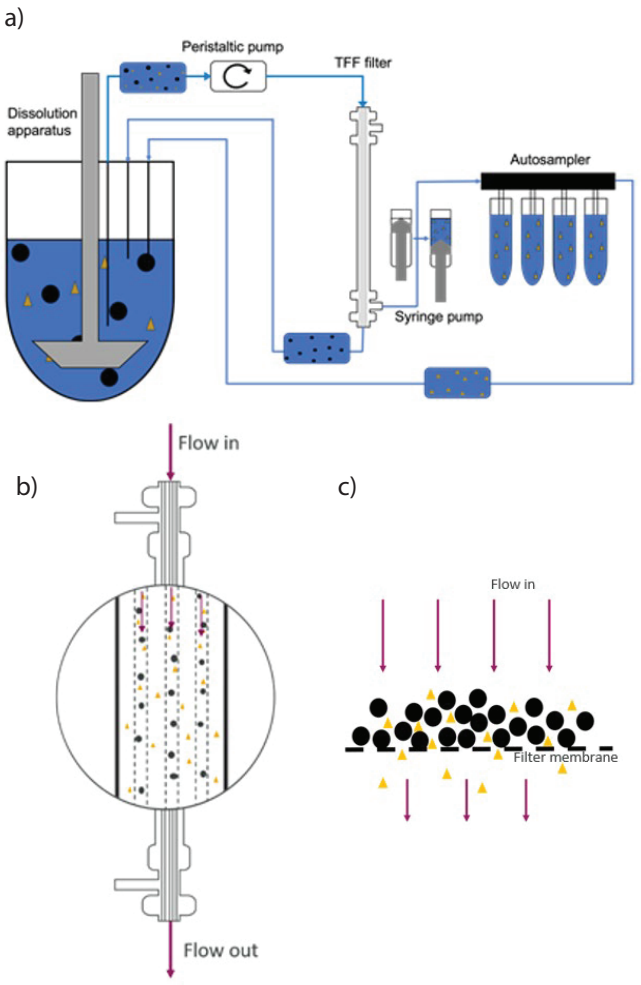

Figure 1. Diagram shows the flow of sample within NanoDis (a), with black circles representing nanoparticles and gold triangles representing the released API. Diagram of TFF filter (b) shows the separation of a nanoparticle and released API through a filter membrane compared to (c) dead end filtration.

Research into the use of TFF for IVRT has already highlighted some advantages over traditional methods such as dialysis techniques, showing that reverse dialysis gives a much slower release of difluprednate in an IVR study (*16*). This difference is thought to be a result of the membrane permeation in the dialysis method being a rate limiting factor (*10, 16*). This demonstrates the ability of TFF technology to produce more timepoint-specific and representative data by reducing the time lag associated with membrane permeation kinetics.

NanoDis is a new instrument recently developed to utilize TFF technology. NanoDis is a fully automated piece of equipment for flow studies, requiring minimal analyst input once the system is running. The combination of the main aspects working across four different stages allow for timepoint-specific sampling and separation of the released drug in solution from nanoparticles, as seen in Figure 2.

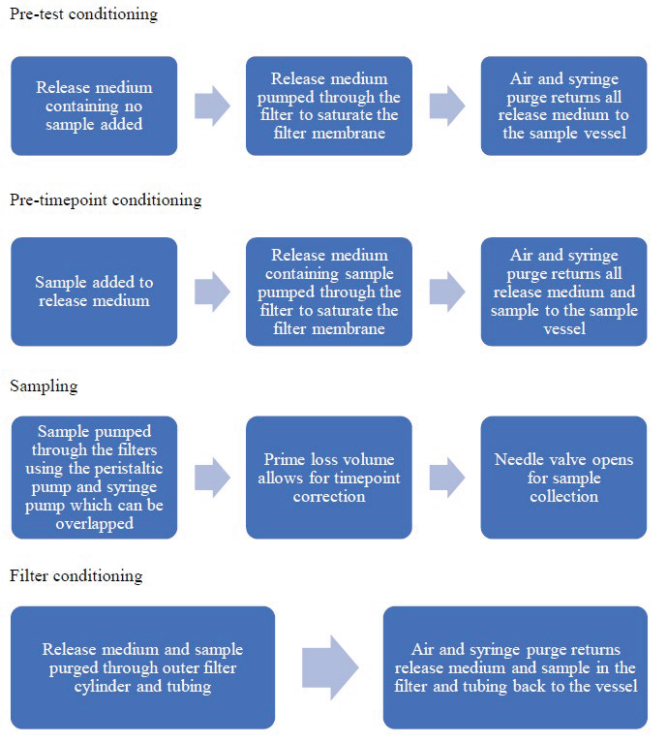

Figure 2. Flow diagram of the key stages completed within the NanoDis sampling cycle.

Since the early studies with the NanoDis, automating the TFF process has been found to eliminate problems around membrane permeation kinetics, thus allowing the accurate measurement of burst release (*17*). In a study by Lombardo et al, the dialysis technique produced a release rate around 25% lower than the NanoDis method at the final time point, and the dialysis method showed a more gradual release at earlier time points (*17*). This gradual release inaccurately demonstrates the burst release phase and mirrors previous findings from adaptive perfusion studies (*16*).

NanoDis has been shown to overcome challenges faced in the dialysis technique when using a polylactic coglycolic acid (PLGA) nanoparticle, to be employed as a recognized approach, but further studies are needed to assess its wider application (*17*). A study of the sample and separate technique with ultracentrifugation for the release of an aurora kinase B inhibitor from a polymeric

nanoparticle achieved slow release of the API of over 1 week (*18, 19*). The nanoparticle is a polylactic acid (PLA) nanoparticle with a polyethylene glycol (PEG) stealth layer, 100 nm in size. There are no permeation kinetic effects associated with the ultracentrifugation method for this nanoparticle, unlike the dialysis technique used for the PLGA nanoparticle; however, the process of taking samples for ultracentrifugation, followed by subsequent analysis such as by liquid chromatography (LC), is long and labor-intensive, providing many opportunities for human error and reducing robustness of the method.

MATERIALS AND METHODS

API and Release Medium Components

BioXtra Tween 20 (Polysorbate-20), butylated hydroxy anisole (BHA), high pressure liquid chromatography (HPLC)-grade trifluoroacetic acid (TFA), sodium chloride (NaCl) pellets, sodium phosphate monobasic ($NaH_2PO_4 \cdot H_2O$), and sodium phosphate dibasic dihydrate ($Na_2HPO_4 \cdot 2H_2O$) were purchased from Sigma Aldrich (St. Louis, MO, USA); methanol and acetonitrile were purchased from VWR chemicals (Radnor, PA, USA); and 2 M sodium hydroxide (NaOH) solution was purchased from Fisher (Pittsburgh, PA, USA). Sample diluent with a composition of 67% v/v water, 33% v/v acetonitrile was prepared. The polymeric nanoparticle product containing a poorly soluble API and counter ion were produced by AstraZeneca (Macclesfield, UK).

Preparation of Release Medium

Release medium used throughout the testing was a 100 mM Sorensen's phosphate buffer (pH 6.9) and 10% Tween 20, with the addition of 150 mM NaCl and 0.06 mg/mL BHA (antioxidant). The pH was tested and confirmed to be within ± 0.05 of the target pH of 6.9 using a Mettler Toledo pH meter (calibrated before use).

Filter Compatibility

Interaction between the drug product and filter material was assessed through a manual filtration procedure. By preparing the drug product in water, the sample could be filtered through a 0.45-µm modified polyether sulfone (mPES) filter, and the recovery was assessed against the pre-filtered sample by HPLC. To ensure that the 300 kDa pore size would facilitate the collection of API in the filtrate, the pore size (300 kDa) was assessed to ensure collection of API in the filtrate as follows. Samples containing pure API were prepared at 15%, 75%, 100%, 225%, and 1500% of the 0.02 mg/mL nominal concentration in release medium. These were pumped through the NanoDis system, and API concentration in the filtrate was assessed against the starting concentration in each sample to determine recovery.

Dynamic Light Scattering

A Malvern Zetasizer was used to determine the size of any nanoparticles, using a semi macro cell, a 120-sec equilibration, and 173° backscatter. The material refractive index and absorption were previously determined as 1.330 and 0.010, respectively (in-house data).

IVRT with Ultracentrifugation for Sample Isolation

For IVRT, samples were incubated in 50 mL release medium at 45 °C using a Julabo SW23 shaking water bath at 75 rpm. Uncentrifuged 0.25-mL samples were taken at time zero (T_0) to give the total starting concentration of the API, and 3.2-mL samples were taken for ultracentrifugation at different timepoints over 24 hours (or 48 hours for the slower releasing batch) including T_0 to assess the release relative to the starting concentration. For the ultracentrifugation, a Beckman ultracentrifuge (Indianapolis, IN, USA) was used between 55,000 and 110,000 rpm at 4 °C for 30 minutes, with a Beckman Coulter TLA-55 or TLA- 110 rotor. Supernatant (0.25 mL) was sampled following ultracentrifugation and diluted in a 1:4 dilution with sample diluent.

IVRT with NanoDis for Sample Isolation

The NanoDis system is made up of sampling cannulas, a peristaltic pump, TFF filters, and an autosampler, all coordinated by dissolution workstation software. Within the system, there are opportunities for optimization, which may be required for different drug products. This includes the different molecular weight cut offs for the filter, with compatibility of filters between 10 and 500 kDa. This allows the system to facilitate the separation of API and nanoparticles, where the nanoparticles can range from less than 10 nm to excess of 150 nm (*20*). This is an addition to adaptations that can be made to the syringe pump settings, such as reducing the plunger speed or increasing the aspiration dwell time, which can help with more viscous media or that which has high concentrations of surfactant, so is more susceptible to foaming. Filters (300 kDa mPES) from Repligen (Waltham, MA, USA) were purchased. Samples were incubated as per the sample and separate method above; 0.25-mL samples were taken at T_0 to give the initial concentration, and 1-mL samples were taken at different increments over 24 hours (or 48 hours for the slower releasing batch) by the autosampler. A 0.25-mL aliquot of sample was diluted as per the ultracentrifuged samples.

The setup did not incorporate a dissolution bath as the NanoDis was intended for, but instead a water bath was used. The sampling and two return cannulas were placed into the sample with return cannulas suspended above the medium and the sampling cannula left in the medium

to optimize the setup, with parameters set to achieve a 1-mL sampling volume (Table 1).

Table 1. Optimized Parameters in Dissolution Workstation to Achieve a 1-mL Sample Volume with NanoDis Using a 300-kDa Filter

Parameter	Value
Plunger speed	6 mL/min
Aspiration dwell time	5 s
Prime loss	2.5 mL (specific to system tubing volume)
Sampling parameters	
Sample volume	1 mL
Purge volume	7.5 mL
Active channels	6
Waste drop volume	0.3 mL
Samples/filter	0
Pre-test filter conditioning	
Peristaltic flow through duration	240 s
Syringe purge volume	4 mL
Peristaltic air purge duration	20 s
Pre-timepoint filter conditioning	
Peristaltic flow through duration	100 s
Syringe purge volume	2 mL
Timepoint sampling properties	
Filter outer cylinder rinse volume	4 mL
Filter outer cylinder rinse cycles	0
Peristaltic pump sample duration	120 s
Peristaltic syringe overlap	60 s
Peristaltic filter purge duration	30 s
Purge filter toward sample cannula	Yes

Chromatographic Conditions

The release profiles were measured using ultra-performance liquid chromatography-UV (UPLC-UV). API concentration was determined using a C18 reverse phase column (Waters CSH C18 2.1 x 150 mm, 1.7 µm) at 30 °C using 0.1% TFA/water for the aqueous mobile phase and 0.08% TFA/acetonitrile for the organic. The gradient program was ran at 15% to 20% B over 4 mins, 20% to 50% B over 1 min, and 50% to 85% B over 1 min, with a 0.3 mL/min flow rate. The eluent absorbance was monitored at 238 nm. Sample concentration was calculated against an external API reference standard.

RESULTS AND DISCUSSION

There was no adsorption of the drug product to the mPES filter material. Consistently high recoveries were measured (96.2–101.4%) across the replicates, giving confidence in using mPES TFF filters.

The filter size compatibility results can be seen in Figure 3A. Recovery of the API can be seen when solutions of API without nanoparticles were prepared in the release medium and ran on NanoDis using 300 kDa TFF filters, as suggested by the manufacturer for 100-nm nanoparticles. The high recovery for all timepoints across all concentrations illustrates that the API can pass through the pore size in the filters, and there is an absence of filter packing by maintaining this consistently high recovery. The unexpectedly high recovery at T_0 for 15% and 75% can be accounted for through residual API in the needle from previous tests that had not been cleaned out sufficiently; subsequent work optimized the sampling cycle.

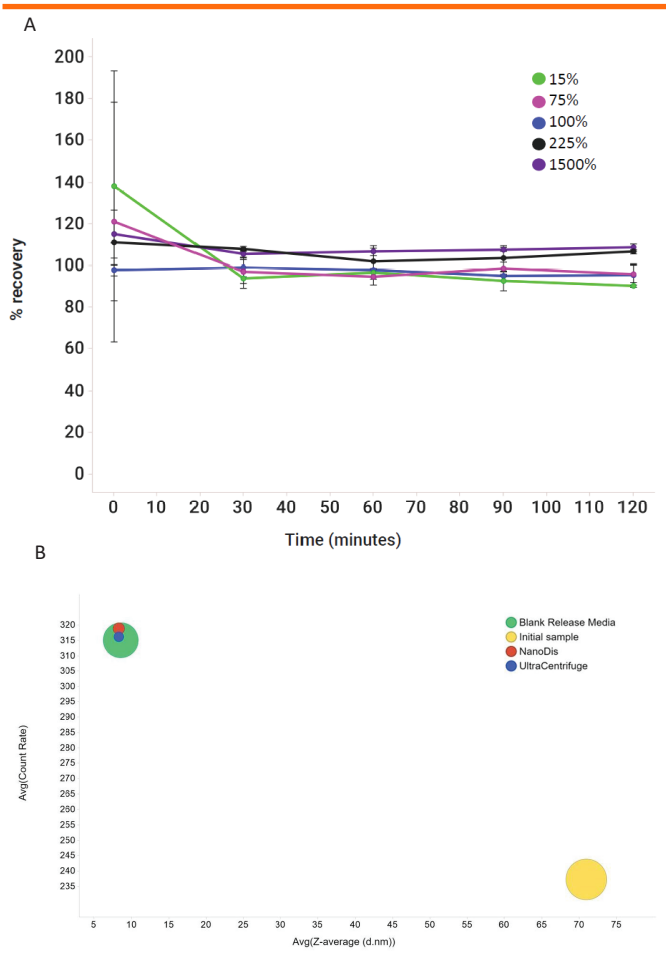

Figure 3. (A) Percent recovery of API (mean ± SD, n = 2) when the drug substance passes through the 300-kDa mPES filter when prepared at different concentrations relative to the nominal concentration of 0.02 mg/mL. (B) Particle size distribution in different samples studied through dynamic light scattering. The nanoparticles are ~ 100 nm and Tween 20 micelles are ~ 10 nm in the release medium (present in blank release medium and with API present). Shape size is representative of SD (n = 3). mPES, modified polyether sulfone.

Dynamic light scattering was used to further validate the filters, providing evidence that while the API can pass through the filter membrane, the nanoparticles are isolated because they cannot pass through the

membrane (Fig. 3B). The data demonstrate that the polymeric nanoparticles (~100 nm) are no longer present in the samples following treatment (i.e., NanoDis or ultracentrifugation). Subsequently, this visualization shows that TFF works as effectively as the ultracentrifugation step for separating nanoparticles from the released drug.

IVRT results are presented in Figure 4A. Similar release profiles were measured with both techniques (NanoDis and ultracentrifugation), with the fastest release kinetics between 2 and 6 hours before slowing down between 6 and 24 hours. A positive bias in ultracentrifugation results after the initial timepoint can be seen, as well as carryover from residual API in the needle of the autosampler seen in the NanoDis results. The carryover could easily be attributed to a poor cleaning method, leaving some material in the needle following completion of the previous tests, which could be overcome by programming a 10-mL injection to be performed with the cleaning medium (10% ethanol in water).

The positive bias identified was a result of the time taken to achieve sufficient sample cooling (from 45 °C to 4 °C) before ultracentrifugation could occur, causing an excess release of API from the nanoparticle, resulting in a falsely high release. The bias could be eliminated through rapid cooling of samples on ice, as shown in Figure 4B, explaining the differences in release profiles in Figure 4A. Furthermore, the ultracentrifugation method has been validated, with its accuracy confirmed by a ^{19}F NMR method (*18*). Due to the incubation being at 45 °C, the cooling to 4 °C would take longer than from 37 °C, which is used in more common IVRT methods including the ^{19}F NMR method.

The current guidelines around IVRT for parenterals were considered to study the applicability of NanoDis, such as its discriminatory power for product variants deliberately manufactured to exhibit different release rates (*21*). As part of the product and process development, several different batches were manufactured to test the NanoDis capabilities to measure slow, intermediate, and fast release rates.

IVRT results of batches with varying release rates are presented in Figure 5. The data demonstrate that NanoDis is capable of discrimination, giving excellent concordance between the profiles from the two techniques. Therefore, NanoDis can satisfy the guidance laid out by regulatory authorities, such as the FDA (*21*). Not only can NanoDis discriminate between different batches, but it can do this more efficiently than the ultracentrifugation method and with greater temporal resolution.

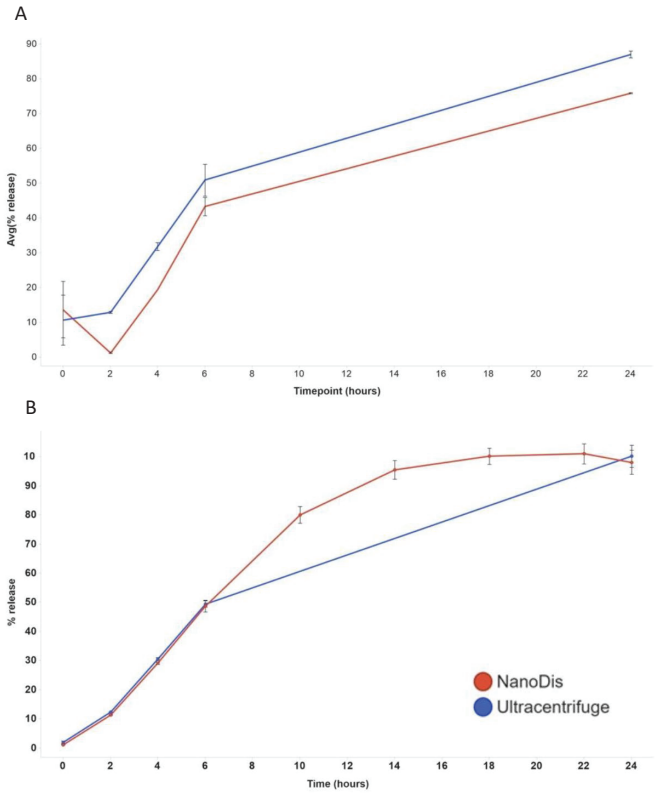

Figure 4. (A) In vitro release profiles (mean ± SD, n = 3) of the API over 24 hours for samples passed through NanoDis (red) and samples undergoing ultracentrifugation (blue). (B) In vitro release profile (mean ± SD, n = 3) of the API over 24 hours using NanoDis (red) or ultracentrifugation following rapid cooling on ice (blue).

CONCLUSION

NanoDis provides the same capabilities of a sample and separate with ultracentrifugation IVRT method, allowing distinct separation of the API and nanoparticle for analysis of drug release, while overcoming many limitations of a manual and laborious process. NanoDis uses an automated approach to remove potential sources of manual error and provide significant time savings. This is in addition to collecting more timepoints and gaining an enhanced understanding and greater temporal resolution of API release from the drug product. NanoDis has promising potential to be implemented more widely for IVRT of long-acting nanomedicine injectables.

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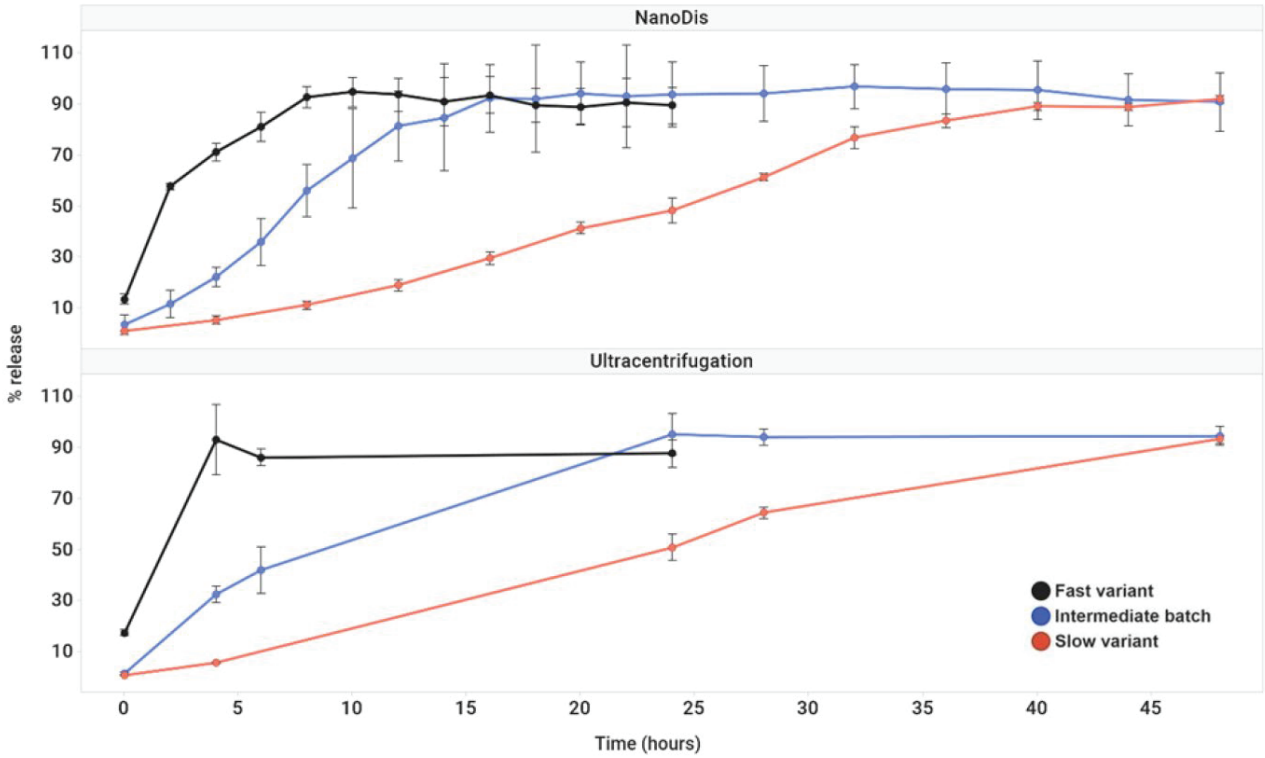

Figure 5. In vitro release profiles (mean ± SD) of the API for fast (black, n = 3), slow (red, n = 3), and intermediate release batches (blue, n = 4) using NanoDis (top) or ultracentrifugation (bottom).

CONFLICTS OF INTEREST

The authors disclosed no conflicts of interest related to this article.

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