Dissolution Technologies

www.dissolutiontech.com Volume 30, Issue 3 | August 2023

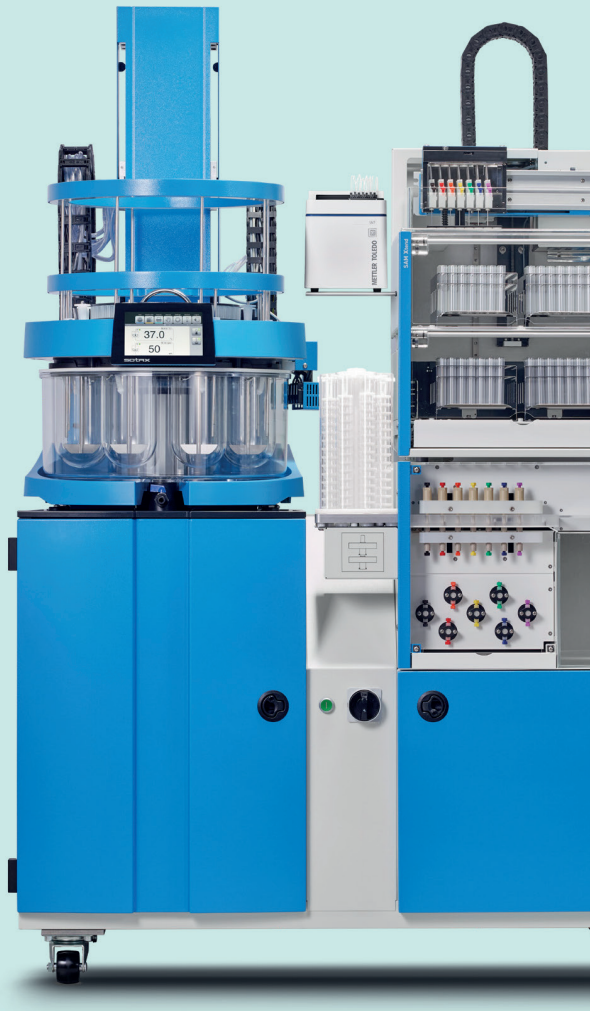
ATF Xtend™ —
Fully Automated Dissolution
Testing System

50 years

sotax

Dissolution.
100 % Unattended.

Schedule your virtual DEMO today!



The new ATF Xtend™ – Automating the dissolution test does not need to be complex.

Controlled media preparation with vacuum degassing, recorded vessel filling, and robust system cleaning have been added to the proven Xtend™ semi-automated dissolution platform.

With a focus on efficient capture of data flows, this simplified take on automated disso is ready for global deployment!

Solutions for Pharmaceutical Testing

sotax.com

Table of Contents

In This Issue

Advertisers

To Submit Articles

Please check the website for instructions, the articles are peer-reviewed and are submitted through the PeerTrack™ website, https://www.editorialmanager.com/dt.

The scope of articles is limited to dissolution or disintegration topics as the major focus. Articles on formulation development where dissolution is just one test of many should not be submitted.

For inquiries and prescreening, e-mail vagray@rcn.com

***Dissolution Technologies* is indexed in:**

Science Citation Index Expanded®
Journal Citation Reports®/ Science Edition
International Pharmaceutical Abstracts
Chemical Abstracts (CAS)
SCOPUS
EMBASE
CrossRef

Topics for the Next Issue

The **November 2023** issue will include research articles on topics of verapamil HCl tablets (apparatus 2 and 4), bilastine and montelukast fixed combination dosage forms, mifepristone liquid compacts, and budesonide dosage form interchangeability. The issue will also include USP stimuli article on mucosal products, DPVS webinar report, AAPS highlights, Philippines workshop report, and the Q and A feature.

Research Editor
Vivian Gray
vagray@rcn.com

Publisher's Information

PURPOSE: *Dissolution Technologies* is a peer-reviewed quarterly publication reporting current information on dissolution testing. It provides an international forum for open exchange of information on various dissolution topics.

CORRESPONDENCE: Send all correspondence to: *Dissolution Technologies*, Inc., 9 Yorkridge Trail, Hockessin, DE 19707. Phone (302) 235-0621, e-mail: vagray@rcn.com. Website: www.dissolutiontech.com

STAFF: Founder, Cynthia Brown; Managing Director, Vivian Gray; Associate Editor, Valerie Clark; Research Editor, Vivian Gray; Research Editor, William Brown; Communications, Michael Larson; Circulation Manager, Sandra Larson; Layout, Michele Arnold; Publication, Printing, and Distribution Services, Archer Print Group, Sellersville, Pennsylvania.

ISSN 1521-298X

Dissolution Technologies is indexed in:
Science Citation Index Expanded®
Journal Citation Reports®/ Science Edition
International Pharmaceutical Abstracts
Chemical Abstracts (CAS)
SCOPUS
EMBASE
CrossRef
Google Scholar

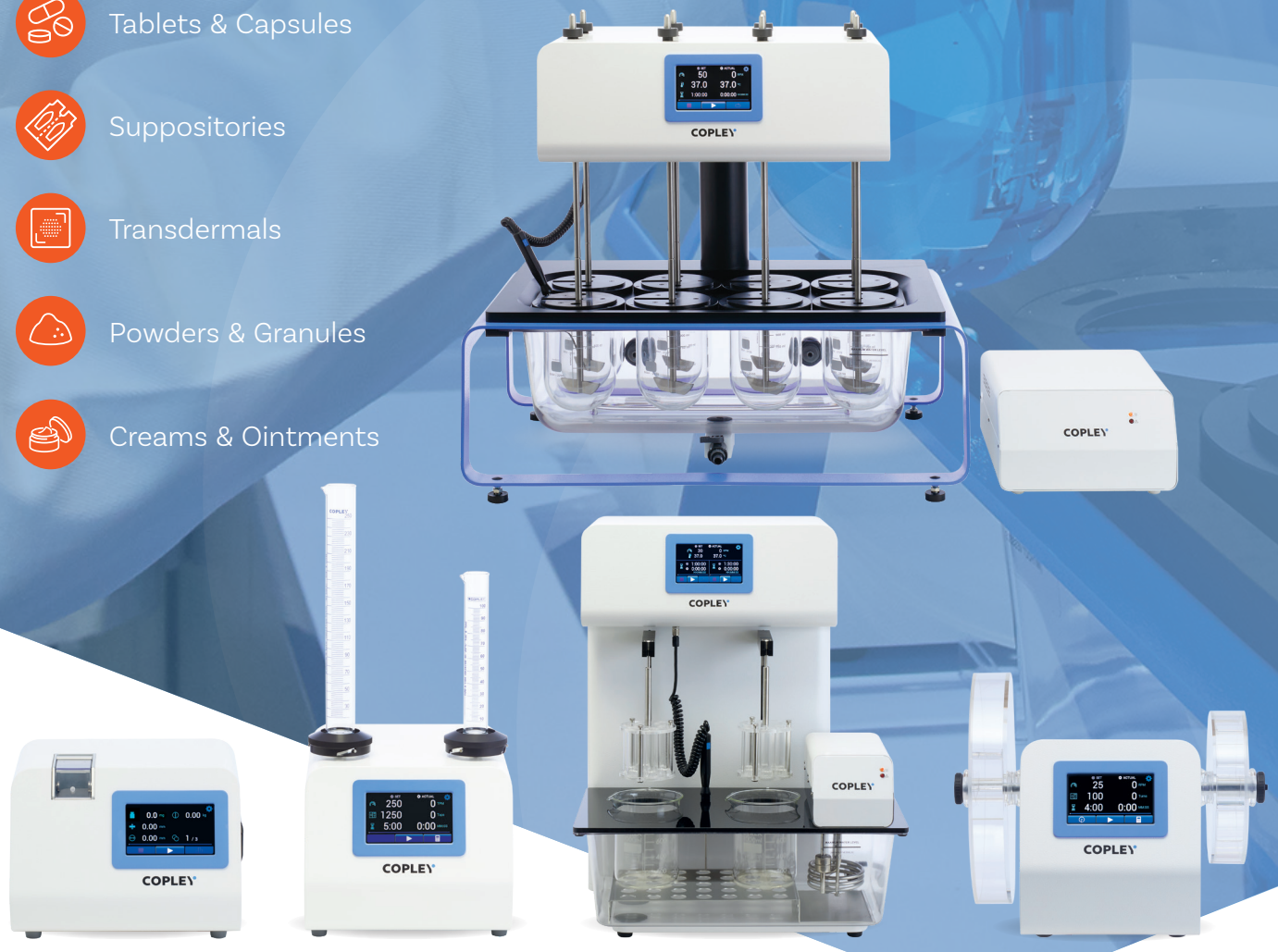
COPLEY

INNOVATIVE
COMPLIANT
TRUSTED

Precision Engineered Systems for Everyday Pharmaceutical Testing

Trusted equipment for routine testing that consistently delivers results

- Tablets & Capsules
- Suppositories
- Transdermals
- Powders & Granules
- Creams & Ointments



+44 (0)115 961 6229 copleyscientific.com sales@copleyscientific.co.uk

dx.doi.org/10.14227/DT300323P126

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

Georgia Taylor-Vine[1], Heather Mead[1], Vasiliki Paraskevopoulou[1], and James Mann[2*]
[1]*New Modalities & Parenteral Development, Pharmaceutical Technology & Development, Operations, AstraZeneca, Macclesfield, UK.*
[2]*Oral Product Development, Pharmaceutical Technology & Development, Operations, AstraZeneca, Macclesfield, UK.*

e-mail: james.mann@astrazeneca.com

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

*Corresponding author

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).

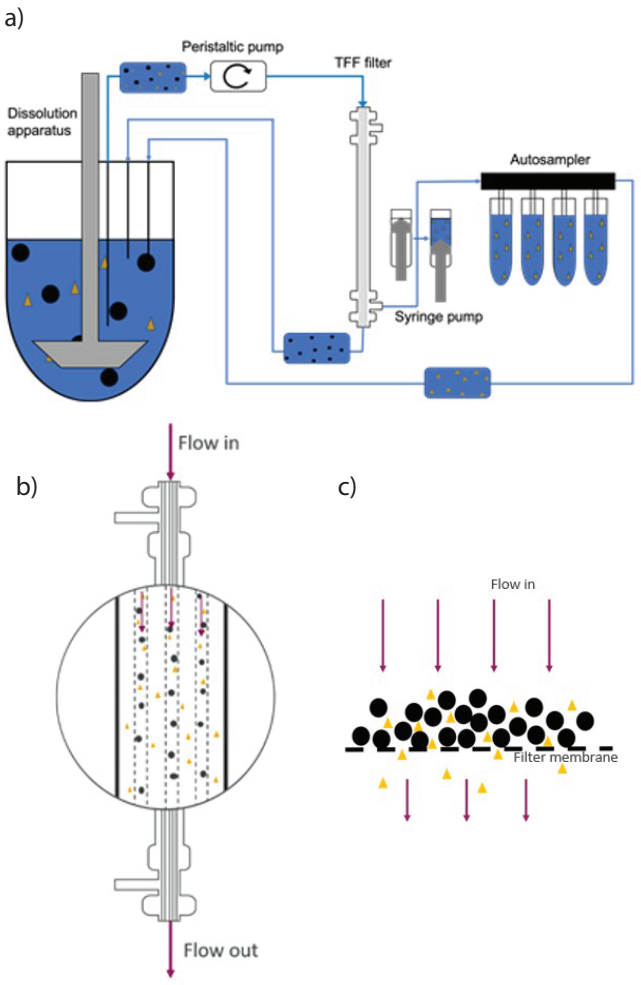


Figure 1. Diagram shows the flow of sample within NanoDis (a), with black circles representing nanoparticles and gold triangles representing the released API. Diagram of TFF filter (b) shows the separation of a nanoparticle and released API through a filter membrane compared to (c) dead end filtration.

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.

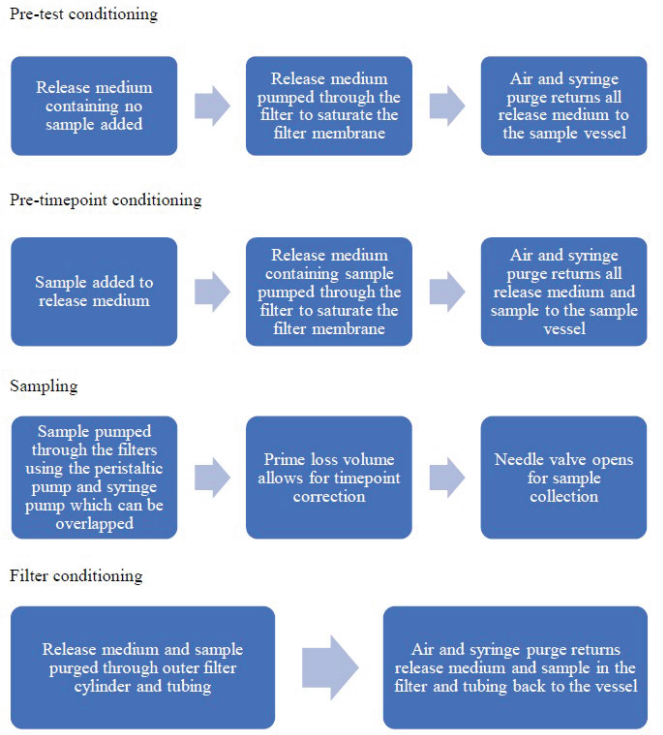


Figure 2. Flow diagram of the key stages completed within the NanoDis sampling cycle.

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.

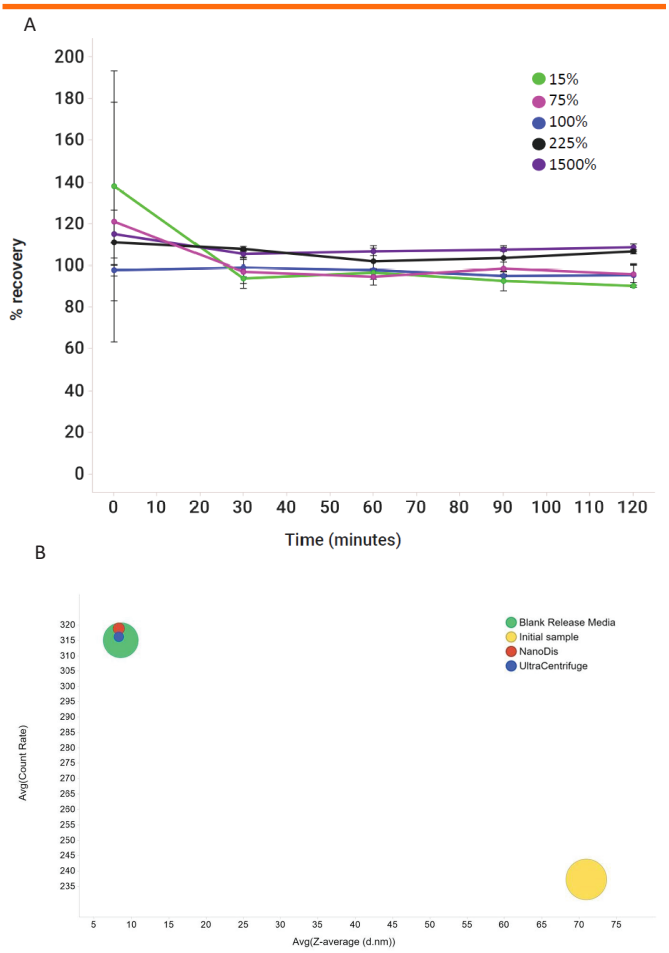


Figure 3. (A) Percent recovery of API (mean ± SD, n = 2) when the drug substance passes through the 300-kDa mPES filter when prepared at different concentrations relative to the nominal concentration of 0.02 mg/mL. (B) Particle size distribution in different samples studied through dynamic light scattering. 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.

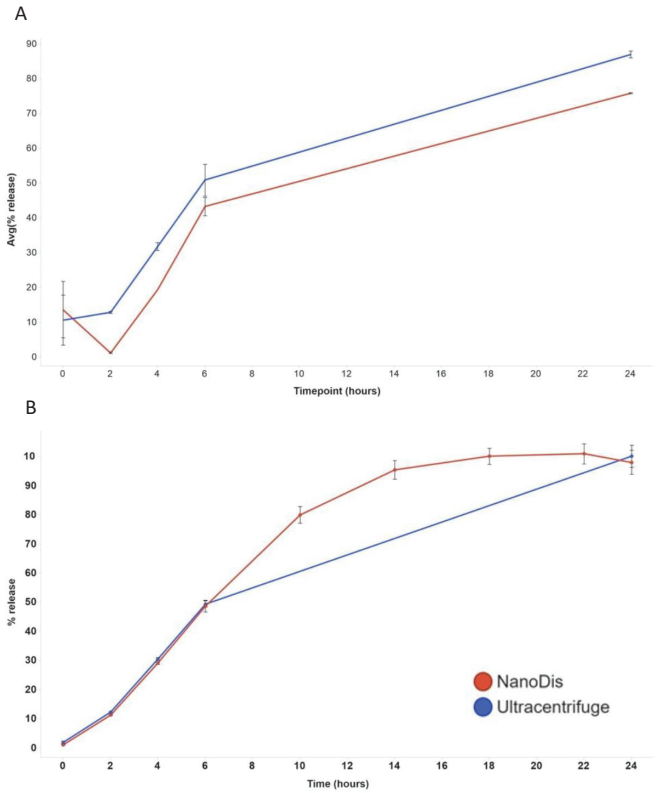


Figure 4. (A) In vitro release profiles (mean ± SD, n = 3) of the API over 24 hours for samples passed through NanoDis (red) and samples undergoing ultracentrifugation (blue). (B) In vitro release profile (mean ± SD, n = 3) of the API over 24 hours using NanoDis (red) or ultracentrifugation following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

ACKNOWLEDGEMENTS

The authors thank Agilent Technologies (Santa Clara, CA, USA) for loaning the NanoDis instrument. Special recognition should also be given to Lorraine Kay, Matt Ainge, and Karen Krauel-Göllner at Agilent Technologies for their support throughout the study.

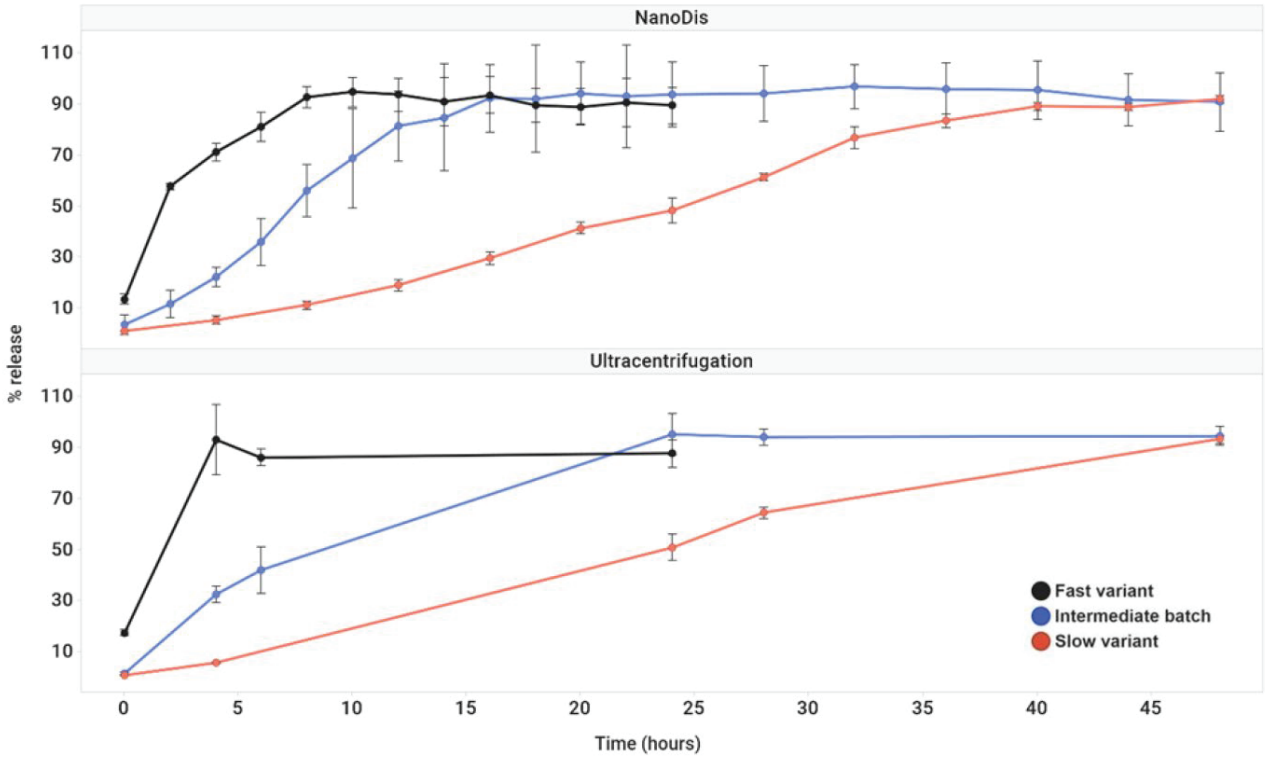


Figure 5. In vitro release profiles (mean ± SD) of the API for fast (black, n = 3), slow (red, n = 3), and intermediate release batches (blue, n = 4) using NanoDis (top) or ultracentrifugation (bottom).

CONFLICTS OF INTEREST

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

REFERENCES

1. <1153> Drug products containing nanomaterials. In USP-NF. The United States Pharmacopeial Convention, Inc., 2022. DOI: 10.31003/USPNF_M99470_02_01.
2. Krukemeyer, M.; Krenn, V.; Huebner, F.; Wagner, W.; Resch, R., History and Possible Uses of Nanomedicine Based on Nanoparticles and Nanotechnological Progress. *J. Nanomed. Nanotechnol.* **2015**, *6* (6): 1000336. DOI: 10.4172/2157-7439.1000336.
3. Yao, Y.; Zhou, Y.; Liu, L.; Xu, Y.; Chen, Q.; Wang, Y.; Wu, S.; Deng, Y.; Zhang, J.; Shao, A. Nanoparticle-based drug delivery in cancer therapy and its role in overcoming drug resistance. *Front. Mol. Biosci*. **2020**, *7*, 193. DOI: 10.3389/fmolb.2020.00193.
4. Schoenmaker, L.; Witzigmann, D.; Kulkarni, J. A.; Verbeke, R.; Kersten, G.; Jiskoot, W.; Crommelin, D. J. A. mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability. *Int. J. Pharm*. **2021**, *601*, 120586. DOI: 10.1016/j.ijpharm.2021.120586.
5. Verbeke, R.; Lentacker, I.; De Smedt, S. C.; Dewitte, H. The dawn of mRNA vaccines: The COVID-19 case. *J. Control. Release* **2021**, *333*, 511–520. DOI: 10.1016/j.jconrel.2021.03.043.
6. Maeda, H.; Bharate, G. Y.; Daruwalla, J. Polymeric drugs for efficient tumor-targeted drug delivery based on EPR-effect. *Eur. J. Pharm. Biopharm*. **2009**, *71* (3), 409–419. DOI: 10.1016/j.ejpb.2008.11.010.
7. Anselmo, A. C.; Mitragotri, S. Nanoparticles in the clinic: An update. *Bioeng. Transl. Med*. **2019**, *4* (3), e10143. DOI: 10.1002/btm2.10143.
8. Wang, M.; Thanou, M. Targeting nanoparticles to cancer. *Pharmacol. Res*. **2010**, *62* (2), 90–99. DOI: 10.1016/j.phrs.2010.03.005.
9. López-Dávila, V.; Seifalian, A. M.; Loizidou, M. Organic nanocarriers for cancer drug delivery. *Curr. Opin. Pharmacol*. **2012**, *12* (4), 414–419. DOI: 10.1016/j.coph.2012.02.011.
10. Gray, V.; Cady, S.; Curran, D.; DeMuth, J.; Eradiri, O.; Hussain, M.; Krämer, J.; Shabushnig, J.; Stippler, E. In vitro release test methods for drug formulations for parenteral applications. *Dissolut. Technol*. **2018**, *25* (4), 8–13. DOI: 10.14227/DT250418P8.
11. <1001> In vitro release test methods for parenteral drug preparations In *USP-NF*. The United States Pharmacopeial Convention, Inc., 2022. DOI: 10.31003/USPNF_M99470_02_01.
12. Wacker, M. G.; Lu, X.; Burke, M.; Nir; Fahmy, R. Nir, I.; Fahmy, R., Testing the in-vitro product performance of nanomaterial-based drug products: view of the USP Expert Panel. *Dissolut. Technol*. **2022**, *29* (1), 6–20. DOI: 10.14227/DT290122P6.
13. Modi, S.; Anderson, B. D. Determination of drug release kinetics from nanoparticles: overcoming pitfalls of the dynamic dialysis method. *Mol. Pharm*. **2013**, *10* (8), 3076–3089. DOI: 10.1021/mp400154a.
14. D'Souza, S. S.; DeLuca, P. P. Methods to assess in vitro drug release from injectable polymeric particulate systems. *Pharm.*

Res. **2006**, *23* (3), 460–474. DOI: 10.1007/s11095-005-9397-8.
15. Schwartz, L.; Seely, K. *Introduction to Tangential Flow Filtration for Laboratory and Process Development Applications*; Pall Corp; 2014; pp 1–10.
16. Patel, D.; Zhang, Y.; Dong, Y.; Qu, H.; Kozak, D.; Ashraf, M.; Xu, X. Adaptive perfusion: An in vitro release test (IVRT) for complex drug products. *J. Control. Release* **2021**, *333*, 65–75. DOI: 10.1016/j.jconrel.2021.03.024.
17. Lombardo, S. M.; Günday Türeli, N.; Koch, M.; Schneider, M.; Türeli, A. E. Reliable release testing for nanoparticles with the NanoDis System, an innovative sample and separate technique. *Int. J. Pharm*. **2021**, *609*, 121215. DOI: 10.1016/j.ijpharm.2021.121215.
18. Wren, S.; Collins, L.; Hughes, L. P.; Jones, I. Measuring the Rate of In-vitro Drug Release From Polymeric Nanoparticles by 19F Solution State NMR Spectroscopy. *J. Pharm. Sci*. **2021**, *110* (11), 3546–3549. DOI: 10.1016/j.xphs.2021.08.011.
19. Ashton, S.; Song, Y. H.; Nolan, J.; Cadogan, E.; Murray, J.; Odedra, R.; Foster, J.; Hall, P. A.; Low, S.; Taylor, P.; Ellston, R.; Polanska, U. M.; Wilson, J.; Howes, C.; Smith, A.; Goodwin, R. J. A.; Swales, J. G.; Strittmatter, N.; Takáts, Z.; Nilsson, A.; Andren, P.; Trueman, D.; Walker, M.; Reimer, C. L.; Troiano, G.; Parsons, D.; De Witt, D.; Ashford, M.; Hrkach, J.; Zale, S.; Jewsbury, P. J.; Barry, S. T. Aurora kinase inhibitor nanoparticles target tumors with favorable therapeutic index in vivo. *Sci. Transl. Med*. **2016**, *8* (325), 325ra17. DOI: 10.1126/scitranslmed.aad2355.
20. Tureli, E.; Krauel-Gollner, K. Agilent NanoDis System Method Development Guide; USA, 2020.
21. *Drug Products, Including Biological Products, that Contain Nanomaterials*; Guidance for Industry; U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), 2022.

Simply smart – make your everyday work easier

Proven Dissolution Test Instruments – made with precision and passion –
for everyone who expects precise results and smart handling

Made in Germany

https://riggtek.de

DissoPrep

Dissolution Media Preparation Station

- USP compliant high precision working principle
- High degassing efficiency
- Possibility of using foaming media
- Browser-Interface

Dissilio

Dissolution Tester

- 6 to 16 vessel positions available
- Vessels from other manufacturers can be used for easier cross-validation
- Integrated compartments for secure storage of accessories
- Browser-Interface

Sampilio

Dissolution Autosampler

- Patented small-volume diaphragm pumps for FIFO-sampling compareable to manual sampling
- Lowest cross-contamination and sample times down to 2 min
- Easy connection to Dissolution Testers
- Browser-Interface

Browser-Interface – easy use and live monitoring via Web-Browser on your PC without software installation and time wasting qualification

Many simply smart features that make your everyday work easier

Compliant to common pharmacopeias

ISO 9001:2015 certified for highest quality standards

✉ sales@riggtek.de

RIGGTEK
Dissolution Test Systems

dx.doi.org/10.14227/DT300323P134

Factors Influencing the Selection of Medium for Evaluating Drug Solubility and Dissolution in Bovine Milk

Marilyn N. Martinez[1†*], David Longstaff[1†], Vivek Fellner[2], and Martin Coffey[3]
[1]*US Food and Drug Administration Center for Veterinary Medicine, Office of New Animal Drug Evaluation, Rockville, MD, USA.*
[2]*Animal Science, Nutrition and Biotechnology, North Carolina State University, Raleigh, NC, USA.*
[3]*Research and Development, Bausch and Lomb, Rochester, NY, USA.*
[†]*This work reflects the opinions of the authors and does not reflect or represent the view of the US FDA, Center for Veterinary Medicine.*

e-mail: marilyn.martinez@fda.hhs.gov

ABSTRACT

Milk or milk-containing beverages can be used as vehicles for drug product administration and as a component of human fed-state simulated gastric fluids. Unprocessed bovine milk is also the matrix within which drugs must be solubilized or released when formulations are administered into the bovine mammary gland. Therefore, an appreciation of factors impacting the effect of milk on drug solubility and product dissolution is necessary. Although an off-the-shelf container of cow milk may be adequate for evaluating drug solubility, the composition of milk varies as a function of fat content (e.g., differences in fat content). Differences can occur between bovine breeds, diet, environment, and suppliers. Importantly, it is unclear how to quantify differences in drug solubility across types of milk products versus when the drug is infused directly into the bovine udder. To address these concerns, a two-tiered approach was employed. The first tier involved comparing drug solubility across a range of milk products, including raw (unprocessed) bovine milk obtained from healthy cattle and an aqueous buffer. The second tier, which is the subject of this review, explores publicly available information on the composition of bovine milk and the potential variability of its constituents. The goal of this work is to provide the basis for inclusion of milk as one of the biologically relevant media described in the *United States Pharmacopeia* general chapter on solubility testing.

KEYWORDS: Solubility, complex matrices, bovine milk, bioavailability, dissolution

INTRODUCTION

The *United States Pharmacopeia* (*USP*) general chapter <1236> Solubility Measurements describes the measurement of drug solubility across a range of biorelevant media relevant both to human and veterinary medicine. (*1*). Recently, the issue of cow milk as a medium for testing drug solubility and for evaluating product dissolution has been raised as a point of interest for several reasons (*2*–*8*).

- **Veterinary drug delivery**: When administered into the bovine mammary gland, the drug acts within the udder (typically, minimal systemic absorption) for the purpose of treating bovine mastitis. Bovine mastitis is a major health problem encountered within the US and around the world (*2*). Its importance is reflected in the incidence of clinical and subclinical mastitis within the US: approximately 20–25 cases per 100 cows per year. Clinical mastitis occurs in all dairy herds, even those that are well-managed (*3*). Therefore, there is a tremendous need for safe and effective antimicrobials for treating bovine mastitis.

- **Human drug delivery**: Cow milk is a potential vehicle for delivering drugs to pediatric and geriatric patients (*4*–*6*). Therefore, the issue of drug solubility in milk, or its adsorption to milk proteins and fats, is relevant for both human and animal health. This led the USP to initiate an effort to define the composition of milk in normal and mastitic cattle and the variability that may exist across cow nutritional and health status.

- Milk has been suggested as a component of fed-state simulated gastric fluids (SGFs) (*7*).

[*]*Corresponding author*

- **Pharmacokinetic considerations**: The partitioning and solubilization of environmental contaminants in milk is an important consideration from the perspective of their presence and persistence in milk ingested by human consumers (*8*).

Whether evaluating drug solubility or product dissolution, the challenges associated with evaluations conducted in milk are due to the variability and highly complex nature of this medium. It contains more than 20 proteins along with fats, and there is the potential for preferential binding to casein milk proteins, whey, or fat (*9*). For example, looking at three hydrophobic drug molecules (flunixin, meloxicam [weak acids charged at the pH of milk], and thiabendazole [weak base that is unionized in milk]) confirmed that compound hydrophobicity alone could not explain the disparities in drug solubilization. Rather, solubilization appeared to relate to whether the drug would bind preferentially to either casein (meloxicam and thiabendazole), whey (flunixin), or milk fat (*10*) (Table 1).

Table 1. In Vitro Drug Association with Components of Raw Whole Milk (10)[a]

Drug	Log P	pKa	Log D (pH 6.8) (native pH of milk)	In vitro (measured in raw whole milk)		
				Casein	Whey[a]	Fats
Meloxicam	3.43	4.08 (acidic)	0.71	66%[b]	21%	11%
Flunixin	4.0	5.82 (acidic)	3.08	29%	54%	13%
Thiabendazole	2.92	4.64 (basic)	2.92	50%	29%	18%

[a]Note that the study did not distinguish between drug concentration in the aqueous versus whey components of the milk serum. Therefore, no definitive statement can be made regarding the proportion of drug in serum that is associated with whey proteins.
[b]Fraction of active pharmaceutical ingredients (API) concentration in the specific component tested versus the total concentration present in milk.

With the intent of exploring milk as a drug delivery vehicle for humans, Macheras et al. evaluated the solubility of nine drugs representing a range of aqueous solubilities and extent of binding to milk proteins (equilibrium dialysis with a molecular cutoff of 5000) (*11*). Drug solubility was markedly higher in milk than in buffer (pH 6.5) at all temperatures, and the extent of protein binding tended to correlate with drug lipophilicity. For most drugs, this binding tended to be higher at 15 °C vs 37 °C (especially in the low milk-fat samples). While the magnitude of protein binding was similar in 0.75% vs 3.5% fat content, for most drugs studied by these authors (exception being dicumarol and nitrofurantoin), the observed solubility in whole milk tended to be greater than that in skim milk. These results were interpreted to imply that drug binding to milk proteins is only one of the reasons for higher drug solubility in milk and that the other aqueous phase components may have an important influence on the solubilization of active pharmaceutical ingredients (APIs).

Milk has been used to simulate biorelevant SGFs. For example, the in vitro dissolution of acetaminophen (Biopharmaceutics Classification System [BCS] class I compound) and BCS II compounds danazol and mefenamic acid were previously studied in milk. The 3.5% fat bovine milk was purchased from an Austrian supplier (pH 6.5, buffer capacity of 14 mEq/L/pH). Although the various aqueous media used in the dissolution study did not influence the dissolution of acetaminophen, milk markedly slowed the tablet release rate. In contrast, as compared to that seen using aqueous buffers containing surfactants, milk markedly enhanced the dissolution rate of poorly soluble drugs such as danazol and mefenamic acid (*12*). The same group also studied in vitro dissolution of poorly soluble drugs such as troglitazone, atovaquone, sanfetrinem cilexetil, and an experimental drug (GV150013X) in whole milk (3.5% fat) versus traditional aqueous buffers (USP 23 fasted-state simulated intestinal fluid [FaSSIF] with pancreatin (*13*). In some cases, faster and more complete dissolution was observed in fed-state simulated small intestinal fluid (FeSSIF) versus milk (troglitazone and GV150013X). Conversely, milk appeared to provide a more formulation-dependent dissolution profile for sanfetrinem cilexetil and a markedly faster dissolution of atovaquone as compared to that seen with the other media, including FeSSIF. However, the milk used in these studies cannot be considered standardized media, as would be the case for the other aqueous buffers. The authors raised the issue of potential batch-to batch variability in milk and its potential effects on in vitro dissolution study results (*13*).

Given the drug-specific influence of the various milk constituents and large variability that can occur in milk, depending on the source or commercial processing, there is a need to establish some level of standardization in the milk used for solubility and dissolution testing. To that end, the USP <1236> provides an overview of the concepts and equations relevant to solubility measurements, including a description of experimental methods for assessing drug solubility and species-specific biorelevant media for generating the drug solubility assessments (*1*). To date, the media and methods described in <1236> have pertained to conditions associated with the gastrointestinal (GI) tract. The formulations provided include biorelevant media for humans, dogs, and cattle,

with the aim of expanding to include other veterinary species (e.g., poultry, cats, swine, and horses).

With these points in mind, it is important to define the protein, lipid, and aqueous composition of milk when trying to understand the factors that can influence drug solubility assessment or in vitro dissolution characteristics in this medium. Ultimately, the question is whether the complexity of bovine milk will preclude establishing a particular "recipe" for drug solubility testing, and, if so, what alternative can be used to provide a standardized-like medium. Doing so will impact assessments of drug product performance when administered in milk to human patients, the evaluation of the solubility of drugs intended for bovine intramammary infusion, and the inclusion of milk as a component of fed-state SGF.

The aim of this work is to review milk composition in cows and humans with a goal of expanding *USP* <1236> to include a proposal for a "standardized" bovine milk medium.

The composition of bovine milk was studied from a range of publicly available sources, including published articles and government publications.

Proteins

Cow milk contains more than 20 proteins, the main ones being casein (about 80% of milk proteins) and whey (about 20% of milk proteins). Casein is fractionated into α_{s1}, α_{s2}, β, and κ-casein. The proportion of the various caseins in bovine milk can differ across dairy breeds. However, they all are amphiphilic and present in several conformations when in solution. Their amphiphilic nature renders them relatively insensitive to denaturation. Unlike whey proteins, caseins are insoluble in aqueous media and therefore form micelles. They are characterized by a high capacity for binding phosphorus and calcium. Casein micelles typically have an open structure with serum-filled cavities accessible to small molecules, but the micelle structure itself exhibits pH-dependent behavior. It becomes more compact as the pH drops and swells (becoming less compact) with an increase in pH. Therefore, caseins are being explored as a potential candidate for controlled-release drug delivery (*14*).

Although fat-soluble compounds appearing in milk are believed to associate with the fat fraction, it has been hypothesized that the open structure of native casein micelles provides a better environment for the binding and transport of lipophilic substances. To explore the influence of casein on drug adsorption, Cheema et al. examined three hydrophobic APIs (meloxicam, flunixin, and thiabendazole) (*10*). Interestingly, the outcome showed differences between when the drug enters milk via secretion from plasma (i.e., administered to dairy cattle as per the approved product label) versus when the drug is introduced in vitro by addition to milk samples maintained at room temperature (~25 °C). Although in vivo and in vitro binding to casein was similar for flunixin, OH-flunixin, and OH-thiabendazole (the parent thiabendazole molecule could not be quantified in the in vivo milk samples), statistically significant in vivo/in vitro differences were observed with meloxicam. More than twice the percentage of casein-associated meloxicam was observed in vitro (61% of the amount added to the whole raw milk) vs in vivo (31% of the recovered drug). Conversely, twice the percentage was associated with whey protein in the in vivo versus the in vitro samples (21% in vitro vs 52% in vivo).

In contrast to the insoluble caseins, the major whey proteins are water soluble (*14*). Whey is predominantly made up of proteins β-lactoglobulin (β-LG), which comprises about 50% (g/L relative to total whey proteins), α-lactalbumin (α-LA), which comprises about 26% (g/L relative to total whey proteins), bovine serum albumin (BSA) 8% (g/L relative to total whey proteins), immunoglobulins A, M and C (total = 14% g/L relative to total whey proteins), lactoferrin 2% (g/L relative to total whey proteins), and lactoperoxidase 0.6% (g/L relative to total whey proteins). The primary three proteins in whey are considered to be the two lactoglobulins and BSA.

The challenge facing efforts to define the whey fraction is that this complex mixture is difficult to standardize. Multiple variants of β-LG exist, with the A and B variants being the most common (*15*). The relative amounts of these all-whey proteins can vary per breed and diet, and the stability of the major proteins is variable. There is also a noted difference in the whey protein composition between milk produced by healthy cows and that produced by mastitic cows, with mastitic milk having an increase in albumin and serotransferrin and a decrease in β-LA and α-LA (*16*).

Whey protein isolate (WPI) is a commercially available protein raw material that could be used to represent this protein component in a standardized milk formulation. However, modern milk processing techniques such as ultra-high temperature (UHT) treatment have been demonstrated to reduce the amount of β-LG and alter the tertiary structure of whey protein (*17*). Depending on the methods used to isolate the WPI, there could be problems with assuming that a WPI is representative of

the whey composition in raw milk. Furthermore, other than BSA, none of the other ingredients are available as USP-grade material. The proteins β-LG, α-LA, and BSA share an ability to interact and bind to the milk fatty acids, and the binding affinity of BSA to some fatty acids tends to exceed that of β-LG (*18*). Whether the physicochemical differences seen across the three primary whey proteins will impact drug solubilization and binding has not been adequately evaluated.

The United States Department of Agriculture (USDA) data for whole milk indicates that the total protein content of milk is 3.15% w/w (*19*). Given that the composition of milk proteins is 80% casein and 20% whey, it is proposed that a standardized milk formulation would be targeted to contain 2.52% technical-grade casein and 0.63% BSA. The problem is that there are many factors (breed, diet, stage of lactation, seasonal variation, ruminal fermentation) that can potentially influence milk composition (*20*). This raises the question of whether such variations may affect solubility test results, and if so, how to adjust test conditions to accommodate these variations.

When caseins are isolated from milk, they are typically acidified during the isolation process. As a result, the use of caseins typically requires the addition of a base to adjust the pH back to neutral to swell and rehydrate the caseins. In contrast, sodium caseinate is a readily available casein material that has already been neutralized with sodium hydroxide to convert to sodium salt. Although either form of casein could be used in a standardized milk formulation, the use of sodium caseinate would minimize the need to adjust the pH of the formulation. Again, there are uncertainties that arise when striving to develop some standardized milk medium.

Milk Fat

Milk fat contains approximately 400 different fatty acids. Its relative proportion to the total milk constituents is 3.3–4.4%, depending upon breed, stage of lactation, diet, presence of mastitis, and the ruminal flora (*21*, *22*). Trace fatty acids will likely have little influence on drug solubility owing to the small amount present in milk.

The fat is present in milk as an oil-in-water emulsion formed by the endoplasmic reticulum in the epithelial cells of the mammary gland. When secreted, they are enveloped with the plasma membrane of the epithelial cell. Therefore, membrane-associated materials comprise approximately 2–6% of the globule mass. The milk fat globule membranes contain about 70% membrane proteins, 25% phospholipids, 3% cerebrosides, and 2% cholesterol (all units based upon w/w). This is in contrast with the composition of the milk fat itself, which contains about 98% triglycerides and 2% diacylglycerol. The amount of cholesterol is less than 0.5%, about 0.5–1% phospholipids and 0.1% free fatty acids (*23*).

The remainder (~0.2%) consists of trace amounts of ether lipids, hydrocarbons, fat-soluble vitamins, and other constituents that may be secreted into the milk from the feed. Although cholesterol is a minor component in milk fat membranes, the phospholipid content (25% w/w) may be important for formation of the fat globule. The number of milk fat globules (MFG) is 10^{10} per mL of milk, with a total area of 700 cm^2 per mL of milk, with that total area estimate being a function of the MFG fat content (*21*). Although diacylglycerol, which is a component of both triglyceride biosynthesis and lipolysis, can constitute up to 2% of the lipid portion, diacylglycerol is typically part of the milk fat globule membrane and therefore not anticipated to directly affect the solubility of drugs in milk (*21*).

For a comparison of documented fatty acid composition in cow milk, four data sources from 2007–2020 were used: Månsson (Swedish cows), Zou et al. (Danish cows), and two USDA data sources including Haug et al (*19*, *21*, *24*, *25*) (Table 2). Note that values are reported as %w/w by Månsson, Zou et al., and in the 2007 USDA database (*19*, *21*, *24*), whereas %w/v values are reported by Haug et al (*25*). To facilitate comparison, the 2007 USDA data were converted to %w/w using a specific gravity of 1.030 g/cm^3. The numbers from the Månsson and Zou et al. were calculated to indicate the %w/w in milk using a total fat content of 3.25% per the 2020 USDA data (i.e., the standardization of values to w/w is based on the USDA description of 3.25% milk fat content) (*19*).

As can be seen in Table 2, the data gleaned from the various sources confirms the variability of milk lipid composition. This is not surprising when considering that milk fatty acids are derived both from feed and ruminal microbial activity. Variations may be attributed to breed, diet, stage of lactation, mastitis, and ruminal flora (*22*). According to Table 2, total fatty acids are lower than that described by the USDA (2.9% vs 3.25%). This shortfall is due to the elimination of various trace fatty acids.

Aqueous Phase

Linzell provides a high-level overview of the composition of the aqueous phase of milk (*26*). The concentrations of ions more closely resemble that of the cell than it does plasma, including K+, Ca++, Mg++, citrate--^{5}, and HPO_4--, and low concentrations of Na+, Cl-, and HCO_3. Lactose is the main osmole in milk at around 5% w/w, and

Table 2. Estimates of Milk Fatty Acid Content

	USDA (*19*)	Månsson (*21*)	Zou et al. (*24*)	Haug et al. (*25*)
	% w/w	% w/w	% w/w	% w/w*
Saturated Fatty Acids				
4:0 Butyric acid	0.075	0.143	0.135	-
6:0 Caproic acid	0.075	0.078	0.101	-
8:0 Caprylic acid	0.075	0.046	0.076	-
10:0 Capric acid	0.075	0.088	0.159	-
12:0 Lauric acid	0.077	0.107	0.212	0.077
14:0 Myristic acid	0.297	0.354	0.675	0.291
16:0 Palmitic acid	0.829	0.995	1.041	0.777
18:0 Stearic acid	0.365	0.397	0.133	-
Monounsaturated Fatty Acids, cis				
16:1 Palmitoleic acid	-	0.033	-	-
18:1 Oleic acid	0.812	0.741	0.429	0.777
Polyunsaturated Fatty Acids				
18:2 Linoleic acid	0.12	0.052	0.067	0.117
18:3 alpha linoleic acid	0.075	-	-	0.073
Trans Fatty Acids				
18.1t Vaccenic acid	-	0.068	0.036	-

**Values converted from %w/v to %w/w by assuming a density of 1.03 g/cm^3.*

once secreted cannot ordinarily pass back through the secretory or duct epithelia.

Similar findings were published by Gaucheron (*27*). The mineral fraction, which is a small fraction of milk (about 8–9 g × L^{-1}), contains cations (calcium, magnesium, sodium, and potassium) and anions (inorganic phosphate, citrate, and chloride). In milk, these ions play an important role in the structure and stability of casein micelles (*27*). The mineral content undergoes some variation as a function of the lactation phase. For most ions, fluctuations amount to no more than 20%, but relatively larger differences (40–50%) can occur in terms of sodium, potassium, chloride, and soluble calcium. Nevertheless, given the relatively small fraction of ions versus total bulk volume of milk, for the purpose of assessing the rate and extent of drug solubility in milk, it should be adequate for defining ionic composition in terms of what has been reported in bulk skim milk (Table 3). For the sake of completeness, Table 3 is based on information from Gaucheron in terms of what is found in subclinical mastitis (*27*).

In milk, all macro-elements are distributed differently into diffusible and non-diffusible fractions (essentially casein micelles). Potassium, sodium, and chloride ions are essentially diffusible although calcium, and inorganic phosphate and magnesium are partly bound to the casein micelles. About one-third of calcium, half the inorganic phosphate, two-thirds of magnesium, and over 90% of citrate are in the aqueous phase of milk. A small proportion of calcium is also bound to α-LA (there is one atom of calcium per protein). There is little to no binding of these elements to either lactose or fat. Furthermore, Gaucheron lists nine forms of calcium and magnesium salts that can be seen in a typical milk ultrafiltrate while noting that 1) the addition of one ion can impact the number of other ions in the diffusible phase; and 2) the addition of NaCl to milk leads to a slight decrease in pH and increases in Ca^{2+} concentrations in the diffusible phase.

The majority of calcium and magnesium appear to be in the form of calcium citrate and magnesium citrate. Phosphate and chloride are in the form of sodium and potassium salts, which can be well represented by a mixture of sodium phosphate and potassium chloride (*26*). The only significant differences between the soluble and total ions are for calcium, magnesium, and phosphate and can be represented by addition of these insoluble salts. A buffer composition that approximates these concentrations is shown in Table 4.

DISCUSSION

Optimally, a medium would be standardized to allow for reproducible assessments of in vitro dissolution and drug solubility. However, given the diversity of milk

Table 3. Milk Mineral Composition (mmol/L) and pH

	Healthy (Bulk)	Subclinical Mastitis	Aqueous Buffer
Total Calcium	30.1	29.4	-
Total Calcium	9.5	9.1	9.5
Total Magnesium	5.1	4.9	-
Soluble Magnesium	3.3	3.2	3.3
Total Inorganic Phosphate	20.9	19	-
Soluble Inorganic Phosphate	11.2	9.2	11.25
Total Citrate	9.8	8.8	-
Soluble Citrate	9.2	8.3	9.2
Total Sodium	25.5	34.5	18.1
Total Potassium	36.8	36.1	36.1
Total Chloride	30.3	40.5	36.1
pH	6.72	6.87	6.8

Based on Gaucheron [27].

Table 4. Buffer Composition of Milk (% w/w and mmol/L)

Soluble Buffer Ingredients	w/w (%)	mmol/L
Potassium chloride	0.27%	36.1 mmol/L K, 36.1 mmol/L Cl
Calcium citrate, 4 H_2O[a]	0.18%	3.16 mmol/L = 9.5 mmol/L Ca, 6.3 mmol/L citrate
Phosphoric acid, 85%	0.130%	11.25 mmol/L Phosphate
Magnesium citrate, 9 H_2O[b]	0.067%	1.1 mmol/L = 3.3 mmol/L Mg, 2.2 mmol/L citrate
Citric acid, anhydrous	0.013%	0.7 mmol/L citrate
Sodium hydroxide, 2N (q.s. to pH 6.8)	~1.11%	~22.2 mmol/L Na
Insoluble Buffer Ingredients		
Calcium phosphate	0.23%	7.5 mmol/L = 22.5 mmol/L Ca, 15 mmol/L P
Magnesium phosphate	0.02%	0.7 mmol/L = 2.7 mmol/L Mg, 1.8 mmol/L P

Based on Gaucheron [27].
[a]Estimates based upon molecular weight values for calcium citrate tetrahydrate.
[b]Estimates based upon molecular weight values magnesium citrate tribasic nonahydrate.

composition, such a standardization is not feasible, and any effort to establish a singular definitive recipe would reflect only a single set of conditions (breed, diet, lactation stage, etc.). Moreover, the complexity of the milk matrix renders the genesis of a synthetic milk extremely challenging and subject to variation due to manufacturing procedures and difficulty in obtaining many of the ingredients.

Is Standardization Possible?

Among the many potential concerns that would need to be addressed in the development of a standardized medium is how closely the casein micellar structure mimics that which is found in cow milk. While technically, one could generate a 3D structure analysis on these proteins in the two media, the most critical issue for our purposes would be to compare the corresponding estimates of solubility of a range of compounds. Another issue is that milk processing can alter the amount of β-LA. This may impact the relative solubility drug estimates in raw milk (relevant to bovine mastitis), processed milk (relevant to the use of milk to facility drug administration to humans or to drug solubility in mastitic milk) or the proposed synthetic milk medium. This can be assessed by testing drug solubility in all three media during the initial validation of the proposed medium.

Therefore, it is likely that the best possible solution will be to obtain a commercially available milk source. However, the question remains how the various sources may compare to raw milk obtained from a lactating cow. To that end, we engaged in studies to evaluate that possibility. Our first set of collaborative studies, conducted by Dr. Fang Zhao and colleagues at St. John Fisher University, Rochester, New York, involved such an evaluation (*28*). That study involved an assessment of the solubility of a

range of APIs in buffer solution, pH 6.8, raw bovine milk, and commercially obtained skim milk, whole milk, and reconstituted dehydrated whole milk. The second set of comparisons employed the same media but focused specifically on intramammary infusion, examining the solubility of two related compounds that are approved for use in the treatment of bovine mastitis: cephapirin sodium (highly soluble) and cephapirin benzathine (low solubility) (*29*). These data suggest that an off-the-shelf milk product can be used to assess drug solubility across a range of commodities containing bovine milk and as well as for assessing/comparing the solubility of compounds intended for intramammary infusion (*28*, *29*).

Using Milk as a Dissolution Medium

Of particular interest has been the use of milk as a vehicle for drug administration when evaluating the dissolution of pediatric formulations. This may be particularly important when the medicine is formulated as granules or crushed tablets (*30*). In these situations, it has been of value to use milk as a component of the dosage form that is added to the biorelevant in vitro dissolution medium. The inclusion of milk in the sample of drug plus vehicle added to the dissolution vessel successfully reflected the higher oral bioavailability of the low solubility drug, montelukast, and provided a higher correlation with the in vivo oral bioavailability reported in fed infants as compared to in vitro dissolution testing conducted when infant (pediatric milk-based) formula or applesauce was used as the administering vehicle.

In some studies, the milk is gradually digested over time to reflect what occurs in vivo (*31*). In fact, it has been suggested that the lack of attention to the use of milk as a drug delivery vehicle may be a function of the lack of attention given to the importance of post-ingestion milk digestion on the solubility and dissolution of highly lipophilic drugs (*32*). Along those lines, to identify effective ways to administer this antiparasitic drug to infants in tropical countries, Eason et al. examined the dissolution of the poorly soluble drug, praziquantel (BCS class 2 drug) in milk or infant formula and the consequence of adding pancreatic lipase to these dissolution on drug dissolution (*33*). These results were compared to the solubility and in vitro dissolution of praziquantel in SGF and in simulated intestinal fed and fasted fluids (FeSSIF and FaSSIF, respectively). Despite a positive food effect on oral bioavailability, the solubility of praziquantel in undigested milk was slightly lower than that in either FeSSIF or in 0.1 M HCl + 2 mg/mL sodium lauryl sulfate (SLS). Drug solubility in infant formula was similar to that in the aqueous media containing SLS or bile salts (FeSSIF). However, upon digestion with pancreatic lipase, praziquantel solubility in milk and formula increased to more than 3-fold that of the aqueous media. The impact of milk digestion on drug solubility was reflected in the marked rise in the percent drug released into the dissolution medium (100% dissolved), with the profound impact of digestion occurring in milk (*33*). This work showed that the amount of bile salt needed to effectively solubilize and dissolve praziquantel is markedly greater than the bile salt concentration known to be present in infants. Accordingly, given the digestion of milk (or infant formula) as it moves down the gastrointestinal tract, milk can serve as an effective vehicle to dose this drug to infants.

When used for evaluating highly soluble drugs, the use of milk can delay drug release as compared to that seen using aqueous media (*34*). This was shown for several acetaminophen formulations where the milk medium contained 3.5% fat (without digestion). Although three acetaminophen (paracetamol) formulations (two immediate-release tablets, one uncoated and one film-coated tablet, and one suspension) exhibited rapid release in simulated gastric fluid, FaSSIF, and FeSSIF, dissolution was markedly slower in milk. Moreover, only minor differences in product dissolution rates were seen in the aqueous buffers, but substantial formulation-associated differences in dissolution rates were seen when tested in milk. In terms of blood level data, when administered to fasted dogs with 200 mL water, the two tablet formulations were found to have differences in T_{max}. However, they were equivalent when administered to dogs with 200 mL milk despite observed differences in gastric tablet disintegration rates seen in a subset of fistulated canine subjects. The authors concluded that the bioequivalence in milk was a result of the delayed gastric emptying in these dogs, which then camouflaged the effects on differences in in vivo dissolution. Importantly, it was only in milk that the formulation effects were seen when the paracetamol tablets administered to dogs with water (*34*).

The influence of gastric contents in the fed state has led to an examination of how dietary constituents may influence drug dissolution in the fed human stomach. The effect of various food constituents (e.g., casein, egg albumin, and gelatin) on the intrinsic in vitro dissolution of two BCS II drugs (itraconazole and ketoconazole) was examined in SGF media containing milk (3.6%, 1.7% and 0.1% milk fat) or SGF plus other food ingredients such as egg albumin, wheat gluten, glucose, starch, bovine gelatin, glycine, leucine, and aspartic acid (35, 36). The drug solubility

in each dissolution medium was determined using a modified shaker-flask method. The intrinsic dissolution rate (15–240 min) for ketoconazole was similar in SGF plus whole milk, SGF plus partly skimmed milk, and SGF-plus skim milk, whereas dissolution for itraconazole in part SGF-part skim milk was significantly higher than in the other milk media. A markedly lower intrinsic dissolution rate for ketoconazole occurred in the presence of SGF plus other food ingredients, but unlike ketoconazole, itraconazole solubility and dissolution was substantially higher in SGF plus high concentrations of albumin or gelatin. For these two compounds, although casein has excellent emulsifying properties, casein-containing media only slightly increased the solubility or the in vitro dissolution rate as compared to that seen with the milk-media, indicating that the effect of milk was not attributable to its casein content. The positive influence of albumin on itraconazole solubility exceeded that observed for ketoconazole (when ketoconazole is measured between 15-240 min) whereas that of gelatin was greater for ketoconazole as compared to itraconazole. The addition of amino acids had a greater effect on the solubilizing of ketoconazole than of itraconazole. Observed drug-related differences in the relationships between milk-containing media or food ingredients on solubility and in vitro dissolution appear to be related to drug lipophilicity (itraconazole greater than ketoconazole) and charge (itraconazole is mono-ionic at pH 3 while ketoconazole is positively charged at pH 3).

The interest in bovine milk as a vehicle for drug delivery (especially in pediatric and geriatric patients) and its use in predicting drug solubility and formulation-associated product dissolution in the fed stomach points to the need to: 1) understand the composition of milk; and 2) to explore the possibility of defining a way to minimize the variability in milk composition that can occur across sources of the milk. This review can facilitate inter- and intra-laboratory consistency in milk-associated study results.

CONCLUSION

Understanding the complexity of the milk medium, including the magnitude to which solubility and in vitro dissolution evaluations can differ as a function of the inherent variability in milk composition, provides valuable information with respect to the potential challenges that need to be considered when milk is the matrix within which a drug must be solubilized. Efforts to develop a single standardized milk matrix for testing drug solubility would need to address many potential sources of variability; however, this review provides the necessary information for adding milk as a matrix for testing drug solubility within *USP* <1236>. Furthermore, recent drug solubilization collaborative studies suggest that a close estimate of drug solubility can be obtained by using off-the-shelf whole milk as the surrogate matrix.

FUNDING

No specific funding was provided to support this work.

CONFLICT OF INTEREST

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

REFERENCES

1. <1236> Solubility Measurements. In *USP–NF*. United States Pharmacopeial Convention, Inc., 2022.
2. Ibrahim, N. Review on mastitis and its economic effect. *Canadian J. Sci. Res*. **2017**, *6* (1), 13–22. DOI: 10.5829/idosi.cjsr.2017.13.22.
3. Cobirka, M.; Tancin, V.; Slama, P. Epidemiology and classification of mastitis. *Animals* (*Basel*) **2020**, *10* (12), 2212. DOI: 10.3390/ani10122212.
4. Kamal, S.S.; Kaur, D.; Singh, S.; Sharma, A.; Katual, M. K.; Garg, A. K.; Kumar, R. An Investigative and explanatory review on use of milk as a broad-spectrum drug carrier for improvement of bioavailability and patient compliance. *J. Young Pharm*. **2016**, *8* (2), 72–75. DOI: 10.5530/jyp.2016.2.3.
5. Taylor, S.; Glass, B. D. Altering dosage forms for older adults. *Aust. Prescr*. **2018**, *41* (6), 191–193. DOI: 10.18773/austprescr.2018.063.
6. Charkoftaki, G.; Kytariolos, J.; Macheras, P. Novel milk-based oral formulations: proof of concept. *Int. J. Pharm*. **2010**, *390* (2), 150–159. DOI: 10.1016/j.ijpharm.2010.01.038.
7. Baxevanis, F.; Kuiper, J.; Fotaki, N. Impact of presence of excipients in drug analysis in fed-state gastric biorelevant media. *Eur. J. Pharm. Biopharm*. **2018**, *131* (Oct), 178–188. DOI: 10.1016/j.ejpb.2018.08.004.
8. Shelver, W. L.; Lupton, S. J; Shappell, N. W.; Hakk, H.; Smith, D. J. The distribution of environmental contaminants and pharmaceuticals among skim milk, milk fat, curd, whey and milk protein fractions through milk processing. Presented at ARS-FSIS 2019 meeting. U.S. Department of Agriculture; National Agricultural Library. https://www.nal.usda.gov/sites/www.nal.usda.gov/files/fsrio-meet-the-experts/02-ars-fsis2019-shelver-residuedetection508gk_na_rr_final.pdf (accessed September 21, 2022).
9. El-Agamy, D. I. The challenge of cow milk protein allergy. *Small Rumin. Res*. **2007**, *68* (1-2), 64–72. DOI: 10.1016/j.smallrumres.2006.09.016.
10. Cheema, M.; Hristov, A. N.; Harte, F. M. The binding of orally dosed hydrophobic active pharmaceutical ingredients to casein micelles in milk. *J. Dairy Sci*., **2017**, *100* (11), 8670–8679. DOI: 10.3168/jds.2017-12631.
11. Macheras, P. E.; Koupparis, M. A.; Antimisiaris, S. G. Drug binding

and solubility in milk. *Pharm. Res*. **1990**, *7*, 537–541. DOI: 10.1023/a:1015881103340.

12. Galia.; Nicolaides, E.; Hörter, D.; Löbenberg, R.; Reppas, C.; Dressman, J. B. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs. *Pharm. Res*., **1998**, *15* (5), 698–705. DOI: 10.1023/a:1011910801212.
13. Nicolaides, E.; Galia, E.; Efthymiopoulos, C.; Dressman, J. B.; Reppas, C. Forecasting the in vivo performance of four low solubility drugs from their in vitro dissolution data. *Pharm. Res*., **1999**, *16* (12), 1876–1882. DOI: 10.1023/a:1018959511323.
14. Madureira, A. R.; Pereira, C. I.; Gomes, A. M. P.; Pintado, M. E.; Malcata, F. X. Bovine whey proteins – overview on their main biological properties. *Food Res. Int*. **2007**, *40* (10), 1197–1211. DOI: 10.1016/j.foodres.2007.07.005.
15. Godovac-Zimmermann, J.; Krause, I.; Baranyi, M.; Fischer-Frühholz, S.; Juszczak, J.; Erhardt, G.; Buchberger, J.; Klostermeyer, H. Isolation and rapid sequence characterization of two novel bovine beta-lactoglobulins I and J. *J. Protein Chem*. **1996**, *15* (8), 743–750. DOI: 10.1007/BF01887148.
16. Hogarth, C. J.; Fitzpatrick, J. L.; Nolan, A. M.; Young F. J.; Pitt, A.; Eckersall, P. D. Differential protein composition of bovine whey: A comparison of whey from healthy animals and from those with clinical mastitis. *Proteomics* **2004**, *4* (7), 2094–2100. DOI: 10.1002/pmic.200300723.
17. Qi, P. X.; Ren, D.; Xiao, Y.; Tomasula, P. M. Effect of homogenization and pasteurization on the structure and stability of whey protein in milk. *J. Dairy Sci*., **2015**; *98* (5), 2884–2897. DOI: 10.3168/jds.2014-8920.
18. Le Maux, S.; Bouhallab, S.; Giblin, L.; Brodkorb, A.; Croguennec, T. Bovine β-lactoglobulin/fatty acid complexes: binding, structural, and biological properties. *Dairy Sci. Technol*., **2014**, *94* (5), 409–426. DOI: 10.1007/s13594-014-0160-y.
19. FoodData Central. U.S. Department of Agriculture; Agricultural Research Service. Published Deceember 16, 2019. https://fdc.nal.usda.gov/fdc-app.html#/food-details/746782/nutrients (accessed September 21, 2022).
20. Linn, J. G. Factors affecting the composition of milk from dairy cows. In *Designing Foods: Animal Product Options in the Marketplace*. National Research Council (US) Committee on Technological Options to Improve the Nutritional Attributes of Animal Products; National Academies Press (US), 1988; pp 224-241.
21. Månsson, H.L. Fatty acids in bovine milk fat. *Food Nutr. Res*., **2008**, *52* (52), 1–2. DOI: 10.3402/fnr.v52i0.1821.
22. Markiewicz-Keszycka, M.; Czyzak-Runowska, G.; Lipinska, P.; Wojtowski, J. Fatty acid profile of milk: a review. *Bull. Vet. Inst. Pulawy*. **2013**, *57* (2), 135–139. DOI: 10.2478/bvip-2013-0026.
23. Jensen, R. G.; Ferris, A. M.; Lammi-Keefe, C. J. The composition of milk fat. *J. Dairy Sci*. **1991**,*74* (9), 3228–3243. DOI: 10.3168/jds.S0022-0302(91)78509-3.
24. Zou, X.; Huang, J.; Jin, Q.; Guo, Z.; Liu, Y.; Cheong, L.; Xu, X.; Wang, X. Lipid composition analysis of milk fats from different mammalian species: potential for use as human milk fat substitutes. *J. Agric. Food Chem*. **2013**, *61* (29), 7070–7080. DOI: 10.1021/jf401452y.
25. Haug, A.; Høstmark, A. T.; Harstad, O. M. Bovine milk in human nutrition – a review. *Lipids Health Dis*. **2007**. *6* (1), 25. DOI: 10.1186/1476-511X-6-25.
26. Linzell J. L. Mechanism of secretion of aqueous phase of milk. *Dairy Res*. **1968**, *55* (9), 1316–1322. DOI: 10.3168/jds.S0022-0302(72)85670-4.
27. Gaucheron, F. The minerals of milk. *Reprod. Nutr. Dev*. **2005**, *45* (4), 473–483. DOI: 10.1051/rnd:2005030.
28. Li, S.; Gabriel, J. J.; Martinez, M. N.; Longstaff, D. G.; Coffey, M. J.; Zhao, F. An exploratory study of a simple approach for evaluating drug solubility in milk related vehicles [preprint]. bioRxiv. https://biorxiv.org/cgi/content/short/2022.10.27.514033v1
29. Martinez, M. N.; Zhao, F.; Longstaff, D. G.; Gabriel, J. J.; Coffey, M. J. Evaluating the solubility of compounds intended for intramammary infusion based upon tests conducted across a range of milk matrices. *PLoS One*. **2023**, *18* (7), e0288780. DOI: 10.1371/journal.pone.0288780.
30. Martir, J.; Flanagan, T.; Mann, J.; Fotaki, N. In vivo predictive dissolution testing of montelukast sodium formulations administered with drinks and soft foods to infants. *AAPS PharmSciTech*. **2020**, *21* (7), 282. DOI: 10.1208/s12249-020-01825-7.
31. Fotaki, N.; Vertzoni, M. Biorelevant dissolution methods and their applications in in vitro and in vivo correlations for oral formulations. *Open Drug Deliv. J*. **2010**, *4* (1), 2–13.
32. Boyd, B. J.; Salim, M.; Clulow, A. J.; Ramirez, G.; Pham, A. C.; Hawley, A. The impact of digestion is essential to the understanding of milk as a drug delivery system for poorly water soluble drugs. *J. Control. Release* **2018**, *292*, 13–17. DOI: 10.1016/j.jconrel.2018.10.027.
33. Eason, T.; Ramirez, G.; Clulow, A. J.; Salim, M.; Boyd, B. J. Revisiting the dissolution of praziquantel in biorelevant media and the impact of digestion of milk on drug dissolution. *Pharmaceutics* **2022**, *14* (10), 2228. DOI: 10.3390/pharmaceutics14102228.
34. Kalantzi, L.; Polentarutti, B.; Albery, T.; Laitmer, D.; Abrahamsson, B.; Dressman, J.; Reppas, C. The delayed dissolution of paracetamol products in the canine fed stomach can be predicted in vitro but it does not affect the onset of plasma levels. *Int. J. Pharm*. **2005**, *296* (1-2), 87–93. DOI: 10.1016/j.ijpharm.2005.02.028.
35. Ghazal, H. S.; Dyas, A. M.; Ford, J. L.; Hutcheon, G. A. The impact of food components on the intrinsic dissolution rate of ketoconazole. *Drug Dev. Ind. Pharm*. **2015**, *41* (10), 1647–1654. DOI: 10.3109/03639045.2014.983114.
36. Ghazal, H. S.; Dyas, A. M.; Ford, J. L.; Hutcheon, G. A. In vitro evaluation of the dissolution behaviour of itraconazole in bio-relevant media. *Int. J. Pharm*. **2009**, *366* (1-2), 117–123. DOI: 10.1016/j.ijpharm.2008.09.003.

aaps PharmSci 360

OCT 22–25 2023

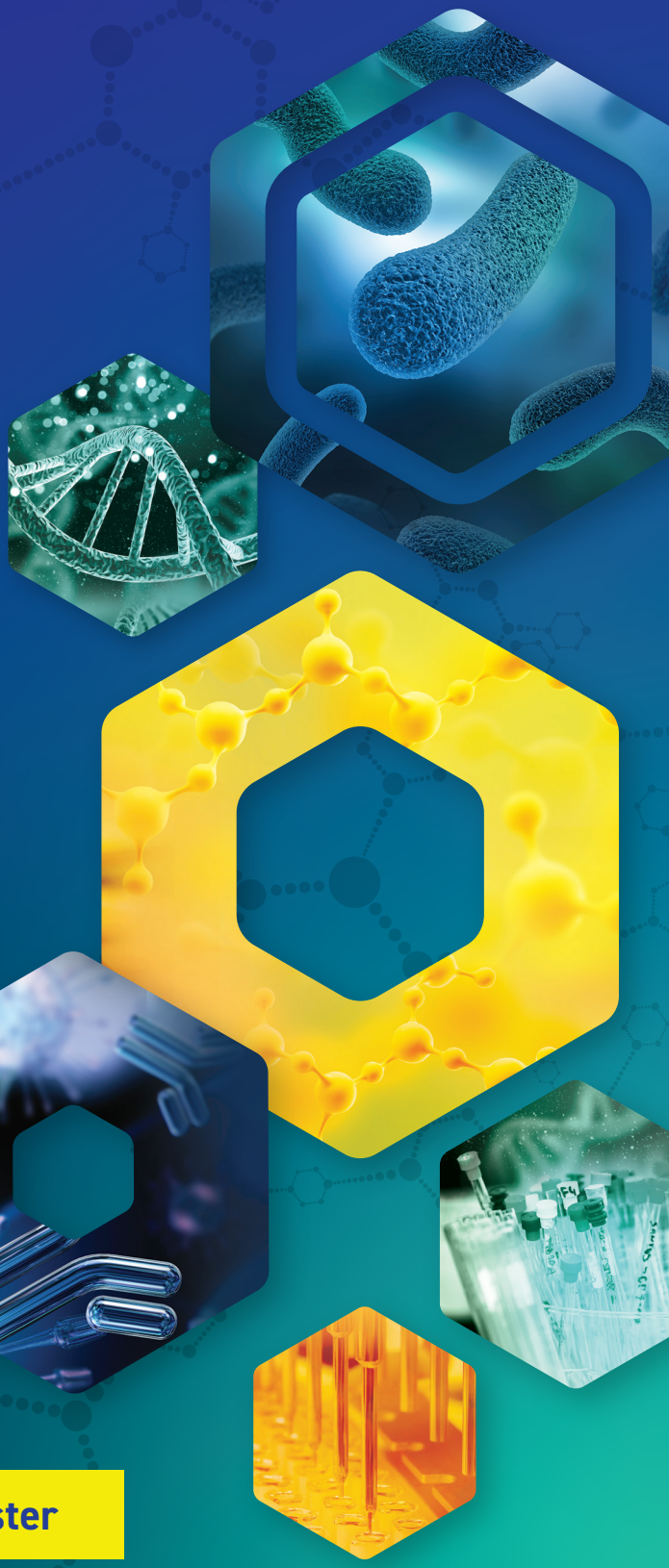
ORANGE COUNTY CONVENTION CENTER
ORLANDO, FL

REGISTER TODAY

TO LEARN ABOUT CUTTING-EDGE RESEARCH, SHARE YOUR SCIENCE, AND GROW YOUR NETWORK!

LEARNING AND INTERACTIVE EVENTS, HUNDREDS OF SCIENTIFIC POSTERS, CAREER DEVELOPMENT, AND NETWORKING OPPORTUNITIES!

- DISCOVERY BASIC RESEARCH
- PRECLINICAL AND TRANSLATIONAL SCIENCES
- BIOANALYTICS
- MANUFACTURING AND ANALYTICAL CHARACTERIZATION
- FORMULATION AND DELIVERY



Register NOW! aaps.org/pharmsci/register

dx.doi.org/10.14227/DT300323P144

Biorelevant Dissolution of Dipyridamole and Piroxicam Using an Automated UV/Vis Spectrophotometric and Potentiometric Dissolution Testing Platform

Ragna Berthelsen[1], Susan Weng Larsen[1], Anette Müllertz[1,2], Karl Box[3], and Jesper Østergaard[1*]
[1]*Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark.*
[2]*Bioneer:FARMA, Copenhagen, Denmark.*
[3]*pION Inc, Forest Row, East Sussex, UK.*

e-mail: jesper.ostergaard@sund.ku.dk

ABSTRACT

The objective of this study was to investigate the implications of changing dissolution parameters, including pH ramp time, absence and presence of simulated intestinal fluid (SIF), and the addition of a partitioning phase, using an automated dissolution-testing platform. The molar absorption coefficients, pKa, and dissolution characteristics of dipyridamole and piroxicam were investigated in the UV/Vis spectrophotometric and potentiometric platform, inForm (Pion Inc). Dissolution of dipyridamole and piroxicam from 10-mg compacts (tablets) was studied at pH 2.0 and 6.5 in fasted-state SIF version 2 (v2) and in biphasic media using decanol as the partitioning phase. Transfer through the gastrointestinal tract was simulated by shifting pH from 2.0 to 6.5 during dissolution testing. Dipyridamole (pK_a 6.0) dissolved rapidly at pH 2.0; however, changing the pH to 6.5 brought dipyridamole into a supersaturated state, from which it precipitated. Precipitation was slower in the presence of SIF, and higher dipyridamole concentrations were maintained in solution compared with simple buffer systems. In the biphasic dissolution assay, rapid distribution of dipyridamole into the lipophilic partitioning phase minimized drug precipitation. For piroxicam (pK_a1 1.9; pK_a2 5.3), the dissolution rate increased with increasing pH. The inclusion of SIF and introduction of a partition phase had limited influence on piroxicam dissolution. The automated platform facilitated efficient exploration of dissolution conditions. Tailoring of dissolution assays including pH gradients, SIF, and biphasic partitioning enabled detailed drug characterization, increased biorelevance, and possibly in vivo predictability. The use of a biphasic dissolution assay had a large impact on the in vitro dissolution of dipyridamole. The incorporation of an absorptive sink might be key for unraveling the supersaturation and dissolution behavior of weakly basic drug compounds.

KEYWORDS: Biorelevant medium, biphasic dissolution, supersaturation, dissolution, pH shift, InForm

INTRODUCTION

To reach systemic circulation after oral administration, an active pharmaceutical ingredient (API) must dissolve in the gastrointestinal (GI) fluids and subsequently be absorbed across the intestinal membrane. The physicochemical characteristics of the API, e.g., solubility, pK_a, solid form, and lipophilicity, together with the properties of the drug delivery system and the physiological environment of the GI tract (e.g. pH, content of bile salts, and gastric emptying rate) determine the rate and extent of drug dissolution and absorption (*1-4*). Many oral drugs are absorbed in the upper part of the small intestine, and thus have to be in solution in a slightly acidic to neutral environment (pH 5.4-6.5) (*5*). Biopharmaceutics Classification System (BCS) class II compounds are poorly soluble and may dissolve slowly in the small intestine, but have a high permeability, and consequently exhibit solubility and/or dissolution-limited absorption (*6*). For such APIs, it may be important to cover the pH range experienced during transit from the acidic stomach to the near neutral small intestine during in vitro dissolution testing. In particular, the pH change during transit from the stomach to the small intestines may lead to a significant decrease in solubility of basic APIs. Weak bases, fully ionized and highly soluble in the acidic environment, may upon transit into the small intestine

*Corresponding author

precipitate upon transient supersaturation (*3*, *7*–*9*). As the extent and duration of in vivo supersaturation have great influence on the bioavailability, it is crucial to capture these phenomena in an in vivo relevant manner during in vitro dissolution studies (*3*, *7*, *10*–*14*). Biphasic dissolution assays have been proposed to mimic the dissolution and absorption events taking place in vivo (*12*, *15*, *16*).

In the present study, the dissolution behavior of dipyridamole (PubChem CID: 3108) and piroxicam (PubChem CID: 54676228) was investigated using an automated UV/Vis spectrophotometric and potentiometric dissolution testing platform. The pH and composition of the dissolution medium were adjusted to simulate the conditions of the human GI tract. Transport of API into a lipophilic phase during dissolution was used to simulate drug absorption. The objective of the study was to investigate the implications of changing defined dissolution testing parameters; pH, pH ramp time, absence and presence of simulated intestinal fluid (SIF), and absence and presence of a partitioning phase, on the dissolution behavior of dipyridamole and piroxicam.

METHODS

Materials

Dipyridamole, methanol, N-methyl-2-pyrrolidinone (NMP), and piroxicam (anhydrate) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride, 0.50 M HCl, and 0.50 M NaOH were from Fisher Scientific (Leics, UK); n-Decanol was obtained from Alfa Aesar (Heysham, UK); and SIF-v2 powder was purchased from biorelevant.com (Croydon, UK). Purified water was obtained from a Purite Select deionization unit (Ondeo Industrial Solutions, Grangemouth, UK)

Instrumentation

Determination of molar absorption coefficients and pKa values, as well as the dissolution studies on dipyridamole and piroxicam were performed using the inForm instrument from Pion Inc. (Forest Row, UK), which is an automated platform based on potentiometric titration as well as UV-metric measurements. Figure 1 shows a schematic presentation of the 100-mL measurement vessel (46-mm inner diameter, 75-mm height) containing two fiber-optic UV probes, a pH electrode, temperature probe, flat blade type overhead stirrer, compact holder, and capillaries for dispensing acid, base, and media. Temperature was maintained using a Peltier element. The autosampler, having two robotic arms, allows for automated washing, sample handling, and measurement procedures. The instrument was controlled using inForm software version 1.1.3.6 (Pion Inc.).

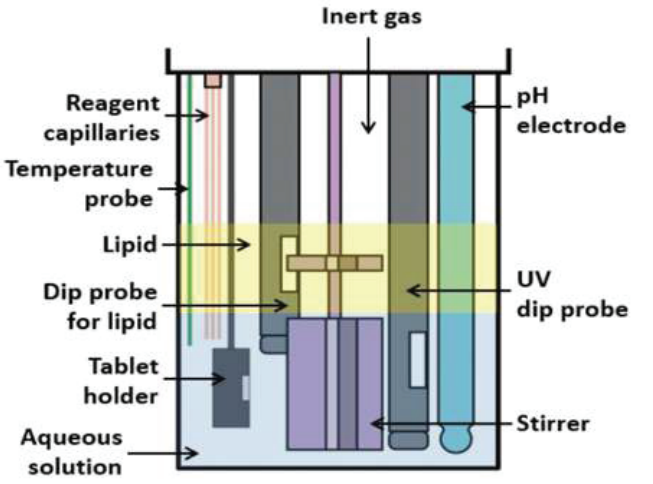


Figure 1. Schematic representation of potentiometric and UV-metric measurement vessel used for determination of molar absorption coefficients, pK_a and dissolution experiments.

Molar Absorption Coefficient and p*K*a Determination

Molar absorption coefficients, as a function of wavelength (185–750 nm) and pH ranging from 2.0–12.0, were determined at 37 °C for dipyridamole and piroxicam. An API solution in NMP at a concentration of 20 mM was added to 36 mL of an ionic strength-adjusted buffer solution (I = 0.172 M) containing acetate, phosphate, and sodium chloride. For both dipyridamole and piroxicam, experiments were performed in neat aqueous medium (three titrations in each experiment, n = 3) as well as in methanol-buffer solutions (45, 30, and 22% methanol). Drug concentrations in the solutions ranged from 12–350 µM and 4–27 µM for dipyridamole and piroxicam, respectively. During the measurements, the sample solution was stirred at a rate of 300 rpm. Potentiometric titrations from high to low pH and low to high pH were performed by the addition of 0.50 M HCl and 0.50 M NaOH, respectively. UV/Vis spectra were recorded during the titrations using the fiber optic probe with a light path of 10 or 20 mm connected to the inForm diode array spectrometer.

Molar absorption coefficients of the APIs in decanol were determined using the fiber optic probe by addition of aliquots of API stock solution in NMP to 40 mL of decanol while stirring at 300 rpm, providing dipyridamole and piroxicam concentrations ranging from 50–250 µM and 14–71 µM, respectively.

Preparation of Compacts

Compacts with a surface diameter of 3 mm, comprising 7.5–10.8 mg of drug substance, were prepared in stainless steel dies using a manual screw press (Pion Inc.). The dimensions of the compact holding dies were 3, 12,

and 6 mm for the inner diameter, outer diameter, and thickness, respectively; the back side was sealed with a silicon rubber stopper. Compacts were prepared under a weight of 120 kg applied for 6 min. The compacts were visually inspected and ensured to have a smooth surface free of visible defects.

Dissolution Studies

The dissolution behavior of dipyridamole and piroxicam from 3-mm diameter compacts was investigated at 37 °C. Experiments were initiated by lowering the compact into 40 mL pre-heated dissolution medium at 37 °C while stirring the medium at 100 rpm. UV/Vis spectra and solution pH levels were recorded every 30 s. Drug dissolution was investigated in buffered solutions at pH 2.0 and pH 6.5 (I = 0.15–18 M), in FaSSIF v2 (prepared from SIF-v2 powder and added as a 10x concentrate), and in biphasic medium with decanol as the organic layer. The aqueous dissolution medium comprised of acetate-phosphate buffer (0.10 M sodium acetate, 0.10 M sodium dihydrogen phosphate) for maintaining pH with NaCl (0.15 M) added for ionic strength adjustment. The dissolution was investigated utilizing a range of different experimental conditions as summarized in Table 1. In general, drug dissolution was followed for 30 min after which the experiment was terminated or the dissolution conditions altered in terms of pH, media change and/or addition of an absorptive/partitioning phase. Transfer through the GI tract was simulated by shifting pH from 2.0 to 6.5 during dissolution. The pH was either ramped from acidic to neutral pH over 60 s or changed gradually (linear relationship of pH against time) over a period of 30 min. In selected experiments, a partitioning phase consisting of decanol (30 mL) was added to simulate an absorptive step. The nominal interfacial area between the aqueous and decanol phases was 16.6 cm^2. The decanol phase was also subjected to agitation (100 rpm) and the amount of drug substance partitioning into the decanol phase was measured using a second fiber optic probe (light path 10 mm).

Data Processing

Analysis of the dissolution experiments was based on the Noyes-Whitney equation:

$$\frac{dC}{dt} = k(S - C) \qquad (1)$$

where S represents the solubility of the drug substance, C is the drug substance concentration in solution at time t, and k is a constant. Integration of Eq. (1) provides:

$$C = S(1 - e^{-kt}) \qquad (2)$$

To account for a temporal offset, the exponential Eq. (2) was modified as follows:

$$C = S(1 - e^{-k(t-t_0)}) \qquad (3)$$

where t_0 allows for the temporal offset (*17, 18*). The intrinsic dissolution rate, J, was calculated according to (*18*):

$$J = \frac{dC}{dt}\frac{V}{A} = \frac{V}{A}kS \qquad (4)$$

where V is the volume of the dissolution medium and A is the surface area of the compressed drug disk.

The precipitation rate, $\frac{dM}{dt}$, was determined by fitting a first order expression to the relevant part of the concentration – time profile:

$$C = C_{\text{onset}}\, e^{-k'(t-t_{\text{onset}})} \qquad (5)$$

followed by substitution of the empirical precipitation rate constant k':

$$\frac{dM}{dt} = \frac{dC}{dt}V = -k'VC_{\text{onset}} \qquad (6)$$

where C_{onset} is the drug substance concentration in solution at the time where precipitation starts (is detected), t_{onset}.

Table 1. Outline of Dissolution and Partitioning Studies Performed[a]

Experiment no.	Sector 1	Sector 2	Sector 3
I	Buffer pH 6.5	-	-
II	Buffer pH 2.0	Buffer pH 6.5	-
III	Buffer pH 2.0	Linear pH gradient	Buffer pH 6.5
IV	Buffer pH 2.0	Linear pH gradient + addition of FaSSIF v2	FaSSIF v2 pH 6.5
V	Buffer pH 2.0	Buffer pH 6.5 + decanol partition phase	-
VI	Buffer pH 2.0	Linear pH gradient + decanol partition phase	Buffer pH 6.5 + decanol partition phase
VII	Buffer pH 2.0	Linear pH gradient + addition of FaSSIF v2 + decanol partition phase	FaSSIF v2 + decanol partition phase

[a]Dissolution experiments were conducted at 37 °C and 100 rpm. The duration of each sector was 30 min.
FaSSIF v2: fasted-state simulated intestinal fluid, version 2.

Data processing was conducted using Sirius Refine Software version 1.1.3.6 (Pion Inc.). Absorbance spectra were converted to drug concentration or the absolute sample amount of drug substance dissolved using the molar absorption coefficients determined using inForm. Subsequently, k, S, and J (k', C_{onset}, and $\frac{dM}{dt}$) were calculated utilizing a refinement process in which, k, S, and t_0 (k', C_{onset}, and t_{onset}) were varied to minimize the root mean square deviation between the modelled and measured drug substance concentrations.

Statistical comparison of intrinsic dissolution rates as well as maximum concentrations (estimated solubilities) was conducted by use of a two-way ANOVA test followed by multiple comparison using the Tukey method ($\alpha = 0.05$). The statistical tests were performed using GraphPad Prism, 9.2.0 (GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

Both model compounds, dipyridamole and piroxicam, are categorized as BCS class II drugs and are relatively well-characterized in the literature with respect to their in vitro and in vivo behavior (*3*, *12*, *19–26*).

Absorbance Spectra, Molar Absorption Coefficients and pK_a

Figure 1 shows a schematic presentation of the inForm potentiometric and UV-metric measurement vessel, which together with the autosampler allow unattended absorbance measurements and subsequent determination of molar absorption coefficients, pK_a, and dissolution rates of up to 20 samples. The application of UV/Vis fiber optic probes and multivariate chemometric approaches to pK_a determination and dissolution testing is well-established (*27–33*). Thus, performance verification was limited to the assessment of the pK_a values for dipyridamole and piroxicam.

For dipyridamole, a mean ± SD (n = 3) pK_a of 6.04 ± 0.14 (fully aqueous medium) and 6.24 ± 0.02 (mixed solvent using Yasuda-Shedlovsky [Y-S] extrapolation) was determined at 37 °C and 25 °C, respectively (I = 0.17 M). Dipyridamole is a weak base having two basic functional groups (pK_a 6.2 and ~0.8 at 25 °C and I = 0.15 M) (*18*). Only the least acidic pK_a was within the pH range of the conducted UV titration. The pK_a determined was consistent with most published values, e.g., 6.23 (25 °C; I = 0.15 M) and 6.22 (25 °C; I = 0.15 M), with exception of 4.93 (37 °C; I = 0.15 M) (*34–36*). For piroxicam, the mean ± SD pK_a values were determined as 1.94 ± 0.03 and 5.28 ± 0.02 (n = 2, Y-S extrapolation) and 1.84 ± 0.03 and 5.29 ± 0.02 (n = 4, Y-S extrapolation) at 37 °C and 25 °C, respectively (I = 0.17 M). These values correlated well with the published pK_a values, e.g., 1.88 and 5.23 (25 °C; I = 0.15 M), 1.88 and 5.29 (25 °C; I = 0.15 M), 1.89 and 5.34 (25 °C; I = 0.15 M), and 5.34 ± 0.02 (37 °C and I = 0.15 M) (*17*, *18*, *34*, *37*). Overall, the results indicated that the UV-metric measuring technique was reliable and robust.

Dipyridamole Dissolution

Figures 2 and 3 display the dissolution profiles for dipyridamole obtained applying the experimental conditions listed in Table 1, with the measured drug concentration as a function of time. When dipyridamole dissolved at pH 6.5 (Fig. 2), the intrinsic dissolution rate was low ($3.7 \times 10^{-3} \pm 0.4 \times 10^{-3}$ mg/min cm^{-2}) and the maximal concentration reached within 30 min was low (0.22 ± 0.02 µg/mL). This low concentration corresponds well to the fact that dipyridamole is a weak base with a pKa of 6.0, and therefore is predominantly neutral at pH 6.5, displaying poor aqueous solubility. In addition, the low concentration measured in the aqueous buffer at pH 6.5 is consistent with reported solubilities of 8.1 ± 0.4 µg/mL (50-mM phosphate buffer, pH 6.5) and 6.9 ± 0.2 µg/mL (phosphate buffered saline [PBS], pH 6.8) (*38*, *39*). At pH 2.0 (Fig. 3), the intrinsic dissolution rate of dipyridamole was much higher (overall mean 6 ± 2 mg/min cm^{-2}), leading to an average maximum concentration of dissolved drug of 163 ± 24 µg/mL, corresponding to 75% ± 8% w/w of the dose dissolved after 30 min of dissolution. Based on the dissolution profiles obtained at pH 2.0, the dipyridamole solubility was estimated to 245 ± 42 µg/mL in this medium at 37 °C using Eq. (3). There was no statistical difference between the intrinsic dissolution rates, the estimated solubility, or the maximum concentration measured in aqueous buffer at pH 2.0 when determined after applying the different experimental settings (Table 1). As the first sector settings were analogous for the studies II-VII (dissolution at pH 2), the results were expected to be similar, and the lack of a statistical difference simply indicates that the model design and data analysis were robust. The estimated solubility of dipyridamole in aqueous buffer pH 2.0 also correlates well with published values (e.g., 234 ± 27 µg/mL in diluted simulated gastric fluid at pH 2.0 and 37 °C) (*38*).

The results from the dissolution experiments conducted using different experimental conditions reveal several interesting points. When the pH ramped from 2.0 to 6.5 within 60 s (Fig. 3a), dipyridamole precipitated instantaneously. However, when the pH was shifted gradually using a linear gradient over 30 min (Fig. 3b), precipitation was not observed before the pH shift was complete, displaying a 30-min lag phase. The observed dipyridamole precipitation rate in aqueous buffer pH

6.5 was found to be independent of how the pH was shifted (setting II and III), with an average value of 1.3 ± 0.4 mg/min for the empirical precipitation rate constant (Fig. 3a and b). In terms of in vivo relevance, the gradual pH shift mimics the fasted-state gastric emptying profile more closely ($t_{½}$ = 13 ± 1 min for 240 mL of water) (*40*). Because the gradual pH shift merely introduced a lag phase for the drug precipitation, which was initiated at pH 6.5 producing a similar precipitation rate as compared to experiments using a fast pH shift, it may be argued that the slow pH shift, at least in this case, complicated the dissolution model without adding supplementary information. The use of a more physiologically relevant dissolution medium (FaSSIF v2) compared to aqueous buffer at pH 6.5 (experiments IV vs III) led to a significant decrease in the drug precipitation rate ($p < 0.05$) (Fig. 3c vs 3b). In FaSSIF v2, the drug precipitation rate at pH 6.5 was calculated to be $7 \times 10^{-2} \pm 2 \times 10^{-2}$ mg/min. As a consequence of the slower precipitation rate, the duration of apparent supersaturation in FaSSIF v2 was increased, with 64% ± 2% of the dose still solubilized after 30 min at pH 6.5 (Fig. 3c).

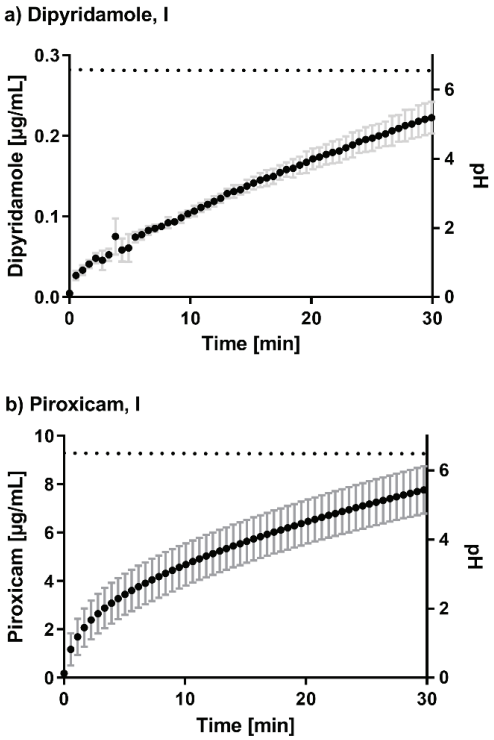


Figure 2. Dipyridamole (a) and piroxicam (b) dissolution profiles obtained at 37 °C with monophasic dissolution in aqueous buffer at pH 6.5. The dotted line indicates the pH, black circles represent the amount of dipyridamole dissolved in the aqueous solutions (mean ± SD, n = 3).

Psachoulias et al. studied the in vivo precipitation of dipyridamole in human adults after administration of the drug in solution directly into the antrum of the stomach (*24*). Drug precipitation was measured after GI transfer by aspirating fluid samples from the ligament of Treitz. Two doses were evaluated (30 and 90 mg), yet the study showed minimal drug precipitation in the small intestine; i.e., the mean precipitated fraction was below 7% (*24*). These results indicate that simulating the GI transfer using simple aqueous buffers (experiments II and III) significantly overestimated the extent of drug precipitation in the small intestine; i.e., only 14% ± 2% of the dose was solubilized after 30 min of dissolution at pH 6.5 (Fig. 3a and b). Furthermore, the results suggest that the use of a biorelevant dissolution media containing bile salts and phospholipids may improve the predictive performance of the in vitro setup. This finding correlates well with studies supporting the use of biorelevant dissolution media for predicting in vivo behavior of orally administrated drugs (*5*, *19*, *41–43*).

In 2004, Kostewicz et al. investigated the influence of hydrodynamics, transfer rates, and composition of the SIF (fed state vs fasted state) on the precipitation behavior of dipyridamole using an in vitro transfer model (*3*). The transfer model mimicked the in vivo passage through the human GI tract by transferring a drug solution in simulated gastric medium into a simulated intestinal medium. Applying varying transfer rates in the range of 0.5–9 mL/min, Kostewicz et al. observed very small differences in the maximum degree of supersaturation achieved in the FaSSIF, indicating no clear dependence on the transfer rate (*3*). In the fed-state medium precipitation was not observed (*3*). The observations of the current in vitro dissolution study are in line with this, as the medium composition significantly affected the rate and extent of drug precipitation, whereas the transfer conditions (pH shift and transfer rate) had limited impact on the drug precipitation.

Biphasic dissolution studies were conducted to investigate the impact of incorporating an absorptive step into the dissolution model. In the present study, decanol was used as the lipophilic phase allowing dissolved drug to partition herein, simulating the in vivo drug removal by absorption into and across the intestinal membrane. The results displayed in Figure 3d–f show that dipyridamole distributes relatively fast into the decanol phase, thereby limiting drug precipitation. The fast drug partitioning into the organic phase, which limited drug precipitation in the aqueous phase, may appear to correlate with the in vivo data presented by Psachoulias et al. (*24*).

Overall, comparison of the dipyridamole dissolution profiles to in vivo data obtained following oral administration of dipyridamole showed that experiments I–III have limited relevance when estimating the in vivo

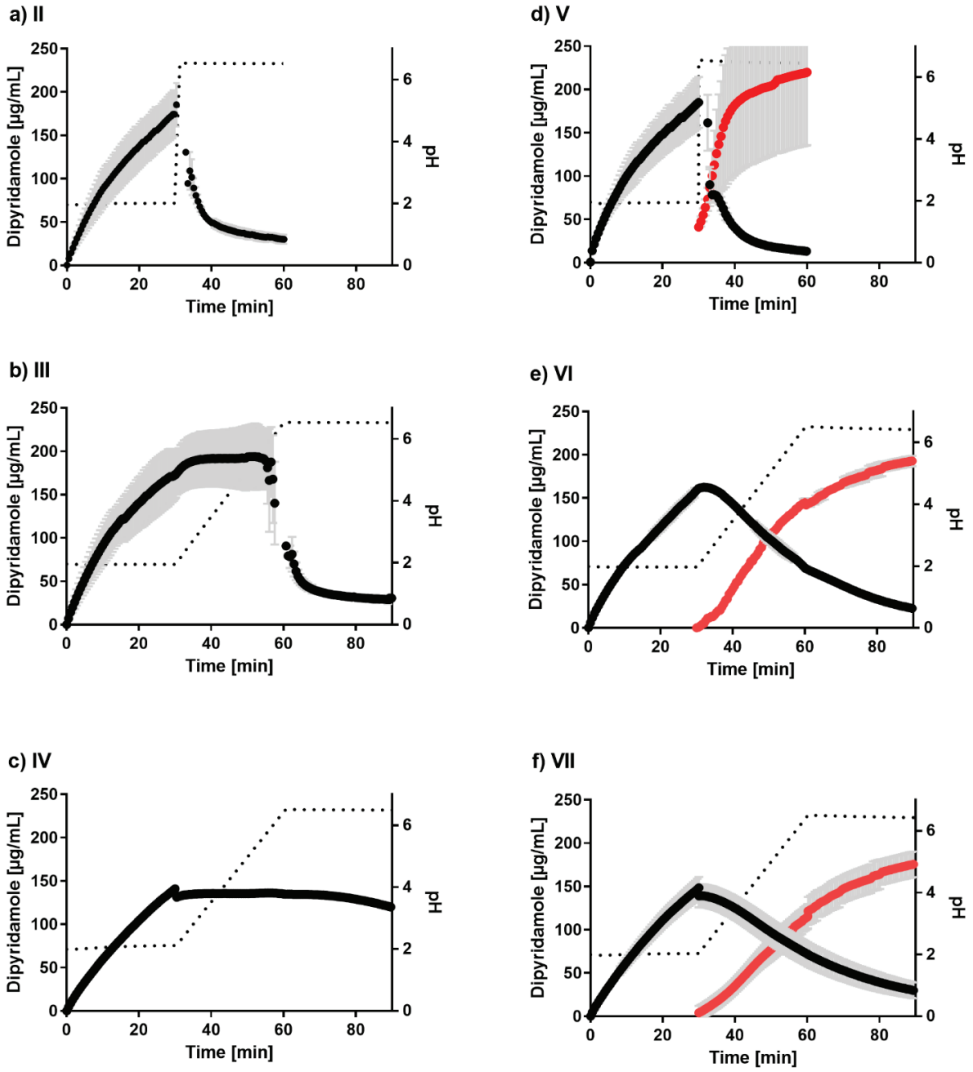


Figure 3. Dipyridamole dissolution profiles obtained at 37 °C at the conditions described in Table 1 (experiments II-VII in a–f, respectively). Monophasic dissolution in aqueous buffers at pH 2.0 and pH 6.5 with a fast pH shift (a: II), slow pH shift (b: III), and slow pH shift from buffer pH 2.0 to FaSSIFv2 pH 6.5 (c: IV). Biphasic dissolution with similar aqueous media and pH shifts (d-f: V-VII).
Dotted lines indicate the pH, black circles represent the amount of dipyridamole dissolved in the aqueous solutions, and red circles represent dipyridamole dissolved in decanol (mean ± SD, n = 3–4).

performance of dipyridamole, as these single-phase settings all led to very low drug concentrations in the SIF following 30 min of dissolution (< 50 µg/mL, Figs. 2, 3a, and 3b). Based on the dissolution profiles depicted in Figure 3, it appears that experiments IV–VII produced in vivo-relevant results, as very small amounts of drug precipitation were observed using those settings (Fig. 3c–f), i.e., using biorelevant media to simulate the intestinal fluid or using a biphasic dissolution setup. A recent study by Klumpp and Dressman demonstrated how physiologically based pharmacokinetic (PBPK) model output is dependent on dissolution data using glibenclamide and dipyridamole as case examples (*25*). The authors found that dissolution input from one-step dissolution testing in simulated gastric medium led to a close simulation of the pharmacokinetic profile of dipyridamole (*25*). Using a two-step dissolution model, with immediate transfer from gastric to intestinal medium (FaSSIF v2), the authors observed immediate precipitation to a drug concentration of 36 ± 1 µg/mL (*25*). The resultant PBPK model showed that the simulated pharmacokinetic profiles are very sensitive to calculated precipitation rate constants. Based on actual human plasma data, little precipitation occurs in vivo, especially as dipyridamole is a highly permeable drug. Therefore, biphasic dissolution (or a combined dissolution permeation model as described

by Mizoguchi et al.) is recommended when estimating the in vivo performance of weak bases resembling dipyridamole (*44*).

Piroxicam Dissolution

Figure 2b and 4 show the dissolution profiles for piroxicam obtained from the experimental conditions in Table 1 (experiments I–VII). The effects of changing the medium during dissolution experiments were less pronounced for piroxicam as compared to dipyridamole and opposite in terms of pH-dependence of the dissolution rate (Fig. 4 vs Fig. 3). Upon shifting the pH, instantaneously or gradually applying the linear gradient over 30 min (Fig. 4a and b), an increase in piroxicam dissolution rate was observed. The intrinsic dissolution rates of piroxicam were 0.11 ± 0.02 mg/min cm^{-2} and 0.29 ± 0.03 mg/min cm^{-2} at pH 2.0 and 6.5, respectively. The observed lower dissolution rate of piroxicam at pH 2 vs pH 6.5, is consistent with the increase in degree of ionization, which in turn increases the solubility and dissolution rate of piroxicam in the given dissolution medium. When comparing Figure 2b and Figure 4, it is apparent that including a gastric step simply delayed the dissolution process. Following 30 min of dissolution at pH 6.5, irrespective of whether gastric dissolution was included or not, the same amount of piroxicam was dissolved, i.e., 311 ± 39, 315 ± 15, and 321 ± 32 µg/mL for experiments I, II, and III, respectively.

The use of FaSSIF v2 as compared to neat aqueous buffer at pH 6.5 had a negligible effect on the dissolution rate of

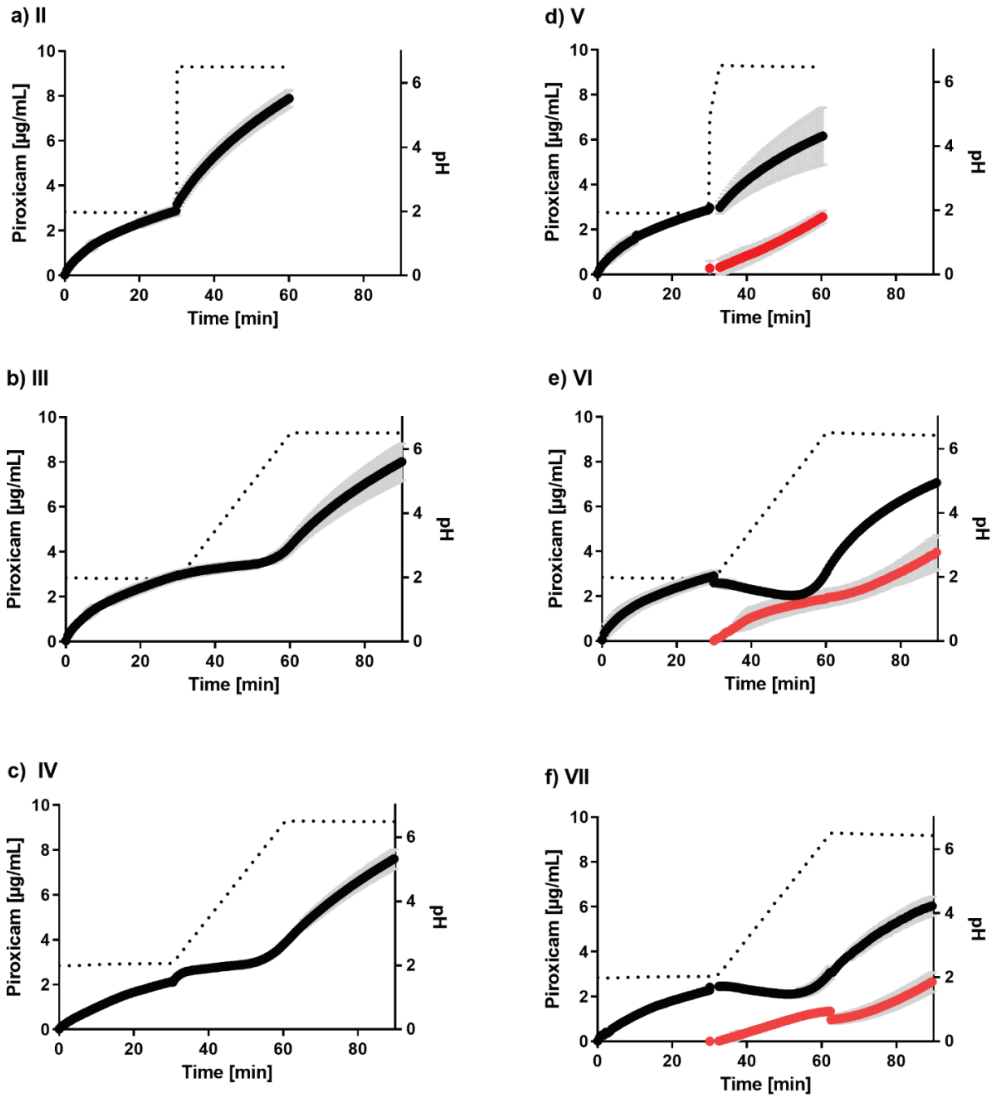


Figure 4. Piroxicam dissolution profiles obtained at 37 °C with the conditions described in Table 1 (experiments II-VII in a–f, respectively). Monophasic dissolution in aqueous buffers at pH 2.0 and pH 6.5 with a fast pH shift (a: II), slow pH shift (b: III), and slow pH shift from buffer pH 2.0 to FaSSIFv2 pH 6.5 (c: IV). Biphasic dissolution with similar aqueous media (d-f: V-VII).
Dotted lines indicate pH, black circles represent the amount of piroxicam dissolved in the aqueous solutions, and red circles represent piroxicam dissolved in decanol (mean ± SD, n = 3–4).

piroxicam (experiment III and IV, Fig. 4b and c). At pH 6.5, 94% of the piroxicam is negatively charged (calculated based on the Hendersson-Hasselbalch equation) and the remainder is present as a zwitter-ion/neutral species. The piroxicam anion has been shown to have very little affinity for the micelles formed by surfactants, so the lack of difference observed when comparing the dissolution profile of piroxicam in phosphate buffer pH 6.5 and FaSSIF v2 pH 6.5 (Fig. 4b and c) is not surprising (*45*). The present results also correlate well with results presented by Khadra et al. in a study, where the effects of composition of SIF on equilibrium solubility for BCS class II compounds were investigated (*46*). The authors found that pH was the most important factor leading to increased solubility; none of the other investigated SIF parameters (i.e., content of sodium oleate, bile salts, and buffer concentrations) had a significant effect on the solubility of piroxicam. Therefore, pH is the single most important factor affecting the solubility and dissolution rate of piroxicam in simulated gastric and intestinal fluids.

Biphasic dissolution studies, with decanol as the lipophilic phase, were also conducted with piroxicam. As apparent from Figure 4d and f, piroxicam partitioned into the decanol phase only to a limited extent. In line with expectations, the predominantly net negatively charged molecule did not interact appreciably with a lipophilic partitioning phase nor with the bile salts and phospholipids of the biorelevant medium (FaSSIFv2).

Collectively, the utilization of GI biorelevant media and biphasic dissolution conditions is of larger significance for the dissolution behavior of the basic molecule dipyridamole as compared to a weakly acidic compound such as piroxicam (Figs. 3 and 4). For piroxicam, the simplest dissolution setup (experiment I) produced similar results as the most complicated setup (experiment VII), i.e., 311 ± 39 and 324 ± 10 µg/mL, respectively. Therefore, a simple dissolution setup may be recommended for evaluating the oral performance of piroxicam (*20*).

CONCLUSIONS

Using the automated instrument platform, inForm, the dissolution behavior of dipyridamole and piroxicam was investigated while varying testing conditions in terms of pH, dissolution medium, and the presence of a partitioning phase. The weak base dipyridamole dissolved rapidly at pH 2.0; shifting the pH to 6.5 during dissolution testing brought dipyridamole into a supersaturated state, from which it precipitated. Upon addition of FaSSIF v2, dipyridamole precipitation was slower, and a higher concentration was maintained in solution. Utilizing the biphasic dissolution assay, rapid distribution of dipyridamole into the decanol phase minimized precipitation. For piroxicam, the dissolution rate increased with increasing pH. The inclusion of FaSSIF v2 and the introduction of a partition phase had a limited effect on the dissolution behavior of piroxicam consistent with ionization properties of the drug. The automated system allows for tailoring of the dissolution assays in an efficient manner, enabled detailed drug characterization, and possibly increased biorelevance and in vivo predictability. The incorporation of an absorptive sink into dissolution experiments may be important for unraveling the supersaturation and dissolution behavior of weakly basic drug compounds.

FUNDING

This project received funding from the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement no. 644056.

CONFLICT OF INTEREST

Karl Box is an employee of Pion Inc. The other authors disclosed no conflicts of interest related to this article.

REFERENCES

1. Ashford, M. Bioavailability - physicochemical and dosage form factors. In *Aulton's Pharmaceutics. The Design and Manufacture of Medicines*, 4th ed.; Aulton, M., Taylor, K. M. G., Eds.; Elsevier Ltd: Edinburgh, 2013; pp 314–333.
2. Ashford, M. Gastrointestinal tract - physiology and drug absorption. In *Aulton's Pharmaceutics. The Design and Manufacture of Medicines*, 4th ed.; Aulton, M., Taylor, K. M. G., Eds.; Elsevier Ltd: Edinburgh, 2013; pp 296–313.
3. Kostewicz, E. S.; Wunderlich, M.; Brauns, U.; Becker, R.; Bock, T.; Dressman, J. B. Predicting the precipitation of poorly soluble weak bases upon entry in the small intestine. *J. Pharm. Pharmacol*. **2004**, *56* (1), 43–51. DOI: 10.1211/0022357022511.
4. Mudie, D. M.; Amidon, G. L.; Amidon, G. E. Physiological parameters for oral delivery and in vitro testing. *Mol. Pharm*. **2010**, *7* (5), 1388–1405. DOI: 10.1021/mp100149j.
5. Jantratid, E.; Janssen, N.; Reppas, C.; Dressman, J. B. Dissolution media simulating conditions in the proximal human gastrointestinal tract: an update. *Pharm. Res*. **2008**, *25* (7), 1663–1676. DOI: 10.1007/s11095-008-9569-4.
6. Amidon, G. L.; Lennernäs, H.; Shah, V. P.; Crison, J. R. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm. Res*. **1995**, *12* (3), 413–420. DOI: 10.1023/A:1016212804288.
7. Carlert, S.; Pålsson, A.; Hanisch, G.; von Corswant, C.; Nilsson, C.; Lindfors, L.; Lennernäs, H.; Abrahamsson, B. Predicting intestinal precipitation--a case example for a basic BCS class II drug. *Pharm. Res*. **2010**, *27* (10), 2119–2130. DOI: 10.1007/s11095-010-0213-8.

8. Taupitz, T.; Dressman, J. B.; Klein, S. In vitro tools for evaluating novel dosage forms of poorly soluble, weakly basic drugs: case example ketoconazole. *J. Pharm. Sci*. **2013**, *102* (10), 3645–3652. DOI: 10.1002/jps.23666.
9. Plum, J.; Bavnhøj, C. G.; Eliasen, J. N.; Rades, T.; Müllertz, A. Comparison of induction methods for supersaturation: Amorphous dissolution versus solvent shift. *Eur. J. Pharm. Biopharm*. **2020**, *152*, 35–43. DOI: 10.1016/j.ejpb.2020.04.017.
10. Bevernage, J.; Brouwers, J.; Brewster, M. E.; Augustijns, P. Evaluation of gastrointestinal drug supersaturation and precipitation: strategies and issues. *Int. J. Pharm*. **2013**, *453* (1), 25–35. DOI: 10.1016/j.ijpharm.2012.11.026.
11. Bevernage, J.; Brouwers, J.; Annaert, P.; Augustijns, P. Drug precipitation-permeation interplay: supersaturation in an absorptive environment. *Eur. J. Pharm. Biopharm*. **2012**, *82* (2), 424–428. DOI: 10.1016/j.ejpb.2012.07.009.
12. Frank, K. J.; Locher, K.; Zecevic, D. E.; Fleth, J.; Wagner, K. G. In vivo predictive mini-scale dissolution for weak bases: Advantages of pH-shift in combination with an absorptive compartment. *Eur. J. Pharm. Sci*. **2014**, *61*, 32–39. DOI: 10.1016/j.ejps.2013.12.015.
13. Sun, D. D.; Lee, P. I. Haste Makes Waste: The Interplay Between Dissolution and Precipitation of Supersaturating Formulations. *AAPS J*. **2015**, *17* (6), 1317–1326. DOI: 10.1208/s12248-015-9825-6.
14. Gan, Y.; Zhang, X.; Xu, D.; Zhang, H.; Baak, J. P.; Luo, L.; Xia, Y.; Wang, J.; Ke, X.; Sun, P. Evaluating supersaturation in vitro and predicting its performance in vivo with Biphasic gastrointestinal Simulator: A case study of a BCS IIB drug. *Int. J. Pharm*. **2020**, *578*, 119043. DOI: 10.1016/j.ijpharm.2020.119043.
15. Phillips, D. J.; Pygall, S. R.; Cooper, V. B.; Mann, J. C. Overcoming sink limitations in dissolution testing: a review of traditional methods and the potential utility of biphasic systems. *J. Pharm. Pharmacol*. **2012**, *64* (11), 1549–1559. DOI: 10.1111/j.2042-7158.2012.01523.x.
16. Shi, Y.; Gao, P.; Gong, Y.; Ping, H. Application of a biphasic test for characterization of in vitro drug release of immediate release formulations of celecoxib and its relevance to in vivo absorption. *Mol. Pharm*. **2010**, *7* (5), 1458–1465. DOI: 10.1021/mp100114a.
17. Avdeef, A.; Tsinman, O. Miniaturized rotating disk intrinsic dissolution rate measurement: effects of buffer capacity in comparisons to traditional wood's apparatus. *Pharm. Res*. **2008**, *25* (11), 2613–2627. DOI: 10.1007/s11095-008-9679-z.
18. Gravestock, T.; Box, K.; Comer, J.; Frake, E.; Judge, S.; Ruiz, R. The " dissolution" method: a low volume, in vitro apparatus for assessing the dissolution/precipitation behaviour of an active pharmaceutical ingredient under biorelevant conditions. *Anal. Methods* **2011**, *3* (3), 560–567. DOI: 10.1039/c0ay00434k.
19. Kostewicz, E. S.; Brauns, U.; Becker, R.; Dressman, J. B. Forecasting the oral absorption behavior of poorly soluble weak bases using solubility and dissolution studies in biorelevant media. *Pharm. Res*. **2002**, *19* (3), 345–349. DOI: 10.1023/A:1014407421366.
20. Shohin, I. E.; Kulinich, J. I.; Ramenskaya, G. V.; Abrahamsson, B.; Kopp, S.; Langguth, P.; Polli, J. E.; Shah, V. P.; Groot, D. W.; Barends, D. M.; Dressman, J. B. Biowaiver monographs for immediate release solid oral dosage forms: piroxicam. *J. Pharm. Sci*. **2014**, *103* (2), 367–377. DOI: 10.1002/jps.23799.
21. Heigoldt, U.; Sommer, F.; Daniels, R.; Wagner, K. G. Predicting in vivo absorption behavior of oral modified release dosage forms containing pH-dependent poorly soluble drugs using a novel pH-adjusted biphasic in vitro dissolution test. *Eur. J. Pharm. Biopharm*. **2010**, *76* (1), 105–111. DOI: 10.1016/j.ejpb.2010.05.006.
22. Mitra, A.; Fadda, H. M. Effect of surfactants, gastric emptying, and dosage form on supersaturation of dipyridamole in an in vitro model simulating the stomach and duodenum. *Mol. Pharm*. **2014**, *11* (8), 2835–2844. DOI: 10.1021/mp500196f.
23. Psachoulias, D.; Vertzoni, M.; Butler, J.; Busby, D.; Symillides, M.; Dressman, J.; Reppas, C. An in vitro methodology for forecasting luminal concentrations and precipitation of highly permeable lipophilic weak bases in the fasted upper small intestine. *Pharm. Res*. **2012**, *29* (12), 3486–3498. DOI: 10.1007/s11095-012-0844-z.
24. Psachoulias, D.; Vertzoni, M.; Goumas, K.; Kalioras, V.; Beato, S.; Butler, J.; Reppas, C. Precipitation in and supersaturation of contents of the upper small intestine after administration of two weak bases to fasted adults. *Pharm. Res*. **2011**, *28* (12), 3145–3158. DOI: 10.1007/s11095-011-0506-6.
25. Klumpp, L.; Dressman, J. Physiologically based pharmacokinetic model outputs depend on dissolution data and their input: Case examples glibenclamide and dipyridamole. *Eur. J. Pharm. Sci.* **2020**, *151*, 105380. DOI: 10.1016/j.ejps.2020.105380.
26. Eriksen, J. B.; Messerschmid, R.; Andersen, M. L.; Wada, K.; Bauer-Brandl, A.; Brandl, M. Dissolution/permeation with PermeaLoop™: Experience and IVIVC exemplified by dipyridamole enabling formulations. *Eur. J. Pharm. Sci*. **2020**, *154*, 105532. DOI: 10.1016/j.ejps.2020.105532.
27. Allen, R. I.; Box, K. J.; Comer, J. E.; Peake, C.; Tam, K. Y. Multiwavelength spectrophotometric determination of acid dissociation constants of ionizable drugs. *J. Pharm. Biomed. Anal*. **1998**, *17* (4-5), 699–712. DOI: 10.1016/S0731-7085(98)00010-7.
28. Gemperline, P. J.; Cho, J. H.; Baker, B.; Batchelor, B.; Walker, D. S. Determination of multicomponent dissolution profiles of pharmaceutical products by in situ fiber-optic UV measurements. *Anal. Chim. Acta* **1997**, *345* (1-3), 155–159. DOI: 10.1016/S0003-2670(97)00095-0.
29. Lu, X.; Lozano, R.; Shah, P. In-situ dissolution testing using different UV fiber optic probes and instruments. *Dissolut. Technol*. **2003**, *10* (4), 6–15. DOI: 10.14227/DT100403P6.
30. Nir, I.; Lu, X. In situ UV fiber optics for dissolution testing – what, why, and where we are after 30 years. *Dissolut. Technol.* **2018**, *25* (3), 70–77. DOI: 10.14227/DT250318P70.
31. Tam, K. Y.; Takács-Novák, K. Multiwavelength spectrophotometric determination of acid dissociation constants: Part II. First derivative vs. target factor analysis. *Pharm. Res*. **1999**, *16* (3),

374–381. DOI: 10.1023/A:1018817617432.
32. Tam, K. Y.; Takacs-Novak, K. Multi-wavelength spectrophotometric determination of acid dissociation constants: a validation study. *Anal. Chim. Acta* **2001**, *434* (1), 157–167. DOI: 10.1016/S0003-2670(01)00810-8.
33. Wiberg, K. H.; Hultin, U. K. Multivariate chemometric approach to fiber-optic dissolution testing. *Anal. Chem*. **2006**, *78* (14), 5076–5085. DOI: 10.1021/ac0602928.
34. Dohoda, D.; Tsinman, K.; Tsinman, O.; Wang, H.; Tam, K. Y. Spectrophotometric pKa determination of ionizable pharmaceuticals: Resolution of molecules with weak pH-dependent spectral shift. *J. Pharm. Biomed. Anal*. **2015**, *114*, 88–96. DOI: 10.1016/j.jpba.2015.05.009.
35. Bendels, S.; Tsinman, O.; Wagner, B.; Lipp, D.; Parrilla, I.; Kansy, M.; Avdeef, A. PAMPA - excipient classification gradient map. *Pharm. Res*. **2006**, *23* (11), 2525–2535. DOI: 10.1007/s11095-006-9137-8.
36. Avdeef, A.; Tsinman, K.; Tsinman, O.; Sun, N.; Voloboy, D. Miniaturization of powder dissolution measurement and estimation of particle size. *Chem. Biodivers*. **2009**, *6* (11), 1796–1811. DOI: 10.1002/cbdv.200900082.
37. Takács-Novák, K.; Tam, K. Y. Multiwavelength spectrophotometric determination of acid dissociation constants part V: microconstants and tautomeric ratios of diprotic amphoteric drugs. *J. Pharm. Biomed. Anal*. **2000**, *21* (6), 1171–1182. DOI: 10.1016/S0731-7085(99)00238-1.
38. Matsui, K.; Tsume, Y.; Amidon, G. E.; Amidon, G. L. In vitro dissolution of fluconazole and dipyridamole in gastrointestinal simulator (GIS), predicting in vivo dissolution and drug-drug interaction caused by acid-reducing agents. *Mol. Pharm*. **2015**, *12* (7), 2418–2428. DOI: 10.1021/acs.molpharmaceut.5b00135.
39. Baghel, S.; Cathcart, H.; O'Reilly, N. J. Understanding the generation and maintenance of supersaturation during the dissolution of amorphous solid dispersions using modulated DSC and 1H NMR. *Int. J. Pharm*. **2018**, *536* (1), 414–425. DOI: 10.1016/j.ijpharm.2017.11.056.
40. Mudie, D. M.; Murray, K.; Hoad, C. L.; Pritchard, S. E.; Garnett, M. C.; Amidon, G. L.; Gowland, P. A.; Spiller, R. C.; Amidon, G. E.; Marciani, L. Quantification of gastrointestinal liquid volumes and distribution following a 240 mL dose of water in the fasted state. *Mol. Pharm*. **2014**, *11* (9), 3039–3047. DOI: 10.1021/mp500210c.
41. Jantratid, E.; Dressman, J. Biorelevant dissolution media simulating the proximal human gastrointestinal tract: an update. *Dissolut. Technol*. **2009**, *16* (3), 21–25. DOI: 10.14227/DT160309P21.
42. Sunesen, V. H.; Pedersen, B. L.; Kristensen, H. G.; Müllertz, A. In vivo in vitro correlations for a poorly soluble drug, danazol, using the flow-through dissolution method with biorelevant dissolution media. *Eur. J. Pharm. Sci*. **2005**, *24* (4), 305–313. DOI: 10.1016/j.ejps.2004.11.007.
43. Higashino, H.; Hasegawa, T.; Yamamoto, M.; Matsui, R.; Masaoka, Y.; Kataoka, M.; Sakuma, S.; Yamashita, S. In vitro-in vivo correlation of the effect of supersaturation on the intestinal absorption of BCS Class 2 drugs. *Mol. Pharm*. **2014**, *11* (3), 746–754. DOI: 10.1021/mp400465p.
44. Mizoguchi, M.; Kataoka, M.; Yokoyama, K.; Aihara, R.; Wada, K.; Yamashita, S. Application of an in vitro dissolution/permeation system to early screening of oral formulations of poorly soluble, weakly basic drugs containing an acidic pH-modifier. *J. Pharm. Sci*. **2018**, *107* (9), 2404–2410. DOI: 10.1016/j.xphs.2018.05.009.
45. Jinno, J.; Oh, D.; Crison, J. R.; Amidon, G. L. Dissolution of ionizable water-insoluble drugs: the combined effect of pH and surfactant. *J. Pharm. Sci*. **2000**, *89* (2), 268–274. DOI: 10.1002/(SICI)1520-6017(200002)89:2<268:AID-JPS14>3.0.CO;2-F
46. Khadra, I.; Zhou, Z.; Dunn, C.; Wilson, C. G.; Halbert, G. Statistical investigation of simulated intestinal fluid composition on the equilibrium solubility of biopharmaceutics classification system class II drugs. *Eur. J. Pharm. Sci*. **2015**, *67*, 65–75. DOI: 10.1016/j.ejps.2014.10.019.

dx.doi.org/10.14227/DT300323P154

Dissolution Profile of Calcium Supplements in Brazil: A Critical Analysis and Formulation Proposal

Rosana Pereira da Silva[1], Michele George Issa[1], Vinícius Danilo Nonato Bezzon[2], Alexandre Gomes Rodrigues[3], and Humberto Gomes Ferraz[1*]

[1]*Department of Pharmacy, Faculty of Pharmaceutical Sciences, University of Sao Paulo, Sao Paulo, Brazil.*
[2]*Centre for Natural and Human Sciences, Federal University of ABC, Sao Paulo, Brazil.*
[3]*Alphaomega Labor, Leipzig, Germany.*

e-mail: sferraz@usp.br

ABSTRACT

Low calcium intake is common worldwide and may lead to osteoporosis. Therefore, calcium supplementation is a vital resource to prevent fractures in patients with osteoporosis. The present study aims to assess whether the dissolution profiles of calcium tablets available in the Brazilian pharmaceutical market are equivalent and interchangeable. Seven commercial samples from the local pharmaceutical market and an experimental formulation containing calcium carbonate from seaweed *Lithothamnium calcareum* were evaluated. In addition to the dissolution test, the tablets were characterized according to average weight, hardness, disintegration time, and calcium content. Moreover, we determined the polymorphic forms of calcium present in the tablets by employing x-ray diffraction. We related the data of these quality attributes by applying principal component analysis (PCA). The results revealed that the formulation containing calcium carbonate from the seaweed *L. calcareum* outperformed the other products from the market, with a complete dissolution within 10 min. Statistically significant differences in dissolution efficiency were noted. The disintegration times for all samples varied greatly from 12 s to 14 min. Polymorphic forms were identified in two samples, and the calcium content of the commercial samples was out of pharmacopeial specification. Thus, the products cannot be considered equivalent. It is recommended to evaluate the manufacturing processes for these supplements.

KEYWORDS: dissolution profile, calcium carbonate, calcium citrate, *Lithothamnium calcareum*, osteoporosis

INTRODUCTION

Intake of inadequate milk or milk derivates may lead to calcium deficiency, which may develop into osteoporosis, a medical condition in which the bones lose density and quality, becoming more prone to fractures. It affects one-third of women and one-fifth of men over the age of 50 worldwide (*1*). In Brazil, this number is about 10 million, causing pain and making daily life more challenging. After a hip fracture, 20–24% of patients with osteoporosis die within a year, and 60% of patients require assistance 1 year later; this illustrates the potential severity of osteoporosis. Estimates of emotional suffering and economic losses are around $200 million in Brazil. A healthy lifestyle should be pursued for prevention, including adequate calcium intake (*2*, *3*).

The average calcium intake in the diet is inadequate worldwide. In South America, the population consumes on average 400–600 mg/day, despite the recommended dose of 1000 mg/day for an adult (19–50 years old) (*1*, *4*).

Therapeutic options to combat osteoporosis have increased, including dietary calcium supplements in the pharmaceutical form of coated or chewable tablets (*5*, *6*). However, calcium in food supplementation can come from several sources, such as biogenic calcium carbonate ($CaCO_3$), such as *Lithothamnium calcareum* seaweed, $CaCO_3$ from oysters, and the mineral $CaCO_3$, which is the most traditional. In addition, $CaCO_3$ contains higher elemental calcium (Ca^{++}) content (40%) compared to other calcium salts (*7*, *8*).

*Corresponding author

A daily calcium intake (700–1200 mg/day) with 800 IU or more of vitamin D is recommended to prevent fracture in adults over 50 years old. This combination is also important for patients at high risk for fractures and those who use medication to treat osteoporosis, such as bisphosphonates (*9*). Despite some controversy, there is a consensus that the calcium-vitamin D association is beneficial for patients with low calcium and/or osteoporosis (*10*).

Calcium dissolution from formulations containing calcium can be challenging due to its absorption by the body, regardless of dosage. This question was addressed by Brennan et al., who evaluated 27 commercial samples containing calcium in the USA and found that 67% of these did not present adequate dissolution (*11*). This is the only study addressing the dissolution profile of products containing calcium.

The dissolution of calcium carbonate formulations and its different salts can be affected by several factors, such as those related to quality attributes (hardness, disintegration, content, dissolution efficiency, among others) (*12–15*). A useful way to identify and relate characteristics, such as the quality attributes of pharmaceuticals formulations, is to apply principal component analysis (PCA). In addition to being an exploratory method, the PCA technique is capable of separating important information from the collected data. PCA can objectively detect several variables in a given set of data and group individuals according to their variation (*16*). A comparison of variance (ANOVA) was also performed with the dissolution efficiency (DE) data, and the formulations were grouped by Tukey's test to confirm significant differences in the calcium release of the analyzed samples.

The objective of the present study is to assess and evaluate the dissolution profile of calcium tablets available in the Brazilian pharmaceutical market and compare with a calcium carbonate formulation from the seaweed *L. calcareum*.

METHODS

Samples

Seven samples of calcium tablets from different manufacturers were acquired from pharmacies in the city of São Paulo, Brazil. The samples were identified by alphabetical letter, composition, calcium per tablet, and expiration date, respectively, as shown below:

- A - oyster calcium carbonate, 500 mg, Oct 2017
- B - calcium carbonate, 600 mg, Dec 2017
- C - oyster calcium carbonate, 500 mg, Feb 2018
- D - calcium citrate malate, 500 mg, Dec 2018
- E - calcium carbonate, 600 mg, Dec 2018
- F - calcium citrate, 600 mg, Feb 2019
- G - calcium citrate malate, 250 mg, Aug 2017

All products were evaluated prior to the expiration date.

In addition, an experimental calcium carbonate formulation (500 mg of elemental calcium) from the seaweed *L. calcareum* was produced. The *L. calcareum* used was previously characterized as described in da Silva et al. (*17*). The process used to prepare this formulation was wet granulation, with conditions established in a Mixer Torque Rheometer (*18*).

Reagents

The analytical grade reagents used were hydrochloric acid (LabSynth, São Paulo, Brazil), sodium hydroxide (LabSynth), and edetate disodium (Merck, Darmstadt, Germany). Polyethylene cannula filters with 45-µ porous ultrahigh molecular weight (Quality Lab Accessories, PA, USA) were used to filter the aliquots during the dissolution tests. Other reagents included ultrapurified water (Merck Millipore, Darmstadt, Germany) and hydroxynaphthol blue (Dinâmica Química, São Paulo, Brazil).

Physical Characterization of Tablets

The tablets were visually inspected (by the naked eye), and their external characteristics such as color, odor, tablet shape, surface aspects, whether coated or uncoated, were described. The samples were characterized according to the *Brazilian Pharmacopoeia* for tablet pharmaceutical forms, including average weight, hardness test, thickness, diameter, and disintegration time (*19*).

X-Ray Diffraction

Powder x-ray diffraction analyses were conducted in a Panalytical Empyrean diffractometer (Malvern Instruments, Malvern, UK). Previously, the tablets were crushed in a mortar with a pestle until a homogeneous powder formed. A chrome-steel planetary spray container (dry) was selected. The instrumental parameters employed were Cu radiation obtained with a voltage of 45 kV and a current of 40 mA. Angular range analyzed from 2–65° (2θ) in the angular step of 0.02° (2θ), and time per step was 150 s. Data were collected in reflection mode in Bragg-Brentano geometry.

To identify polymorphs, the Cambridge Structural Database (CSD) and Inorganic Crystal Structure Database

(ICSD) were used to access structural models (i.e., Crystallographic Information Framework [CIF]). Rietveld refinement was used to confirm the polymorphic phase and quantify the present phases (*20*). The TOPAS-Academic V7 program was employed, in which network parameters of the unit cells, crystallite size, and adjusted background were refined using the Chebyshev polynomial function with eight terms (*21*). The structures used during refinement can be found in the mentioned databases with the ICSD codes 40109 (magnesian calcite), 252901 (aragonite), 150 (calcite), 21017 (talcum), and 248960 (brucite), and CSD code LACTOS01 (∝-lactose).

Calcium Content

Calcium quantification in the tablets was performed as recommended by the *United States Pharmacopeia* (*22*). For the measurement, a digital burette (Bürette II, Gerbershausen, Germany) and quantitative filter of 18.50 ± 0.15 cm (Framex, Blumenau, Brazil) with average filtration speed of 140 s were used. The reagents were prepared as described in the *USP* method.

The quantification of dissolved calcium was performed using Flame Atomic Absorption Spectrometry (Varian SpectrAA 50B, CA, USA). The instrumental parameters applied were acetylene air pressure at 1.5 bar, compressed air pressure at 3 bar, current intensity (40 mA) of the cathode lamp (Photron Hollow-HAG0054, Victoria, Australia), manual burner height adjustments, slit opening of 1.0, and wavelength of 422 nm. For the calculation of calcium quantification, the linearity result was considered with the coefficient of determination (*R*) of 0.998. The solution was prepared at a concentration of 1000 µg/mL (in triplicate) and subsequently diluted to 25, 50, 75, 100, 150, and 200 µg/mL. A 99% content standard (Dinâmica Química) was used for this procedure.

Dissolution Tests

The dissolution tests were conducted using USP apparatus 2 (paddle) and 708-DS Dissolution Apparatus equipment (Agilent Technologies Inc., Santa Clara, CA, USA) for 60 minutes. The dissolution medium was 750 mL of hydrochloric acid 0.01 N at 37 ± 0.5 °C and 75 rpm. Samples (5 mL aliquots, in triplicate) were collected at intervals of 5, 10, 15, 20, 30, 45, and 60 min, without medium replacement, and the aliquots were filtered through 45-µ porous cannula filters. Subsequently, calcium was quantified with dilutions from 4 to 30 times in an atomic absorption spectrometer.

The dissolution profiles were derived from the results obtained using Microsoft Excel software. DE was calculated with the aid of the Microsoft Excel DDSolver add-in (Simulations Plus), as described by Zhang and collaborators (*23*). The DE parameter was calculated using the following equation (*24*): $DE\% = \int 0ty \times dty_{100} \times t \times 100\%$, where **y** is the percentage of drug (*d*) dissolved at time *t*.

Statistical Analysis

Cal Initially, PCA was performed using the data of hardness, disintegration, content, and the percentage of dissolved calcium at 15 ($Q\%_{15min}$) and 45 min ($Q\%_{45min}$), in addition to DE. Statistica (version 13.5.0.17, TIBCO Software Inc., Tulsa, OK, USA) was used for the analyses. After standardizing the data, new variables were created, and those with higher eigenvalues (CPA1 and CPA2) were selected for the construction of two-dimensional graphs.

For comparative effect, a one-way analysis of variance (ANOVA) was performed with the DE data, and the formulations were grouped using the Tukey test. Normality of the data was tested using the Anderson-Darling method, requiring the transformation of this method by applying the Johnson model. Action Stat (version 3.6.331.450 build 7 – 2019, Estatcamp, São Carlos, Brazil) was used for these analyses.

RESULTS AND DISCUSSION

Sample Characterization

The calcium tablets presented no apparent defects, with a varied shape, with or without coating. As shown in Table 1, the lowest weight corresponded to the tablet in the form of citrate (product F). The highest weight (product B) also presented the highest amount of calcium (600 mg). Interestingly, the weight of product G contained only 250 mg of calcium in citrate malate. Product G contains a high amount of excipients and the lowest calcium dose compared to other products studied here.

The weight, size, and consequently, the volume of the pill can represent considerable discomfort for the patient when ingested, impacting adherence to the treatment. Thus, immediate-release formulations are needed to disintegrate rapidly in the gastric fluid. The disintegration time varied from 12 s to 14 min, regardless of hardness values. Products C and D required a longer disintegration time, exceeding 9 min, while product G disintegrated in 14 min. In contrast, the formulation of *L. calcareum* $CaCO_3$ and products B and F stood out, disintegrating in under 1 min.

The calcium content of the tablets should be 90–115% of the label claim to comply with USP specifications (*22*).

Table 1. Sample Characterization

Product	Weight, g (*n* = 20)	Hardness, Kgf (*n* = 10)	Thickness, cm (*n* = 20)	Diameter, cm (*n* = 20)	Disintegration Time, min:sec (*n* = 6)	Ca^{++} Content, mg (%) (*n* = 9)
L. calcareum $CaCO_3$*	1.77 ± 0.30	8.00 ± 0.20	0.61	1.20	0:39 ± 0:01	529.42 (106.01)
A*	1.69 ± 0.05	25.67 ± 0.93	0.48	1.94	6:24 ± 0:12	538.65 (107.73)
B**	1.92 ± 0.01	6.50 ± 0.11	0.51	1.99	0:12 ± 0:01	602.06 (100.34)
C*	1.58 ± 0.03	20.50 ± 0.17	0.51	1.73	9:13 ± 0:12	562.82 (112.56)
D*	1.59 ± 0.03	18.70 ± 0.79	0.76	2.16	9:63 ± 0:32	434.39 (86.87)
E**	1.78 ± 0.03	13.20 ± 1.47	0.61	1.94	1:68 ± 0:61	605.87 (100.97)
F**	1.26 ± 0.01	14.57 ± 1.06	0.58	1.68	0:27± 0:02	578.21 (96.36)
G***	1.88 ± 0.02	8.87 ± 1.35	0.47	1.72	14:00 ± 0:35	255.48 (102.19)

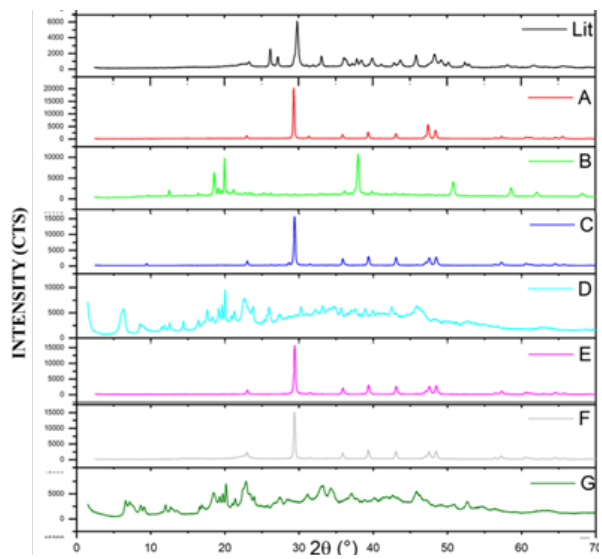
Data are expressed as mean ± SD.
**500 mg; **600 mg; ***250 mg*

Product D contained only 86.87% calcium. In these cases, the manufacturer should review their procedures to conform to the specifications.

X-Ray Diffraction Analysis

Refinement of the crystalline structures and quantification of the crystalline phases was possible for all samples of *L. calcareum* $CaCO_3$, A, B, C, E, and F, because all were identified in the databases (Table 2). Samples D and G presented several phases with high peak overlap, which prevented their identification, but they contain a significant amount of amorphous phase. Both samples have broad peaks and undefined characteristics, such as the calcite peak or another polymorphic phase identified in the samples, which indicates the absence of crystallinity (*25*, *26*).

Table 2. X-ray Diffraction Patterns, Calcium Type, and Distribution of Crystalline Phases

Comparison of XRD Patterns for Sample Standards	Product	Type of Calcium	Crystalline Phases and Mass Ratio (wt% ± SD)
	*L. calcareum* $CaCO_3$	Calcium carbonate	Aragonite (50.8 ± 6.0) and magnesian calcite (49.2 ± 6.0)
	A	Oyster calcium carbonate	Aragonite (1.83 ± 3.0) and calcite (98.20 ± 4.0)
	B	Calcium carbonate	Calcite (0.99 ± 2.0) and brucite (99.01 ± 2.0)
	C	Oyster calcium carbonate	Aragonite (2.82 ± 4.0) and calcite (96.82 ± 5.0)
	D*	Calcium citrate malate	Not determined
	E	Calcium carbonate	Aragonite (0.6 ± 3.0) and calcite (99.4 ± 3.0)
	F	Calcium citrate	Calcite (100%)
	G*	Calcium citrate malate	Not determined

**Products D and G were not determined due to no crystalline phase found.*
XRD: x-ray diffraction.

The crystalline phases constituted by aragonite-calcite are typical of biogenic samples, i.e., samples from the ocean, which corresponds to that indicated in products A and C from oyster calcium carbonate and in the formulation of *L. calcareum* $CaCO_3$, which is from marine origin containing only calcium carbonate (*27*). However, the manufacturer of product E failed to indicate the marine origin of its product, because we could detect a small proportion of aragonite in the product. Interestingly, aragonite is the dominant phase in L. calcareum $CaCO_3$, representing an advantage, as this phase is metastable and much more soluble than magnesian calcite, which is thermodynamically stable (*28*). On the contrary, product F consists of 100% calcite, which is a disadvantage concerning calcium solubility, as can be seen from its behavior in the dissolution test.

Dissolution Profiles

Dissolution profiles were very different among the calcium tablets and *L. calcareum* $CaCO_3$ formulation (Fig. 1, Table 3). The formulation of L. calcareum CaCO3 stood out with a quick calcium release, dissolving entirely within 10 min, followed by product C, A, B, and E. In contrast, products D, F, and G presented slower dissolution, with D and F having a marked deficiency in calcium dissolution. For product D, the explanation can be linked to two aspects: the high disintegration time (9:63 ± 0:32) and low calcium content (86.87%). Product F, in contrast, exhibited a disintegration time of only 12 s; however, DE was only 68%, which is unfavorable. In this case, the tablet can break down very quickly, but its calcium content does not dissolve in the same proportion. This phenomenon can be attributed to the raw material characteristics, i.e., calcium citrate was included in the formulation (*12*, *13*).

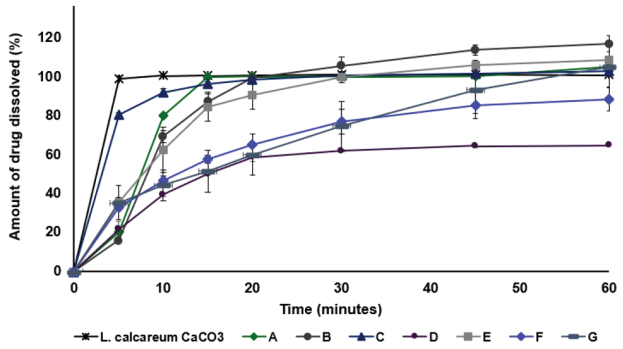


Figure 1. Dissolution profiles of calcium and Lithothamnium calcareum $CaCO_3$ samples (n = 3).

Product G presented an inadequate disintegration time (14 min), the highest of all samples studied here. The excipients used in the formulation development, especially the binder, likely influenced the tablet disintegration (*14*). The lack or insufficient amount of a disintegrant can also contribute to such inadequate performance (*15*).

It is important to highlight that the samples investigated contained different calcium salts, which influenced the dissolution profile. Cartensen et al. demonstrated that calcium salts do not have the same dissolution behavior; however, they concluded that the in vitro dissolution data for different calcium salts are similar to in vivo results (i.e., bioavailability) (*29*). Even though these data were for calcium salts and not for dosage, such findings corroborate the data presented here. Therefore, we can attribute the differences in dissolution profiles to different calcium salts.

As shown in Table 3, the tested products were not equivalent based on the average percentage of dissolved calcium. DE was greater than 90% in only three formulations. Therefore, the most similar formulations to $CaCO_3$ from *L. calcareum* were products B and C. In addition to having rapid calcium release, these products are different in their origin: B is calcium carbonate and C it is oyster calcium carbonate.

Table 3. Dissolution of Calcium Within 60 Minutes

Product	Dissolved Calcium (%), mean ± SD	Dissolution Efficiency (%)
L. calcareum $CaCO_3$	101.05 ± 2.15	96.90
A	104.97 ± 3.16	88.36
B	116.98 ± 4.32	91.87
C	102.84 ± 2.42	93.86
D	64.73 ± 0.96	53.64
E	108.87 ± 0.16	87.42
F	88.56 ± 6.01	68.00
G	105.04 ± 10.77	70.44

Statistical Analysis

PCA results are shown in Table 4. In Figure 2, it is noticeable that the two new variables created from the distribution presented (PCA1 and PCA2) were able to retain 84.01% of the original information contained in the input factors (hardness, disintegration, content, $Q\%_{15min}$, $Q\%_{45min}$, and DE). Concerning PCA1, the samples approximated the results from the dissolution test (Fig. 1). The samples that had a slower release (F and D), as in the case of product G, were closer together. The type of salt used also influenced the grouping in PCA1. All samples on the left side contain calcium carbonate, and those on the right contain citrate

or citrate malate. The isolation of product D is most likely due to lower calcium content.

In the case of PCA2, hardness and disintegration showed a greater influence on the grouping of the samples. However, as shown in Table 4, the coating appears to influence disintegration time and hardness.

The Anderson-Darling test was performed to assess the normality of the DE data and analyze the ANOVA, obtaining a *P*-value of 0.0009. When considering a significance level of 0.05, the previous transformation of data was necessary. A new test was performed after applying the Johnson model, resulting in a higher *P*-value (0.536), thus attesting to normality. The *P*-value obtained with ANOVA ($p < 0.05$) confirmed statistically significant differences in calcium release from the samples analyzed. With the Tukey test, it was possible to perform the grouping and understand where this difference is located.

The results were similar to the PCA, the only difference being separation of *L. calcareum* $CaCO_3$ from the other samples. This was due to the responses adopted for each analysis (i.e., other data points were considered in the PCA, such as $Q\%_{15min}$).

According to the statistical analyses, the tested products cannot be considered equivalent or interchangeable due to products D, F, and G failing to meet USP specifications for calcium content (D and F) and dissolution of calcium (D, F, and G).

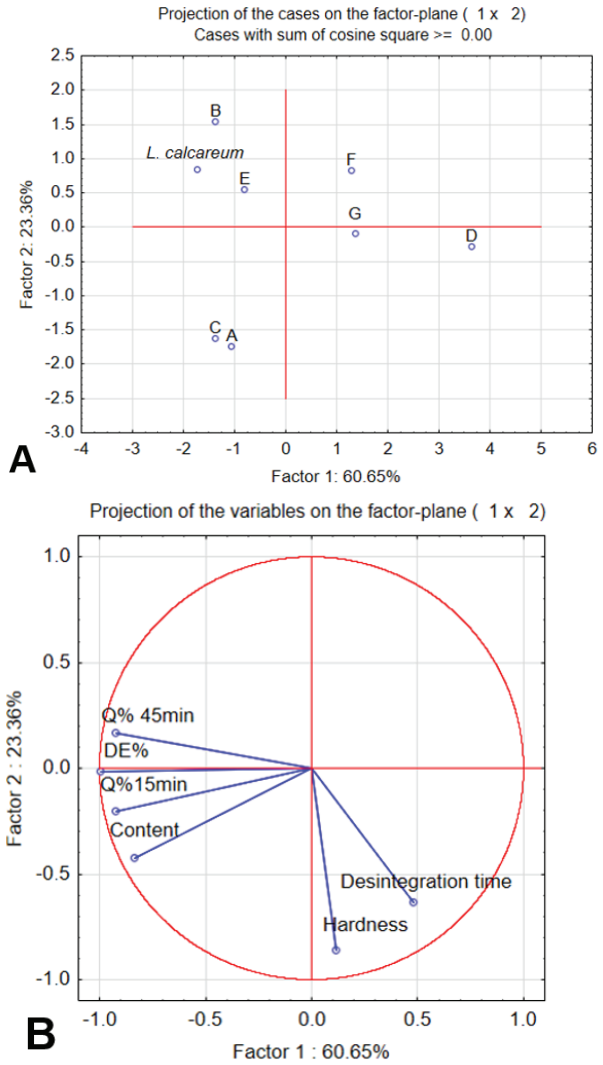


Figure 2. Graphs of principal component analysis. (A) Distribution of samples for comparison. (B) 2D graph of principal components; both factors 1 and 2 correspond to 84.01% of the information contained in the original variables.

Table 4. Results for the Four Groups Suggested by PCA.

Product	Type of Salt	Dose (mg)	Coating	Hardness (Kgf)	Disintegration Time (s)	Content (%)	Cumulative Drug Release (%)		DE%
							15 min	45 min	
Group 1									
L. calcareum $CaCO_3$	Carbonate	500	No	8	39	106.01	100.94	101.26	96.90
B	Carbonate	600	No	6.5	12	100.34	87.23	113.78	91.87
E	Carbonate	600	Yes	13.2	128	100.97	84.63	106.17	87.42
Group 2									
A	Carbonate	500	Yes	25.87	384	107.73	100.06	100.49	88.36
C	Carbonate	500	Yes	20.5	553	112.56	96.15	101.56	93.86
Group 3									
F	Citrate	600	No	14.57	27	96.36	57.56	85.45	68.00
G	Citrate	250	Yes	8.87	840	102.19	51.40	93.30	70.44
Group 4									
D	Citrate malate	500	Yes	18.7	603	86.87	49.99	64.41	53.64

PCA: principal component analysis; DE: dissolution efficiency.

CONCLUSION

The formulation of *L. calcareum* $CaCO_3$ exhibited a much higher dissolution rate, probably due to the presence of aragonite in high concentrations and low disintegration time. Statistical analysis of dissolution profiles revealed the existence of different groups, ranging from products with an outstanding profile to products with a marked deficiency in the release of calcium. Therefore, calcium supplements found in the Brazilian market are not equivalent. In some cases, manufacturers should review their formulations and manufacturing processes to improve relevant quality aspects.

ACKNOWLEDGMENTS

The authors thank Lithocalcio Industria, Comércio, Importação & Exportação Ltda, Brazil for the generous donation of calcium carbonate from the seaweed *L. calcareum*.

FUNDING

The authors disclosed no funding for this research.

CONFLICT OF INTEREST

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

REFERENCES

1. What is osteoporosis? International Osteoporosis Foundation website. 2020. https://www.iofbonehealth.org/what-is-osteoporosis (accessed August 14, 2020).
2. Heidari, B.; Hajian-Tilaki, K.; Babaei, M. Effectiveness, and safety of routine calcium supplementation in postmenopausal women. A narrative review. *Diabetes Metab. Syndr*. **2020**, *14* (4), 435–442. DOI: 10.1016/j.dsx.2020.04.016. Erratum *Diabetes Metab. Syndr*. **2020**, *15* (1), 470. Erratum *Diabetes Metab. Syndr*. **2020**, *16* (5), 102505.
3. Capozzi, A.; Scambia, G.; Lello, S. Calcium, vitamin D, vitamin K2, and magnesium supplementation and skeletal health. *Maturitas*. **2020**, *140*, 55–63. DOI: 10.1016/j.maturitas.2020.05.020.
4. Balk, E. M.; Adam, G. P.; Langberg, V. N.; Earley, A.; Clark, P.; Ebeling, P. R.; Mithal, A.; Rizzoli, R.; Zerbini, C. A. F.; Pierroz, D. D.; Dawson-Hughes, B.; International Osteoporosis Foundation Calcium Steering Committee. Global dietary calcium intake among adults: a systematic review. *Osteoporos. Int*. **2017**, *28* (12), 3315–3324. DOI: 10.1007/s00198-017-4230-x. Erratum *Osteoporos. Int*. **2018**, *29*, 1223.
5. Langdahl, B. L. New treatments of osteoporosis. *Osteoporos. Sarcopenia*. **2015**, *1* (1), 4–21. DOI: 10.1016/j.afos.2015.07.007.
6. Golob, A. L.; Laya, M. B. Osteoporosis screening, prevention and managment. *Med. Clin. North Am*. **2015**, *99* (3), 587–606. DOI: 10.1016/j.mcna.2015.01.010.
7. Al Omari, M. M. H.; Rashid, I. S.; Qinna, N. A.; Jaber, A. M.; Badwan, A. A. Calcium carbonate. *Profiles Drug Subst. Excip. Relat. Methodol*. **2016**, *41*, 31–132. DOI: 10.1016/bs.podrm.2015.11.003.
8. Hickman, G. J.; Belton, D. J.; Newick, R.; Perry, C. C. Barriers to adoption of biogenic carbonates in the food, pharmaceutical & supplement sectors. *NFS J*. **2019**, *16*, 1–8. DOI: 10.1016/j.nfs.2019.05.002.
9. Föger-Samwald, U.; Dovjak, P.; Azizi-Semrad, U.; Kerschan-Schindl, K.; Pietschmann, P. Osteoporosis: pathophysiology and therapeutic options. *EXCLI J*. **2020**, *19*, 1017–1037. DOI: 10.17179/excli2020-2591.
10. Harvey, N. C.; Biver, E.; Kaufman, J-M.; Bauer, J.; Branco, J.; Brandi, M. L.; Bruyère, O.; Coxam, V.; Cruz-Jentoft, A.; Czerwinski, E.; Dimai, H.; Fardellone, P.; Landi, F.; Reginster, J.-Y.; Dawson-Hughes, B.; Kanis, J.A.; Rizzoli, R.; Cooper, C. The role of calcium supplementation in healthy musculoskeletal ageing: An expert consensus meeting of the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO) and the International Foundation for Osteoporosis (IOF). *Osteoporos. Int*. **2017**, *28* (2), 447–462. DOI: 10.1007/s00198-016-3773-6.
11. Brennan, M. J.; Duncan, W. E.; Wartofsky, L.; Butler, V. M.; Wray, H. L. In vitro dissolution of calcium carbonate preparations. *Calcif. Tissue Int.* **1991**, *49* (5), 308–312. DOI: 10.1007/BF02556251.
12. Markl, D.; Zeitler, J. A. A review of disintegration mechanisms and measurement techniques. *Pharm. Res*. **2017**, *34* (5), 890–917. DOI: 10.1007/s11095-017-2129-z.
13. Mehtani, D.; Seth, A.; Sharma, P.; Maheshwari, R.; Abed, S. N.; Deb, P. K.; Chougule, C. B.; Tekade, R. K. Chapter 9 - Dissolution profile consideration in pharmaceutical product development. In *Dosage Form Design Considerations; Adv. Pharm. Product Dev. Res*. Academic Press, 2018; pp 287–336. DOI: 10.1016/B978-0-12-814423-7.00009-5.
14. Jaya, S.; Chowdaryp, K. P. R.; Rajeswara Rao, P. Effect of binders on the dissolution rate and dissolution efficiency of ritonavir tablets. *Int. Res. J. Pharm. Appl. Sci*. **2012**, *2* (4), 109–113.
15. Li, J.; Wu, Y. Lubricants in pharmaceutical solid dosage forms. *Lubricants* **2014**, *2* (1), 21–43. DOI: 10.3390/lubricants2010021.
16. Hongyu, K.; Sandanielo, V. L. M.; Junior, G. J. de O. [Principal component analysis: theory, interpretations and applications] [in Portuguese]. *Engineer. Sci*. **2016**, *5* (1), 83–90. DOI: 10.18607/ES201653398.
17. da Silva, R. P.; Kawai, G. S. D.; Andrade, F. R. D. D.; Bezzon, V. D. N.; Ferraz, H. G. Characterisation and traceability of calcium carbonate from the seaweed Lithothamnium calcareum. *Solids*, **2021**, *2* (2), 192–211. DOI: 10.3390/solids2020013.
18. da Silva, R. P.; Fante, A. S.; Silva, A. R. P.; Pereira, F. L. S.; Gutierrez, Y. L. R.; Ferraz, H. G. Wet powder rheometry: the best conditions for wet granulation using diluent and binder in calcium carbonate samples. *Powder Technol*. **2022**, *397*, 1–11. DOI: 10.1016/j.powtec.2021.117087.
19. *Brazilian Pharmacopoeia*, 6th ed. Agência Nacional de Vigilância Sanitária – Anvisa, 2019.

20. Rietveld, H. M. A profile refinement method for nuclear and magnetic structures. *J. Appl. Cryst*. **1969**, *2* (2), 65–71. DOI: 10.1107/S0021889869006558.
21. Coelho, A. A. Topas and Topas-Academic: an optimization program integrating computer algebra and crystallographic objects written in C++. *J. Appl. Cryst*. **2018**, *51* (1), 1–9. DOI: 10.1107/S1600576718000183.
22. *USP-NF*. United States Pharmacopeial Convention Inc., Rockville, MD, 2019.
23. Zhang, Y.; Huo, M.; Zhou, J.; Zou, A.; Li, W.; Yao, C.; Xie, S. DDSolver: an add-in program for modeling and comparison of drug dissolution profiles. *AAPS J*. **2010**, *12* (3), 263–271. DOI: 10.1208/s12248-010-9185-1.
24. Costa, P.; Sousa Lobo, J. M. Modeling and comparison of dissolution profiles. *Eur. J. Pharm. Sci*. **2001**, *13* (2), 123–133. DOI: 10.1016/S0928-0987(01)00095-1.
25. Kennedy, M.; Hu, J.; Gao, P.; Li, L.; Ali-Reynolds, A.; Chal, B.; Gupta, V.; Ma, C.; Mahajan, N.; Akrami, A.; Surapaneni, S. Enhanced bioavailability of a poorly soluble VR1 antagonist using an amorphous solid dispersion approach: a case study. *Mol. Pharm*. **2008**, *5* (6), 981–993. DOI: 10.1021/mp800061r.
26. Wiedmann, T. S.; Naqwi, A. Pharmaceutical salts: theory, use in solid dosage forms and in situ preparation in an aerosol. *Asian J. Pharm. Sci*. **2016**, *11* (6), 722–734. DOI: 10.1016/j.ajps.2016.07.002.
27. Zeebe, R. E.; Westbroek, P. A simple model for the CaCO3 saturation state of the ocean: the "Strangelove," the "Neritan," and the "Cretan" Ocean. *Geochem. Geophys. Geosyst*. **2003**, *4* (12), 1–26. DOI: 10.1029/2003GC000538.
28. Orr, J. C.; Fabry, V. J.; Aumont, O.; Bopp, L.; Doney, S. C.; Feely, R. A.; Gnanadesikan, A.; Gruber, N.; Ishida, A.; Joos, F.; Key, R. M.; Lindsay, K.; Maier-Reimer, E.; Matear, R.; Monfray, P.; Mouchet, A.; Najjar, R. G.; Plattner, G-K.; Rodgers, K. B.; Sabine, C. L.; Sarmiento, J. L.; Schlitzer, R.; Slater, R. D.; Totterdell, I. J.; Weirig, M-F.; Yamanaka, Y.; Yool, A. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*. **2005**, *437*, 681–686. DOI: 10.1038/nature04095.
29. Carstensen, J. T.; Jarecki, R.; Ertell, C. Handling of non-sink conditions: in-vitro dissolution rates of some common pharmaceutical calcium salts. *Drug Dev. Ind. Pharm*. **1988**, *14* (14), 1971–1989. DOI: 10.3109/03639048809151999.

dx.doi.org/10.14227/DT300323P162

Dissolution Method Development for Regulatory Approval: A Comprehensive Review and Case Study

Pramod Parshuramkar[1,*], Deepak Khobragade[2], and Pranita Kashyap[3]
[1]*Centaur Pharmaceuticals Pvt. Ltd., Hinjewadi, Pune, India.*
[2]*Datta Meghe Institute of Medical Sciences, Wardha, Maharashtra, India.*
[3]*Dr. R. G. Bhoyar Institute of Pharmaceutical Education and Research, Wardha, Maharashtra, India.*

e-mail: pramodudps@yahoo.co.in

ABSTRACT

In vitro dissolution testing is an important tool for any oral drug product. It is useful for product development and to ensure the in vivo performance of drug products throughout their commercial life without conducting clinical or bioequivalence studies after regulatory approval. It also plays a role in maintaining batch-to-batch consistency, providing quality assurance, supporting biowaiver and post-approval changes, etc. Guidelines from regulatory agencies provide expectations about the dissolution method and acceptance criteria; however, pharmaceutical manufacturers may fail to comply with the requirements or the expectations of the agencies, resulting in dissolution deficiencies. Furthermore, updates in dissolution testing may not be effectively communicated to non-industry scientists who are involved in dissolution or drug delivery research. This article provides a comprehensive review of the current American and European regulatory guidance for solid oral dosage forms followed by a case study to demonstrate how a dissolution method should be developed, which can be used as a framework for any drug product.

KEYWORDS: Dissolution method development, dissolution specification, discriminatory power, dissolution apparatus, dissolution medium

INTRODUCTION

This article focuses on the two main regulatory agencies, the United States Food and Drug Administration (FDA) and the European Medicine Agency (EMA). In general, other health authorities consider the development approach followed by these two agencies appropriate and accept the same dissolution methods with supporting rationale. Both agencies have their own guidance and expectations about the dissolution method and acceptance criteria. The FDA and EMA guidance documents are non-binding recommendations from the agencies, so alternative approaches can also be used and justified provided that the dissolution method has sufficient discriminatory power to assess the critical quality attributes (CQAs) of a drug product. This article provides a comprehensive review of the requirements, expectations, significance, and rationale for selection of dissolution test conditions and acceptance criteria. This article also provides a framework for dissolution method development, including examples and case studies for easy interpretation by pharmaceutical scientists.

United States FDA Guidelines

In 1997, the FDA published two guidances for industry that discuss the dissolution method and specifications for acceptance (*1*, *2*):

- *Dissolution testing of immediate release solid oral dosage forms*
- *Extended-release oral dosage forms: development, evaluation, and application of in vitro/in vivo correlations*

For generic product development, the FDA recommended to consider the *United States Pharmacopeia* (USP) and the dissolution method database maintained by the Office of Generic Drugs (*3*). The historical approach for dissolution method development for generic drugs was as follows (*4*). If the dissolution method is published in the *USP* drug-specific monograph, then directly use the same dissolution method for the generic product. If the dissolution method is not in the USP or no monograph has been published, then refer and follow the method

*Corresponding author

published in the FDA's dissolution method database. If the above-mentioned conditions are not suitable, then develop a new dissolution method.

The FDA's perspective on developing the dissolution method recently changed from a historical approach to a biopharmaceutical approach. Considering this, the FDA published the following guidance since 2017 (*4*–*7*):

- *Waiver of in vivo bioavailability and bioequivalence studies for immediate release solid oral dosage forms based on a Biopharmaceutics Classification System (BCS)*
- *M9 biopharmaceutics classification system-based biowaivers*
- *Dissolution testing and acceptance criteria for immediate release solid oral dosage form drug products containing high solubility drug substances*

Immediate-Release Dosage Forms

For immediate-release (IR) drug products, the BCS should be considered when selecting the dissolution method and acceptance criteria (*4*). The dissolution acceptance criteria should be 80% of drug release within 30 min.

For drug products containing highly soluble drug substances, the following dissolution methods should be used (*7*).

- *Method A*: Basket apparatus; 0.1 N HCl medium, 500 mL volume; 100 rpm agitation speed; without surfactant.
- *Method B*: Paddle apparatus; 0.1 N HCl medium, 500 mL volume; 50 rpm agitation speed; without surfactant. A sinker can be added as per the need, and agitation speed can be increased to 75 rpm with justification.

With appropriate justification, other test conditions can be used and accepted by regulatory agencies. For IR drug products containing highly soluble drug substances, the dissolution test can be replaced with the disintegration test in the finished product specifications with adequate justification (*8*).

For drug products containing poorly soluble drug substances, both *USP* and FDA databases should be used as a starting point to see what conditions have already been approved. The selection of the dissolution method should be based on its feasibility and discriminatory power for the proposed drug product. A new method can be developed and validated if the USP and or FDA methods are not available or found inadequate. The selection of the time point should be where not less than (NLT) 80% of the drug is dissolved.

Extended-Release Dosage Forms

Irrespective of the method availability in the USP or FDA dissolution method database, it is expected that a product-specific discriminatory dissolution method should be developed, thoroughly evaluated, and validated for extended-release (ER) dosage forms. When setting the product specifications, a minimum of three time points should be selected to cover the initial, middle, and final phases of the dissolution profile. Dissolution acceptance criteria for the initial and middle time points should be based on a mean target value ± 10%. The last time point should cover at least 80% of the drug release. The target value is based on the mean drug release of the lot/batch used in the clinical study.

Fixed-Dose Combination Products

Fixed-dose combination (FDC) drug products can be the combination of two or more drug substances with similar or different release mechanisms (IR and/or ER). For FDC drug products, both USP and FDA databases should be used as a starting point to see the conditions that have already been approved for the FDC or the single component drug products (*9*).

The development of a dissolution method for FDC drug products is challenging due to the differences in physicochemical properties of the drug substances. Individual dissolution methods can be developed for each drug substance in the FDC product; however, it is expected to have a single dissolution method because of the analytical efficiency, time and cost savings, feasibility during the commercial-release testing, and reduction in the burden during the stability study. Essentially, the method should be robust and reproducible during routine quality control testing.

IR-FDC drug products comprising multiple highly soluble drug substances can be evaluated with a similar approach as an IR drug product containing a single component. Similarly, the dissolution test can be replaced with the disintegration test with adequate justification.

In IR-FDC drug products comprising substances with different solubilities, precedence should be given to the poorly soluble component over the highly soluble component because its dissolution is rate-limiting in the in vivo absorption. The selection of the time point should be where NLT 80% of the drug is dissolved.

In FDC drug products comprising substances with different release mechanisms, precedence should be given to the ER component, followed by the IR component if it is poorly soluble. When setting the acceptance criteria, depending on the release mechanisms of each component, a similar approach for IR or ER drug products containing a single component can be followed.

Delayed-Release Dosage Forms

Delayed release (DR) dosage forms commonly have an enteric coating. There can be other DR mechanisms based on the rationale behind the product design (e.g., to protect against irritation of the stomach mucus membrane, to prevent acidic degradation of the drug, or for targeted drug delivery in the gastrointestinal (GI) tract [i.e., colon targeting]). DR dosage forms can be non-disintegrating (coated tablets) and disintegrating (tablets or capsules containing coated multiple-unit pellet systems).

In general for conventional DR dosage forms, a minimum of two time points is required to meet the specifications. The first point controls the drug release in the acid stage (0.1 N HCl), usually NMT 10% in 2 h, and the second time point controls drug release in the higher pH buffer stage (pH 6.8), usually NLT 80% in 45 min. If DR dosage forms are designed for pulsatile, controlled release, or targeted delivery in the GI tract, the selection of a stage 2 dissolution medium, time points, and acceptance criteria can be set as per the expectations for each release mechanism (*10*, *11*).

European Medicines Agency Guidelines

Just like the FDA follows the *USP*, the EMA follows the *European Pharmacopeia* (*EP*). However, *EP* only has monographs for drug substances, and not drug products. Recently, *EP* has started to publish drug product monographs, including dissolution methods for drug products.

The *British Pharmacopoeia* (*BP*) includes both drug substances and drug product monographs. Earlier BP monographs were acceptable for developing drug products for European territories; however, in February 2020, the UK withdrew from the European Union and become a "third country" (*12*). So, drug products that are developed for the European market must follow and comply with EP and EMA specifications and guidance.

In addition to the general chapters by the EP for the dissolution testing, EMA has published guidelines that discuss dissolution method expectations and acceptance criteria (*13*, *14*):

- *Guideline on quality of oral modified release products*
- *Reflection paper on the dissolution specification for generic oral immediate release products*

For all dosage forms, a product-specific dissolution method with discriminatory power should be developed and validated irrespective of method availability in any public database.

Immediate-Release Dosage Forms

For IR dosage forms, the dissolution method should be developed irrespective of the drug solubility class. Dissolution acceptance criteria should be 75–85% drug release in a given period of time. The target value is the mean drug release of the lot/batch used in the clinical study minus 10%.

Extended-Release Dosage Forms

When setting the product specifications for ER dosage forms, a minimum of three time points should be selected. The first time point is to eliminate dose dumping or to ensure the loading dose (20–30% drug release). The second time point is to define the drug-release pattern (50% drug release), and the acceptance criteria should be ± 10% to the mean target value. The last time point is to ensure at least 80% of the drug release. The target value is the mean drug release of the lot/batch used in the clinical study.

Fixed Dose Combination Products

In FDC drug products comprising drug substances with different solubilities and/or release mechanisms, precedence should be given to the ER component and/or poorly soluble components. Acceptance criteria can be derived using the same principles recommended by the EMA or *EP* for individual IR or ER drug products containing a single component.

Delayed-Release Dosage Forms

For conventional DR dosage forms, expectations of the EMA are the same as the FDA. *USP* general chapter <711> and *EP* general chapter <2.9.3> have been harmonized. For non-conventional DR dosage forms, selection of a stage 2 dissolution medium, time points, and acceptance criteria can be set according to the expectations of the EMA or *EP* for each release mechanism.

DISSOLUTION METHOD DEVELOPMENT

A product-specific dissolution method should be developed in the sequence of activities given in Figure 1.

Drug Solubility and Solution Stability

An analytical method should be developed for detecting the drug using suitable detection techniques. The solubility of the drug should be determined in aqueous

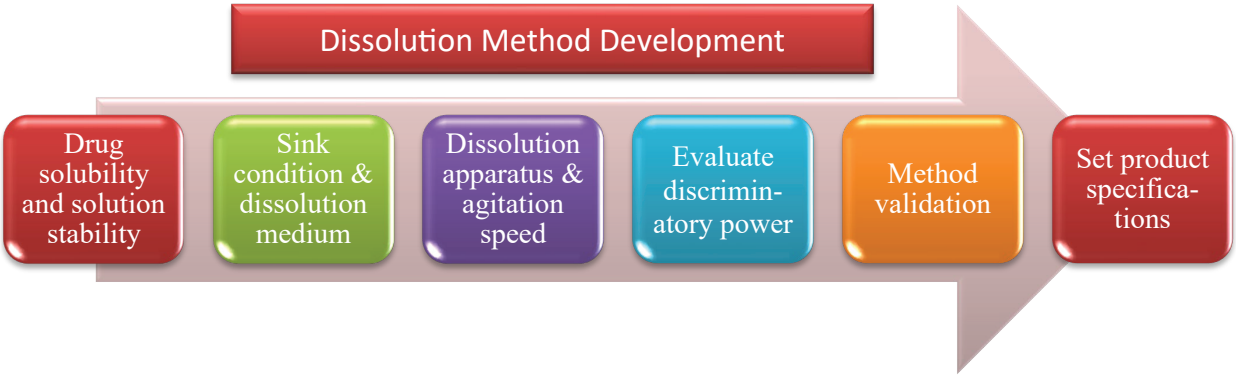


Figure 1. Flow diagram of dissolution method development.

media with a pH in the range of 1–6.8 at 37 ± 1 °C. Solution stability in each medium should be ensured using the stability-indicating assay or impurity method of the analysis. Drug solubility and stability are useful for determining the solubility class and for the selection of dissolution medium. If the highest dose of the drug substance is soluble in 250 mL of the aqueous medium with a pH in the range of 1–6.8, then that drug is considered highly soluble (*5*, *6*, *7*).

For FDC drug products, an in-depth evaluation of the physicochemical properties of each drug substance, like pH solubility, solution stability, and drug-to-drug interaction in the physiological pH range should be performed. In general for FDC drug products, an analytical method with high performance liquid chromatography (HPLC) is preferred over UV-visible spectroscopy to avoid interference in the absorbance at a particular wavelength. However, UV-visible spectroscopy methods are acceptable with the appropriate demonstration of specificity and lack of interference for the active ingredients.

Sink Conditions and Selection of Dissolution Media

To use any aqueous medium as a dissolution medium, it should be capable of maintaining the sink condition and have sufficient solution stability to cover the duration of time required to perform the dissolution test and analyze the sample aliquots. The sink condition is at least three times the volume needed to obtain a saturated solution based on the highest strength of the drug product (*10*, *15*, *16*). The preferred dissolution media volume for USP apparatus 1 and 2 (basket and paddle, respectively) is 500 900, or 1000 mL, and in the worst case, 1800 mL. For the USP apparatus 3 (reciprocating cylinder), media volume can be in the range of 200–300 mL per vessel.

For IR dosage forms containing highly soluble drugs, 500 mL of 0.1 N HCl should be directly used according to the FDA (*7*). In other cases, the choice of the medium should be based on the ability to maintain the sink condition and stability of the solution. If the drug substance has pH-independent solubility and stability, then the preferred dissolution medium can be 0.1 N HCl or purified water. If a drug substance has poor solubility in all pH ranges, then the solubility study can be conducted by adding the minimum effective concentration of the surfactant. The choice and concentration of surfactant should be based on the evaluation and appropriate justification. Commonly, sodium lauryl sulfate, polysorbate 20, and polysorbate 80 are used as surfactants in the dissolution medium. If adequate solubility to satisfy sink conditions exists only over a narrow pH range, then an appropriate buffer should be selected to maintain the pH range.

If FDC drug products contain drug substances with different solubilities, a pH should be selected that meets the sink condition for the low soluble drug. If FDC drug products contain multiple poorly soluble drug substances with different pH solubilities or pH-dependent solution stabilities, then multiple pH media and buffers should be evaluated (even within narrow pH ranges) to accommodate the sink condition and solution stability of multiple drug substances.

Dissolution of conventional DR dosage forms can be performed in 0.1 N HCl followed by a pH 6.8 phosphate buffer. The acid-stage dissolution ensures or validates the efficiency of enteric-coating polymers to avoid drug release or degradation beyond the specified limit, commonly no more than (NMT) 10%. If the drug is insoluble in 0.1 N HCl, then the acid-stage dissolution performance can be checked by developing the acid medium with the addition of surfactants. In some cases, the drug can be degraded in 0.1 N HCl, where the acid stage dissolution can be performed by detecting the degradant products alone or along with the parent drug substance.

Selection of Dissolution Apparatus and Agitation Speed

There are seven compendial dissolution apparatus used in the pharmaceutical industry, depending on the dosage form (*10, 11*):

- USP apparatus 1 (basket): used for tablets, capsules, suppositories, and floating dosage forms
- USP apparatus 2 (paddle): used for tablets, capsules (with or without sinkers), and suspensions
- USP apparatus 3 (reciprocating cylinder): used for IR, ER, and DR tablets
- USP apparatus 4 (flow-through cell): used for implants or when sink conditions cannot be achieved using another apparatus
- USP apparatus 5 (paddle over disc): used for transdermal delivery systems
- USP apparatus 6 (rotating cylinder): used for transdermal delivery systems
- USP apparatus 7 (reciprocating disc): used for transdermal delivery systems and ER tablets

USP apparatus 1 and 2 (baskets and paddle, respectively) is widely used for dissolution testing of solid oral dosage forms, as they are feasible and easily available. In some cases, where a basket or paddle apparatus is not feasible, another USP apparatus can be used. Evaluation of the dissolution apparatus should consider the product design initially, then further considerations should be made based on the observations during the evaluation. The paddle apparatus can be used for IR and ER dosage forms. Sinkers can be used for dosage forms that float or stick to vessel walls. The basket apparatus can be used for dosage forms that tend to float. In certain cases, drug products in the dissolution vessel form a cone or hip if there is a significant amount of insoluble material. In those cases, the agitation speed can be increased or apex vessels can be used with appropriate justification. A non-compendial low-volume apparatus with mini paddles and baskets can be adequately qualified and used with appropriate justification (e.g., low-dose drug products).

The recommended agitation speed is 100 rpm for the basket apparatus and 50 rpm for the paddle apparatus. A paddle with an agitation speed of 75 or 100 rpm can be used with an optimization study and the justification. Sometimes, 50 rpm agitation does not create sufficient hydrodynamics to uniformly disintegrate or dissolve the drug product, resulting in incomplete drug release or unit-to-unit variation. The agitation speed is also important to achieving the discriminatory power of the dissolution method. An increase in agitation speed often reduces the discriminatory capacity of the dissolution method, with a low agitation speed causing the variation.

USP apparatus 3 (reciprocating cylinder) can be used for IR, ER, and DR dosage forms like matrix tablets or formulations containing coated multi-particulate systems, which may not completely disintegrate into fine particles in the earlier rows and pass through the mesh of the cylinder. Apparatus 3 can be useful when drug release is pH-dependent, in which case it becomes appropriate to adjust pH over the course of the dissolution run. Agitation for apparatus 3 is considered in the form of dips per minute (dpm). When developing a dissolution method using apparatus 3, it is necessary to optimize the dips, which generally range from 5–30 dpm.

USP apparatus 4 (flow-through cell) is used for products containing drugs that have limited solubility. For USP apparatus 4, the media flow rate is critically controlled. Standard flow rates are 4, 8, and 16 ml/min. Other flow rates and modified flow-through cells can be used depending on the need and with justification, for example, powder dosage forms.

USP apparatus 7 is useful for ER dosage forms containing coated multi-particulate systems or for osmotic-controlled release delivery systems.

For handling the sequential dissolution in the case of DR dosage forms, two methods are commonly discussed in *USP* <711> and *EP* <2.9.3>.

Method A: Perform the acid-stage dissolution using 750 mL of 0.1 N HCl with a paddle or basket apparatus for 2 h followed by sampling and testing for acid-stage drug release. After 2 h, add 250 mL of 0.20 M tribasic sodium phosphate to each vessel to make 1000 mL of pH 6.8 buffer. If required, the pH adjustment can be done using 2 N HCl/NaOH.

Method B: Perform the acid-stage dissolution using 1000 mL of 0.1 N HCl with a paddle or basket apparatus for 2 h followed by the sampling and testing for acid-stage drug release. After 2 h, drain the 0.1 N HCl from each vessel with careful attention so that the drug product under study should not be lost, and pour 1000 mL of pH 6.8 buffer (previously equilibrated at 37 ± 0.5 °C) in each vessel.

Another option is to directly replace each vessel of 0.1 N HCl with another vessel containing 1000 mL of pH 6.8

buffer (previously equilibrated at 37 ± 0.5 °C) followed by the transfer of drug product from the stage 1 vessels to the stage 2 vessels. In each case, stage 2 dissolution can be performed commonly up to 45 min or on a case-by-case basis as per the adopted dissolution time point, considering the release mechanism or design of the drug product.

Dissolution of DR dosage forms can also be performed using apparatus 3 and 4. The use of apparatus 3 makes it easier for the sequential dissolution as the 0.1 N HCl can be added in the first row and the pH 6.8 buffer in the second row using media volumes in the range of 200–300 mL.

Discriminatory Power Evaluation and Method Validation

Discriminatory power is the ability of the dissolution method to detect changes in the drug product. The rationale behind the requirement for discriminatory power is as follows.

For a new drug product or a new generic drug product, in vivo clinical or bioequivalence (BE) studies are conducted after the completion of the formulation, analytical, and process development (which are submitted in the dossier to the agency for marketing approval). Dissolution specifications are finalized based on the dissolution data of batches used in the in vivo clinical/BE studies. Throughout the commercial life of the drug product, batches are expected to have the same in vivo performance, which is indirectly ensured by using the in vitro dissolution test as a quality control tool (*13*, *14*). Dissolution is identified as a CQA for most formulations (exceptions can be IR dosage forms containing highly soluble drugs) and is often utilized to determine the Proven Acceptable Ranges (PAR) and generate the design space. The study of any individual unit operation or parameter while keeping other parameters constant will give the PAR. By changing more than one factor at a time, multidimensional combinations and interactions of input variables and process parameters can be evaluated. If a factor demonstrates the ability to assure quality, then that factor generates design space (*17*). For example, the factors that can affect dissolution are the granulation process (input raw materials attributes, granulating fluid quantity, particle size distribution of the granules, etc.), lubrication process (lubricant level, lubrication time, etc.), compression process (compression force, tablet hardness, etc.), coating process (weight build-up, spray rate, curing temperature, curing time, etc.), and stability measures (temperature, humidity, hold time, etc.). Therefore, the discriminatory dissolution method is essential for developing a control strategy by controlling Critical Material Attributes (CMAs), fixing the processing equipment, and defining acceptable ranges for the Critical Process Parameters (CPPs). So, with this rationale, the selected dissolution method should be capable of detecting acceptable and unacceptable characteristics that can be possible during the commercial life of the product.

Once a tentative dissolution method (including medium, volume, apparatus, and agitation speed) has been chosen, then the method should be evaluated for discriminatory power by preparing different formulations with meaningful changes to the composition and/or process. The term 'meaningful change' here signifies any change in the raw material, composition, or manufacturing process that is possible during routine operation that may affect the in vivo performance of the product (e.g., differences in particle size or polymorphic forms of the drug substance, differences in lot-to-lot polymer viscosity, changes in functional excipient level like polymer, disintegrant, binder, lubricant level, etc). Manufacturing process changes can be granulation parameters, milling parameters, tablet hardness, polymer coating spray rate, coating weight build-up, curing temperature, curing time, etc. Complete removal of any excipient or change in the process design to prove the discrimination is not supported.

To check the discrimination, a dissolution profile of the final formulation should be compared to a formulation with meaningful changes. The comparison of dissolution profiles may be done using similarity factor analysis, i.e., difference factor (f_1) or similarity factor (f_2). An f_1 value above 15 or an f_2 value below 50 signifies that the dissolution profiles are different (*18*). A difference in the dissolution profile indicates the discriminatory power of the method. Discriminatory power can also be proved if the optimized formulation complies with the proposed dissolution acceptance criteria while formulations with meaningful changes fail to comply with the same. The choice of any one method should be based on the method's comparative discriminatory capacity. To achieve maximum discriminatory power, the dissolution method can be evaluated by varying the media volume, agitation speed, apparatus, etc. Not all formulation or process changes are expected to result in a significant dissolution profile difference, but the dissolution test should be able to discriminate expected differences due to the underlying drug release mechanism(s).

There is a possibility of the dissolution method being over-discriminatory and leading to the rejection of batches

that may not have a concern for in vivo performance. If the manufacturing process is in a state of control that is capable of consistently producing a product that meets specifications, an overly discriminating dissolution test may be justified.

If there is a concern that the process may produce batches that are out of specification, then the best approach to reducing this risk is to establish the IVIVC by performing an in vivo study on the batches produced with the most extreme dissolution profiles, followed by the setting of in vitro dissolution acceptance criteria based on the acceptable and non-acceptable in vivo behavior of the formulation.

The finalized discriminatory dissolution method should be validated as per ICH Q2 guidance and *USP* <1092> (*10*, *19*).

Setting Product Specifications

The dissolution profile of the test batch used in the clinical or BE study should be used to determine the drug product's final specification. In case of IR products where a BCS-based biowaiver is applied, the Q value can be set between 15 and 30 mins. Table 1 and 2 provides the understanding for setting the dissolution specifications for the American and European market, respectively (*2*, *4*, *7*, *13*, *14*, *20*). Hypothetical examples are included in Tables 1 and 2 to make it easy to understand the expectations of the regulatory agencies.

Briefly, the best possible approach to setting the dissolution acceptance criteria for an IR drug product is mean drug release of the clinical/BE batch at a given time point minus 10% (Q). For an ER drug product, the target value is the mean drug release of the clinical/BE batch ± 10% for early time points and minus 10% for the last time

Table 1. Dissolution Specifications for the USA Market (2, 4, 7)

Type	Conditions	Acceptance Criteria (US FDA)	Hypothetical Examples	
			Mean drug release of test lot used in clinical/ BE study	Acceptance criteria
IR	Highly soluble drugs	Single point specification: NLT 80% in 30 min	15 min: 82% 30 min: 93%	30 min: NLT 80%
IR	Complete drug release ≤ 60 min	Single point specification: NLT 80% in specified time interval	15 min: 65% 30 min: 82% 45 min: 93% 60 min: 99%	45 min: NLT 80%
IR	Complete drug release > 60 min	Minimum 2 time points: 1st time point: < 60 min 2nd time point: > 60 min & 80% release	15 min: 35% 30 min: 42% 45 min: 63% 60 min: 72% 75 min: 85% 90 min: 96%	60 min: NLT 60% 90 min: NLT 80%
ER	Conventional ER	Minimum 3 time points (initial, middle, and final phase) & 80% release[a]	1 h: 18% 5 h: 52% 10 h: 93%	1 h: NMT 30% 5 h: NLT 42% & NMT 62% 10 h: NLT 80%
ER	Modified ER with bi-phasic or multi-phasic release	Minimum 3 time points (initial, middle, and final phase) & 80% release[a]	0.5 h: 25% 4 h: 55% 8 h: 96%	0.5 h: NLT 15% & NMT 35% 4 h: NLT 45% & NMT 65% 8 h: NLT 85%
DR	Conventional enteric-coated drugs	Minimum 2 time points: Acid stage: usually NMT 10% in 2 h Buffer stage: usually NLT 80% in given time interval	Acid stage: 2 h: 4% Buffer stage: 15 min.: 65% 30 min.: 82% 45 min.: 93% 60 min.: 99%	Acid stage, 2 h: NMT 10% Buffer stage, 45 min: NLT 80%
DR	DR with ER mechanism	Minimum 1 time point in acid stage and 3 time points in buffer stage. Acid stage: NMT 10% in 2 h Buffer stage: Initial, middle, and final phase & 80% release[a]	Acid stage: 2 h: 4% Buffer stage: 1 h: 22% 2 h: 52% 6 h: 93%	Acid stage, 2 h: NMT 10% Buffer stage: 1 h: NLT 12% & NMT 32% 2 h: NLT 42% & NMT 62% 6 h: NLT 80%

[a]Acceptance based on mean target value ± 10%; mean target value is the mean drug release of the test lot used in the clinical/BE study.
FDA: Food and Drug Administration; IR: immediate release; ER: extended release, DR: delayed release; BE: bioequivalence; NLT: not less than; NMT: not more than.

Table 2. Dissolution Specifications for the Europe Market (13, 14, 20)

Type	Conditions	Acceptance Criteria (EMA)	Hypothetical Examples	
			Mean drug release of test lot used in clinical/BE study	Acceptance criteria
IR	Complete drug release ≤ 45 min	Single point specification: Q value = bio batch mean drug release – 10%. Q value is usually 75–85%. Q value above 85% is considered irrelevant.	15 min: 92% 30 min: 99%	15 min: NLT 80%
			15 min: 79% 30 min: 93%	30 min: NLT 80%
			15 min: 65% 30 min: 82% 45 min: 99%	45 min: NLT 85% i
IR	Complete drug release > 45 min	Minimum 2 time points: 1st time point: < 45 min 2nd time point: > 45 min & 80% release	15 min: 35% 30 min: 42% 45 min: 63% 60 min: 72% 75 min: 85% 90 min: 96%	45 min: NLT 50% 90 min: NLT 85%
ER	Conventional ER drug products	Minimum 3 time points: 1st time point: 20–30 % release 2nd time point: 50% release 3rd time point: ≥ 80% release[a]	2 h: 22% 6 h: 52% 12 h: 93%	2 h: NLT 12% & NMT 32% 6 h: NLT 42% & NMT 62% 12 h: NLT 80%
ER	Modified ER with bi-phasic or multi-phasic release	Minimum 3 time points: 1st time point: 20–30 % release 2nd time point: 50% release 3rd time point: ≥ 80% release[a]	0.5 h: 25% 4 h: 55% 8 h: 96%	0.5 h: NLT 15% & NMT 35% 4 h: NLT 45% & NMT 65% 8 h: NLT 85%
DR	Conventional enteric-coated drugs	Minimum 2 time points: Acid stage: usually NMT 10% in 2 h. Buffer stage: usually NLT 80% in given time interval.	Acid stage: 2 h: 4% Buffer stage: 15 min: 65% 30 min: 82% 45 min: 93% 60 min: 99%	Acid stage, 2h: NMT 10% Buffer stage, 45 min: NLT 80%
DR	DR with ER mechanism	Minimum 1 time point in acid stage and 3 time points in later buffer stage. Acid stage: usually NMT 10% in 2 h. Buffer stage: 1st time point: 20–30 % release 2nd time point: 50% release 3rd time point: ≥ 80% release[a]	Acid stage: 2 h: 4% Buffer stage: 1 h: 22% 2 h: 52% 6 h: 93%	Acid stage, 2 h: NMT 10% Buffer stage: 1 h: NLT 12% & NMT 32% 2 h: NLT 42% & NMT 62% 6 h: NLT 80%

[a]Acceptance based on mean target value ± 10%; mean target value is the mean drug release of the test lot used in the clinical/BE study.
EMA: European Medicines Agency; IR: immediate release; ER: extended release, DR: delayed release; NLT: not less than; NMT: not more than.

point. For a DR drug product, the target in the acid stage is NMT 10% after 2 h, and the buffer stage target depends on the release mechanism (IR or ER) or design of the drug product.

Any deviation from the range specified above can be justified by performing the additional clinical/BE studies using the batches with extreme dissolution profiles. During the stability study, it is expected that the product should meet the acceptance criterion that was derived based on the batches linked to the clinical/BE studies, any change in the dissolution behavior during the stability study can trigger an out-of-specification value followed by an investigation. If needed, to support the change in dissolution data, acceptance criteria can be revised by demonstrating additional BE (i.e., dissolution profile) between the batch with changes vs. the batch used in the early clinical/BE studies (*13*).

CASE STUDY

A dissolution method development case study is presented in the subsequent sections for a better understanding of each element. The case study considers a model drug and ER tablet dosage form; however, the same procedure can be applied to any dosage form.

Materials

Metformin hydrochloride ER tablets (50 mg) was selected as a model drug for this study. Metformin hydrochloride (Harman Finochem), lactose monohydrate (DFE), povidone (BASF), colloidal silicon dioxide (Evonik), magnesium stearate (Petergreven), hypromellose (Lotte),

ethylcellulose (DuPont), triethyl citrate (Stearinerie Dubois), talc (Emerys), and isopropyl alcohol (Runa Chemicals) were obtained from Centaur Pharmaceuticals Pvt Ltd. Hydrochloric acid (37%), sodium hydroxide, potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium acetate, glacial acetic acid were of analytical grade.

Optimized Formulation Development

A formulation was optimized with a reservoir system, and the target was to achieve an ER profile. The tablet core was prepared using granules manufactured using an aqueous wet granulation process, compression using 8.2 mm round punches, and B-tooling tablet press with a target tablet weight of 250 mg. To smooth the core surface and to serve as a barrier between the core and the controlled-release polymer coating, a 3% w/w subcoating was layered over the core tablets. After subcoating, controlled-release polymer coating (15% w/w) was performed using a hydrophilic-hydrophobic polymer combination. The last film coating used an Opadry premix (3% w/w). The formulation composition is listed in Table 3 (formulation #1).

Analytical Method Development

An ultraviolet (UV) spectrophotometer (1800 series, Shimadzu) was used with 1-cm quartz cuvettes. Drug standard solutions with a final concentration of 10 µg/mL were prepared using various buffer solutions (0.1 N HCl, pH 4.5 acetate, pH 6.8 phosphate buffer, and water). Absorbance was measured for each standard solution using the UV spectrophotometer at a wavelength ranging from 200 to 400 nm. The pattern of the spectrum and absorbance maxima was evaluated in each medium. The UV spectrophotometer method was found to be feasible. Spectra with 0.1 N HCl showed a solvent effect, giving a sharp peak close to 200 nm. The absorbance maximum was 233 nm in pH 4.5 acetate, pH 6.8 phosphate buffer, and water. A concentration of 10 µg/mL was finalized to achieve an absorbance of not more than 1.0.

Drug Solubility Study and Solution Stability

The pH-solubility profile of the drug was determined in triplicate at 37 ± 1 °C in aqueous media with a pH in the range of 1–6.8 using the shake-flask method. The drug was added to the 10 mL of the corresponding buffer solution until a saturated solution was formed. Saturated solutions were kept in a shaker maintained at 37 ± 1 °C for 24 h. After 24 h, each solution was filtered (0.45-µ nylon syringe filter, Millipore), followed by dilution using the same buffer solution, and the concentration was

Table 3. Case Study: Composition of Optimized Formulation

Components	Function	Value (mg)
Intragranular		
Metformin Hydrochloride	Drug substance	50
Povidone K 30	Binder	15
Lactose Monohydrate	Diluent	181.25
Purified water	Solvent	q.s.
Extragranular		
Colloidal Silicon Dioxide	Glidant	1.25
Magnesium Stearate	Lubricant	2.5
Subcoating		
Hypromellose – 5 CPS	Film former	7.5
Purified water	Solvent	q.s.
Controlled-release polymer coating:		
Ethyl cellulose – 10 CPS	Release controlling polymer	16.22
Hypromellose – 5 CPS	Pore former and film former	19.7
Triethyl Citrate	Plasticizer	2.7
Isopropyl Alcohol	Solvent	q.s.
Purified water	Solvent	q,s,
Film Coating		
Opadry Premix	Film former with color	8.88
Purified water	Solvent	q.s.
Film coated tablet weight		305

CPS: Centipoise; q. s.: quantity sufficient

determined using UV spectrophotometry. Mean drug solubility was 199, 167, 250, and 200 mg/mL in the 0.1 N HCl, pH 4.5 acetate buffer, pH 6.8 phosphate, and purified water, respectively. The stability of the standard solution in each medium at 37 ± 1 °C was checked for up to 72 h.

Sink Condition and Dissolution Medium

The drug has pH-independent, high solubility, and the sink condition can be maintained in 500 mL, allowing 3 times the unit dose (150 mg) to be sufficiently dissolved. The drug solution with each medium was found stable for up to 72 h. Considering the solubility data, any medium can be taken forward as a dissolution medium. As the drug has pH-independent solubility, water was preferred as the dissolution medium, which was also proven to have discriminatory capacity, as discussed in the later sections.

A standard calibration curve was prepared in purified water with the drug concentration ranging from 2–12 µg/mL. The linear relationship between the drug concentration and absorbance makes water suitable for determining the drug concentration by measuring the absorbance. To obtain the drug concentration within the linear calibration range during dissolution analysis, the dilution factor was adjusted to achieve a final concentration of 10 µg/mL.

Dissolution Apparatus and Agitation Speed

The dissolution apparatus was evaluated by conducting the dissolution test with the optimized formulation using a basket apparatus at 100 rpm and paddle apparatus at 50, 75, and 100 rpm. The dissolution data are presented in Table 4 and Figure 2.

Both apparatus were found feasible and showed uniform drug release. Floating or sticking of the tablet was not observed in the case of the paddle, so a sinker was not required. Although the agitation speed increased with the paddle, the release rate was similar, which could be due to the design of the drug product by the reservoir system. The discriminatory capability of the method can be reduced by increasing the agitation speed; hence, a paddle at 50 rpm and a basket with 100 rpm was considered appropriate for comparison.

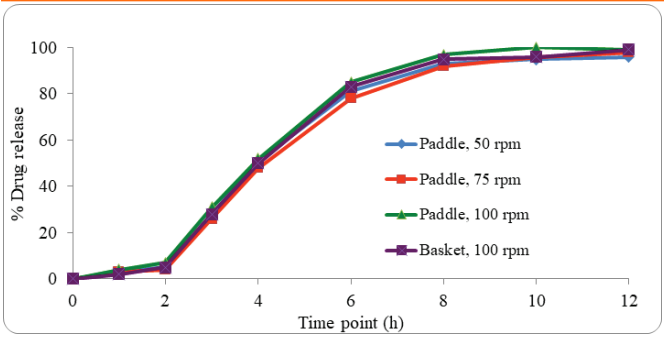


Figure 2. Dissolution profiles (evaluation of test apparatus).

Evaluation of Discriminatory Power

The discriminatory power was evaluated by preparing the different formulations with meaningful changes in the composition like polymer ratio, coating weight build-up, and changes in the manufacturing process like coating spray rate. Dissolution profiles of the formulation with these changes were compared with the dissolution profile of the optimized formulation through f_1 and f_2 calculation. The difference in dissolution profiles is not only measured through these calculations, but also based on the overall dissolution profile. The reason for this is that the f_1 and f_2 values are driven by multiple time points, which may not be necessary to show discrimination. Sometimes, the data can be evaluated by identifying differences at particular time points that are critical to controlling the in vivo performance (i.e., part of the acceptance criteria).

Table 4. Case Study: Dissolution of Optimized Formulation

Time Point (h)	USP Apparatus 2, 50 rpm	USP Apparatus 2, 75 rpm	USP Apparatus 2, 100 rpm	USP Apparatus 1, 100 rpm
0	0	0	0	0
1	3 (1 – 4)	3 (2 – 5)	4 (3 – 7)	2 (1 – 5)
2	5 (3 – 8)	4 (3 – 7)	7 (6 – 10)	5 (4 – 8)
3	28 (23 – 31)	26 (24 – 29)	31 (28 – 35)	28 (25 – 31)
4	51 (46 – 54)	48 (46 – 51)	52 (47 – 56)	50 (48 – 53)
6	81 (75 – 83)	78 (76 – 81)	85 (84 – 88)	83 (81 – 85)
8	93 (90 – 95)	92 (89 – 94)	97 (95 – 100)	95 (91 – 99)
10	95 (93 – 98)	96 (94 – 99)	100 (98 – 102)	96 (94 – 99)
12	96 (95 – 99)	98 (97 – 100)	99 (97 – 101)	99 (99 – 102)

Values are mean (range), n = 12. Dissolution medium was 500 mL of water.
USP: United States Pharmacopeia.

To evaluate the discriminatory power of the dissolution test methods (paddle apparatus at 50 rpm versus basket apparatus at 100 rpm), three formulations trials were developed by changing formulation variables (formulation #2 and #3) or process variables (formulation #4). Formulation trial #2 was manufactured by changing the release-controlling polymer-to-pore former ratio (ethylcellulose: hypromellose) from 42:51 to 39:54 and keeping the coating weight build-up, other components, and process parameters constant with the optimized formulation. Formulation trial #3 was manufactured by keeping the same coating composition and process parameters but increasing the CR polymer coating weight build-up from 15% to 17% w/w. Formulation trial #4 was manufactured by keeping the composition same and only increasing the spray rate to 10–16 g/min from the optimized spray rate of 5–8 g/min, which affects the film property.

Results and Discussion

The results are presented in Table 5 and Figure 3. Both dissolution methods successfully discriminated the slight changes in polymer-to-pore former ratio (Fig. 3A and 3B), polymer coating weight build-up (Fig. 3C and 3D), and polymer spray rate (Fig. 3E and 3F). Although f_2 values were above 50, there were differences in the release at some early and middle time points.

Although both the dissolution methods are discriminatory and equally feasible, the paddle apparatus method is comparatively more discriminatory than the basket apparatus. Thus, the preferred dissolution method for metformin hydrochloride ER tablets is USP apparatus 2 (paddle) at 50 rpm with 500 mL of purified water (37 ± 0.5 °C).

If the optimized formulation (#1) is the same as that used in the clinical or BE study and no in vitro-in vivo

Table 5. Case Study: Dissolution of Optimized Formulation (#1) vs. Formulation Trials[a]

Time Point (h)	Formulation #1	Formulation #2	Formulation #3	Formulation #4
USP Apparatus 2, 50 rpm				
0	0	0	0	0
1	3 (1–4)	5 (2–7)	0	8 (6–11)
2	5 (3–8)	13 (10–15)	2 (1–4)	14 (11–16)
3	28 (23–31)	37 (34–39)	17 (14–21)	42 (38–45)
4	51 (46–54)	68 (66–71)	40 (36–42)	59 (56–62)
6	81 (75–83)	87 (86–91)	77 (76–81)	89 (86–91)
8	93 (90–95)	97 (94–99)	92 (89–95)	99 (96–101)
10	95 (93–98)	99 (97–101)	93 (90–99)	100 (98–101)
12	96 (95–99)	100 (99–102)	96 (92–100)	102 (99–104)
f_1	Ref	32	17	28
f_2	Ref	51	58	52
USP Apparatus 1, 100 rpm				
0	0	0	0	0
1	2 (1–5)	3 (2–3)	1 (0–3)	6 (3–8)
2	5 (4–8)	11 (8–13)	3 (1–6)	12 (10–13)
3	28 (25–31)	34 (30–36)	18 (16–23)	37 (35–39)
4	50 (48–53)	65 (61–67)	39 (35–44)	56 (53–59)
6	83 (81–85)	88 (83–90)	79 (74–82)	89 (83–90)
8	95 (91–99)	99 (98–101)	94 (90–96)	101 (98–101)
10	96 (94–99)	100 (99–102)	99 (98–102)	100 (99–102)
12	99 (99–102)	99 (98–102)	100 (99–103)	101 (98–102)
f_1	Ref	26	15	21
f_2	Ref	55	60	59

Values are mean (range), n = 12. Dissolution medium was 500 mL of water.
USP: United States Pharmacopeia.

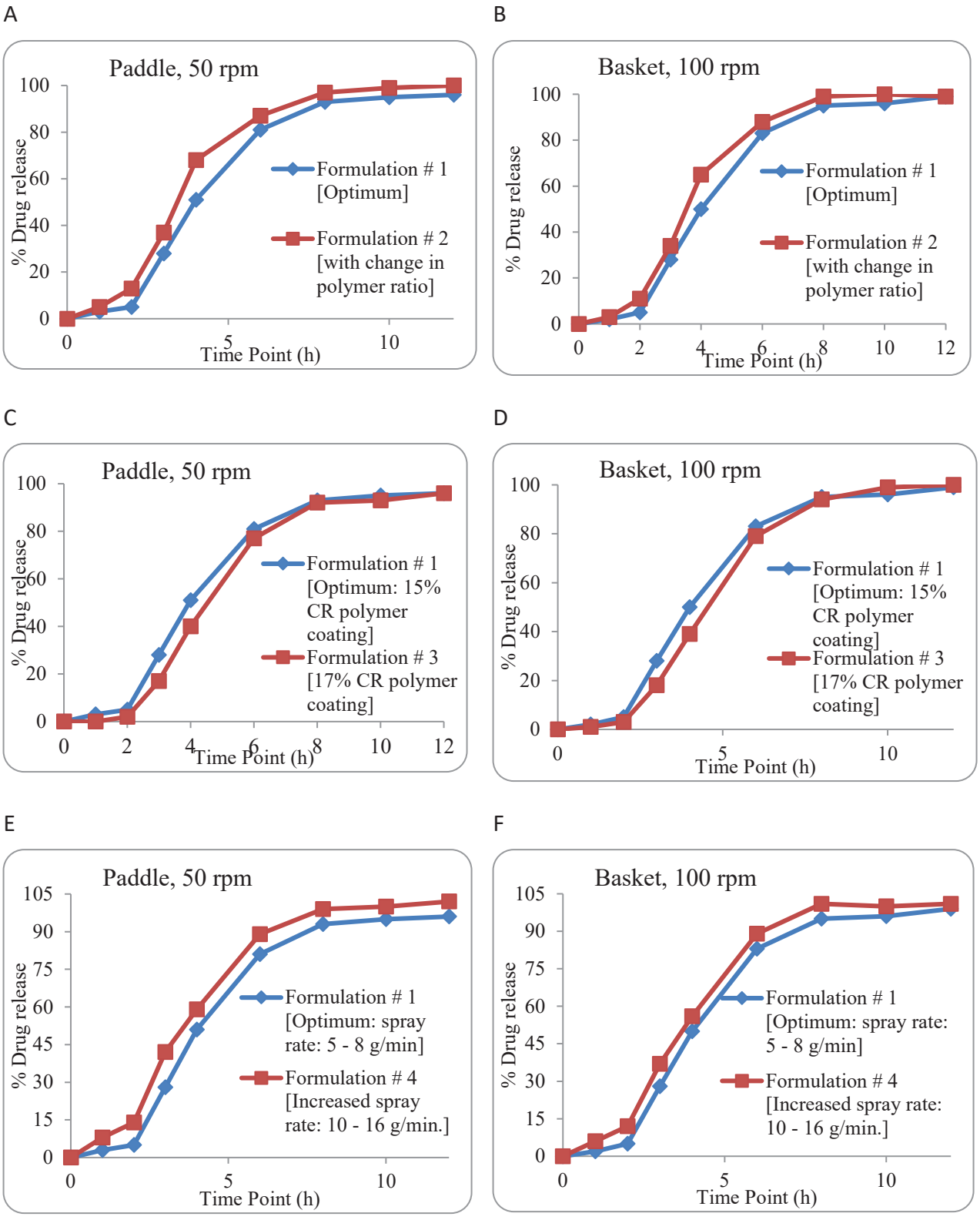


Figure 3. Dissolution of formulations with changes in polymer ratio (A and B), CR polymer coating weight build-up (C and D), and coating spray rate (E and F) using apparatus 2 (A, C, E) and apparatus 3 (B, D, F).

correlation (IVIVC) is established, then the final dissolution specification for the commercial life of the product can be proposed as given in Table 6. The same acceptance criteria can be applied in the American and European markets.

If the dissolution profiles of formulation #1 and #4 (see Table 5) are compared with the derived specifications (Table 6), then the batches are out of specification. This indicates that the dissolution method is capable of discriminating batches with acceptable and non-acceptable release characteristics.

Table 6. Case Study: Derived Specifications for the Optimized Formulation

Time Point (h)	Mean Drug Release (%)	Proposed Acceptance Criteria	
		Option 1	Option 2
1	3	--	--
2	9	NMT 20%	--
3	28	--	NLT 18%, NMT 38%
4	51	NLT 41%, NMT 61%	NLT 41%, NMT 61%
6	81	--	--
8	93	NLT 80%	NLT 80%
10	95	--	--
12	96	--	--

NLT: not less than; NMT: not more than.

SUMMARY

This review showcases the importance of the dissolution test and the specifications for oral solid dosage forms, including a concise summary of regulatory requirements and expectations in the US and Europe. The discussion on dissolution method development, including a case study, provides handy guidance to academics, research scholars, and industry scientists to develop a dissolution method for any new or generic solid oral dosage form.

ACKNOWLEDGEMENT

The authors thank Centaur Pharmaceuticals Pvt. Ltd., Pune, India, for supporting the research work.

FUNDING

The authors disclosed no funding for this work.

CONFLICT OF INTERESTS

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

REFERENCES

1. *Dissolution Testing of Immediate Release Solid Oral Dosage Forms*; Guidance for Industry. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), 1997.
2. *Extended release oral dosage forms: development, evaluation, and application of in vitro/in vivo correlations*; Guidance for Industry. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), 1997.
3. *Dissolution methods*. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Office of Pharmaceutical Quality, Office of New Drug Products, Division of Biopharmaceutics, 2022.
4. Anand, O. Decrease RTR, IR, and CR Regulatory Actions Due to Dissolution Deficiencies, and Increase Approval During First Review Cycle: A Biopharmaceutics Perspective. Presented at Regulatory Education for Industry (REdI): Generic Drug Forum April 3-4, 2019.
5. *Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System*; Guidance for Industry. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), 2017.
6. M9 Biopharmaceutics Classification System-Based Biowaivers; Guidance for Industry. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), 2021.
7. *Dissolution Testing and Acceptance Criteria for Immediate-Release Solid Oral Dosage Form Drug Products Containing High Solubility Drug Substances*; Guidance for Industry. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), 2018.
8. *Specifications: Test procedures and acceptance criteria for new drug substances and new drug products: chemical substances, Q6A*; ICH Harmonised Tripartite Guideline. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 1999.
9. Zhang, L.; Fiske J.; Zhao, H.; Patel, H.; Jennings S. Dissolution Method development for fixed-dose combination drug products –challenges and strategies. *Am. Pharm. Rev*. Published November 30, 2015. https://www.americanpharmaceuticalreview.com/Featured-Articles/181886-Dissolution-Method-Development-for-Fixed-Dose-Combination-Drug-Products-Challenges-and-Strategies/ (accessed July 15, 2023).
10. <1092> The Dissolution Procedure: Development and Validation. In *USP 43–NF 38*. The United States Pharmacopeial Convention, Inc., 2022.
11. <711> Dissolution. In *USP 43–NF 38*. The United States Pharmacopeial Convention, Inc., 2022.
12. Questions and Answers on the United Kingdom's withdrawal from the European Union on 31 January 2020. European Commission, European Union. Published January 24, 2020. https://ec.europa.eu/commission/presscorner/detail/en/qanda_20_104 (accessed July 15, 2023).

13. *Guideline on quality of oral modified release products*; EMA/CHMP/QWP/428693/2013. European Medicines Agency; Committee for Medicinal Products for Human Use (CHMP), 2014.
14. *Reflection paper on the dissolution specification for generic oral immediate release products*; EMA/CHMP/CVMP/QWP/336031/2017. European Medicines Agency; Committee for Medicinal Products for Human Use (CHMP), Committee for Medicinal Products for Veterinary Use (CVMP), Quality Working Party (QWP), 2017.
15. Rohrs, B. R. Dissolution method development for poorly soluble compounds. *Dissolut. Technol*. **2001**, *8* (3), 6–12. DOI: 10.14227/DT080301P6.
16. Phillips, D. J.; Pygall, S. R.; Cooper, V. B.; Mann, J. C. Overcoming sink limitations in dissolution testing: a review of traditional methods and the potential utility of biphasic systems. *J. Pharm. Pharmacol*. **2012**, *64* (11), 1549–1559. DOI: 10.1111/j.2042-7158.2012.01523.x.
17. *Pharmaceutical development, Q8(R2)*; ICH Harmonised Tripartite Guideline. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2009.
18. Kassaye, L.; Genete, G. Evaluation and comparison of in-vitro dissolution profiles for different brands of amoxicillin capsules. *Afr. Health Sci*. **2013**, *13* (2), 369–375. DOI: https://doi.org/10.4314/ahs.v13i2.25.
19. *Validation of Analytical Procedures: Text and Methodology, Q2(R1)*; ICH Harmonised Tripartite Guideline. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2005.
20. <5.17.1> Recommendation on dissolution testing. In *European Pharmacopeia*, 2022.

dx.doi.org/10.14227/DT300323P176

Q+A Question & Answer Section

*The following questions have been submitted by readers of Dissolution Technologies. Margareth R. Marques, Ph.D., and Mark Liddell, Ph.D., United States Pharmacopeia (USP), authored responses to each of the questions. *Note: These are opinions and interpretations of the authors and are not necessarily the official viewpoints of the USP. E-mail for correspondence: mrm@usp.org.*

Q In *USP* general chapter <711> Dissolution it is stated to add enzymes like pepsin and others to prevent cross linking of capsules shell, but when some capsules were analyzed with and without enzyme, no differences between with or without enzyme were found, and no cross-linking happened in medium without enzyme addition. Do you have any comments?

A As stated in the *USP* general chapter <711> Dissolution, enzymes can be added to the dissolution medium when there is evidence of the presence of cross-linking in gelatin capsules. The strongest evidence of cross-linking is observed when capsules do not open. The capsules may lose their shape, appear to be hydrated, but simply do not open and release the contents of the capsule. The presence of cross-linking in gelatin renders the capsule insoluble in aqueous solvents. When this occurs, enzymes are added to digest the cross-linked gelatin, not to prevent the cross-linking. See more information in the *USP* general chapter <1094> Capsules – Dissolution Testing and Related Quality Attributes and the following article: Marques MRC. Enzymes in the dissolution testing of gelatin capsules. *AAPS PharmSciTech*. 2014. doi: 10.1208/s12249-014-0162-3.

Q Can the use of traditional sinkers be completely replaced with the use of the stationary basket as described in the *USP* general chapter <711> Dissolution?

A No, while the stationary basket is a possible option when traditional sinkers are not suitable for a particular formulation, its use must be justified with experimental data obtained with the samples under evaluation.

Q If 0.1 N hydrochloric acid solution is used as dissolution medium, does it need to be standardized?

A No. There is a note at the end of each volumetric solution entry in *USP – NF* stating that if the volumetric solution is used for qualitative purposes such as dissolution medium, the solution does not need to be standardized.

Q Regarding the *USP* general chapter <701> Disintegration, could you provide a definition of what "softening" means?

A During the disintegration test the capsule is going to hydrate upon exposure to the disintegration media. The capsule will lose its shape and may have the appearance/consistency of a gel or soft (malleable) material.

Q The *USP* general chapter <711> Dissolution states "Specimens are to be withdrawn only at the stated times, within a tolerance of ± 2%." Does it mean that the sampling must be finalized by this time?

A For shorter sampling times, say around 5 min (time tolerance is only ± 6 sec), it may not be possible to complete the entire process of pulling the sample and filtering within the sampling time tolerance of ± 2%; however, the sampling and filtering need to be done as fast as practically possible to stop the dissolution process. This may be a particular challenge when using an autosampler with a fixed draw rate. The error in the sampling time will be dependent on both the rate of sample withdrawal and the volume of the sample at a given time point. The dissolution scientist should be aware of the uncertainty that this error may cause in the final dissolution profile.

Q If the acid stage criteria for a particular delayed-release product was met for the A1 level (no individual value exceeds 10% dissolved) but does not meet the buffer stage criteria for B1 level (each unit is NLT Q + 5%), does the acid stage need to be repeated along with the buffer stage? The statement "Continue testing through all levels unless the results of both the *Acid Stage* and *Buffer Stage* conform at an earlier level" is unclear and could be interpreted both ways in which both acid and buffer stages are repeated, or only the buffer stage

is repeated because the acid stage met acceptance criteria at an earlier level.

A In the case of a delayed-release product, a single dissolution test consists of two phases, the acid stage and the buffer stage. As pointed out the acceptance criteria requires that "the results of both the *Acid Stage* **and** *Buffer Stage* conform." Thus, you are going to use new samples and submit them to the acid stage, but you may not need to collect any samples at this stage because you have already verified the lack of release during the acid stage. After the appropriate time in the acid stage, proceed to the buffer stage where you will collect dissolution samples for quantitation.

Q While *USP* general chapter <711> states that the temperature inside the vessel should be 37.0 ± 0.5 °C, the *USP* general chapter <1092> The Dissolution Procedure – Development and Validation states that variations in temperature should be evaluated during the evaluation of robustness. Would successful robustness testing from 36–38 °C allow you to extend the range of the vessel temperatures?

A While it may be the case that the robustness of the temperature range for an individual dosage form may be wider than that of the compendial test requirements, the temperature of dissolution medium and all the other dissolution apparatus parameters must be kept within the compendial ranges when evaluating the robustness of a dissolution method.

Q Is there an optimal way of generating cross-linked capsules to produce consistent results to show the effectiveness and stability of the pepsin in the dissolution media? I found an article where capsule shells were exposed to 37% formaldehyde solution vapors for ~30 minutes in a desiccator, the capsules were filled with the capsule material, and the dissolution was performed. Is this the most efficient way or are there other means of evaluating this?

A Cross-linking is a kinetic process, so time plays a significant role in cross-linking, which may be observed in gelatin capsules. Capsules may need to stay several days under high temperature and high humidity to show cross-linking. Thirty minutes in a formaldehyde environment may not be enough to successfully generate cross-linking in gelatin capsules. The presence and extent of cross-linking may depend on not only the composition of the gelatin capsule but also the components contained within the capsule. As a result, determining ideal conditions to promote cross-linking may vary. See the following papers for additional information on forced cross-linking.

Gold TB, Buice RG Jr, Lodder RA, Digenis GA. Determination of extent of formaldehyde-induced crosslinking in hard gelatin capsules by near-infrared spectrophotometry. *Pharm Res*. 1997;14(8):1046-1050. DOI: 10.1023/a:1012105412735.

Digenis GA, Gold TB, Shah VP. Cross-linking of gelatin capsules and its relevance to their in vitro-in vivo performance. *J Pharm Sci*. 1994; 83:915-921. DOI: 10.1002/jps.2600830702.

Q We sometimes must review the validation of dissolution procedures, and quite often we cannot find the results from filter studies (do the filters release interfering compounds, adequate recovery with standard/sample at different levels, adequate filtration of undissolved particles, etc.) Is this information normally supposed to be documented in the validation, or is this kind of test more adequately documented during development stages?

A Filter evaluation should be done as early as possible in the development process and dissolution method validation project. In many cases, it is needed early on for the solubility assessment of the drug substance and for successive dissolution studies. The filter process may be re-evaluated later depending on the changes made in the formulation/manufacturing process. The filter should be evaluated regarding pore size to ensure that all solids in suspension will be retained. The filter materials should also be evaluated to ensure that there is no drug adsorption and no interference from possible leachables and extractables that may be part of the filter material or housing.

Q+A

Every issue of *Dissolution Technologies* features a Question and Answer section. This section is designed to address general dissolution questions submitted by our readers.

Please send your questions to:
Attn: Q&A
9 Yorkridge Trail, Hockessin, DE 19707
Email: vagray@rcn.com
Submit via our website:
www.dissolutiontech.com

Dissolution Performance Verification Standard – Prednisone

Increased stability, ease of use, and reproductibility

Official May 1, 2023

Learn more at
www.usp.org/dissolution

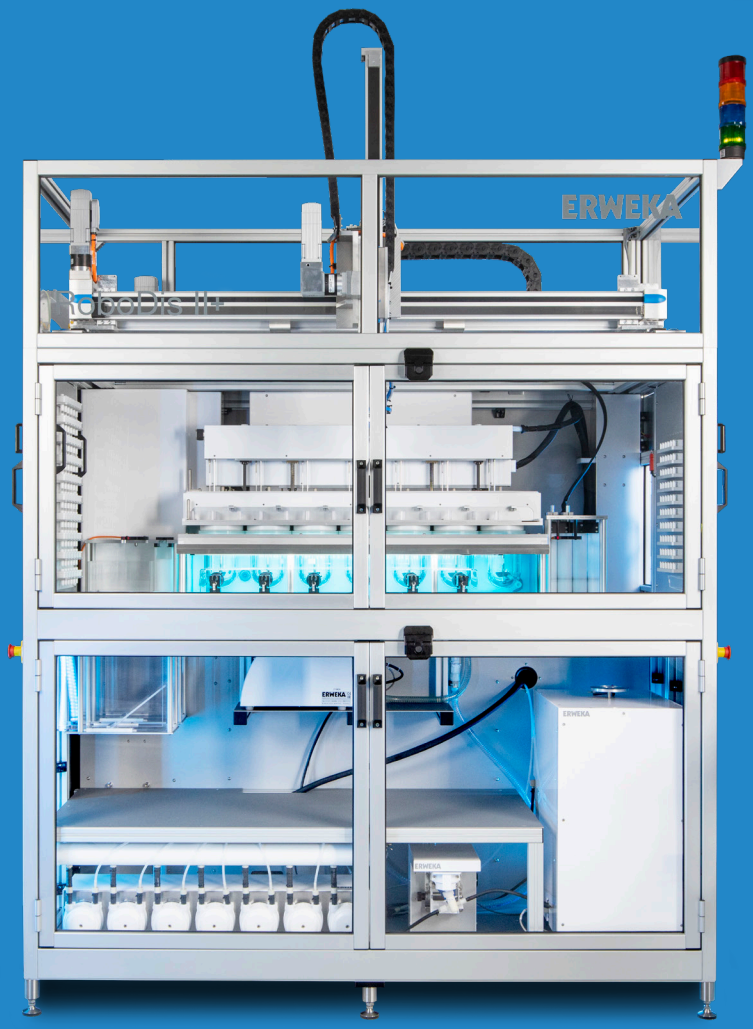
The standard of trust

usp®

Full dissolution automation
The productivity booster
RoboDis II+

For **live online demos** contact us at **sales@erweka.com**

More info about the RoboDis II+ at
www.erweka.com



Features & options:

- **10 or 40 batches in one test run, real 24/7 testing**
- **100% USP/EP/JP compliant**
- **Data integrity with 21 CFR part 11 compliant software**
- **USP methods 1 and 2 exchangeable**
- **Built-in System Suitability Tests (SST)**
- **Fully automated HPLC & UV/Vis**
- **Automatic media preparation and supply**

ERWEKA America Inc.
31 Northfield Avenue
Edison, NJ 08837
USA

sales.usa@erweka.com
+1 (732) 662-1143

ERWEKA
www.erweka.com

Calendar of Events

August 29–31, 2023

FDA/M-CERSI Physiologically Based Biopharmaceutics Modeling, Physiologically Based Biopharmaceutics Modeling (PBBM) Best Practices for Drug Product Quality: Regulatory and Industry Perspectives

Location: University of Shady Grove, Rockville, MD
Registration: https://www.simulations-plus.com/events/physiologically-based-biopharmaceutics-modeling-pbbm-best-practices-for-drug-product-quality-regulatory-and-industry-perspectives/

September 5, 2023

Complimentary Introduction to GastroPlus® Workshop

Location: Online
Registration: https://www.simulations-plus.com/events/complimentary-introduction-to-gastroplus-workshop-10/

October 3, 2023

Complimentary Introduction to GastroPlus® Workshop

Location: Online
Registration: https://www.simulations-plus.com/events/complimentary-introduction-to-gastroplus-workshop-eu-2/

October 12, 2023

Advances in PBPK Modeling and its Regulatory Utility for Oral Drugs Product Development

Location: Online and in person, College Park, MD, USA
For information, visit info@complexgenerics.org

October 22–25, 2023

PharmSci 360 AAPS Meeting

Location: Orlando County Convention Center, Orlando, FL, USA
For information, visit https://www.aaps.org/pharmsci/annual-meeting

October 31, 2023

Complimentary Introduction to GastroPlus® Workshop

Location: Online
Registration: https://www.simulations-plus.com/events/complimentary-introduction-to-gastroplus-workshop-11/

November 13–15, 2023

Eastern Analytical Symposium and Exhibition

Location: Crowne Plaza Princeton-Conference Center, Plainsboro, NJ, USA
For information, visit eas.org

November 23, 2023

Dissolution Discussion Group Quarterly Online Meeting—Dissolution Qualification: The PQ vs MQ debate. What's right for your lab?

Location: DDG Online Meeting at 10:30 am ET
Registration: https://www.agilent.com/chem/dissolution-webinars

December 4, 2023

Complimentary Introduction to GastroPlus® Workshop

Location: Online
Registration: https://www.simulations-plus.com/events/complimentary-introduction-to-gastroplus-workshop-12/

On Demand Events

- ***Clarifying 21 CFR Part 11 & Data Integrity Requirements for Dissolution Testing.***
  www.distekinc.com/watch/clarifying-21-cfr-part-11-and-data-integrity-for-dissolution-testing/
- ***Ocular Administration (OCAT™) in GastroPlus® On Demand***
  https://www.simulations-plus.com/events/gastroplus-additional-dosage-routes-workshop-ocular-administration-ocat-virtual/

- ***Oral Cavity Administration (OCCAT™) in GastroPlus® On Demand***
  https://www.simulations-plus.com/events/gastroplus-additional-dosage-routes-workshop-oral-cavity-administration-occat-virtual/
- ***Pulmonary Administration (PCAT™) in GastroPlus® On Demand***
  https://www.simulations-plus.com/events/gastroplus-additional-dosage-routes-workshop-pulmonary-administration-pcat-virtual/
- ***GastroPlus® ADR – 4 Course Bundle (TCAT™ / OCAT™ / OCCAT™ / PCAT™)***
  https://www.simulations-plus.com/events/gastroplus-adr-4-course-bundle-tcat-ocat-occat-pcat/
- ***GastroPlus® ADR – 5 Course Bundle (TCAT™ / OCAT™ / OCCAT™ / PCAT™ / Injectables)***
  https://www.simulations-plus.com/events/gastroplus-adr-5-course-bundle-tcat-ocat-occat-pcat-injectables/
- ***Transdermal Administration (TCAT™) in GastroPlus®***
  https://www.simulations-plus.com/events/gastroplus-additional-dosage-routes-workshop-transdermal-administration-tcat-virtual/
- ***Injectables (IM, SQ, IA) in GastroPlus® Including Biologics and LAIs***
  https://www.simulations-plus.com/events/gastroplus-additional-dosage-routes-workshop-injectables-incl-lai-biologics-virtual/

EASTERN ANALYTICAL SYMPOSIUM & EXPOSITION 2023

Better life with Analytical Chemistry

SAVE THE DATE & JOIN US

Crowne Plaza Princeton
Conference Center
Plainsboro, NJ
November 13–15, 2023

3 DAYS OF TECHNICAL PROGRAM

Hone your skills with technical sessions and short courses to connect you with experts from the field.

3 DAYS OF EXPOSITION

Discover the latest advances in analytical technology and equipment.

CAREER DEVELOPMENT OPPORTUNITIES

Advance your career with workshops, employment bureau and networking events.

CALL FOR PAPERS 2023

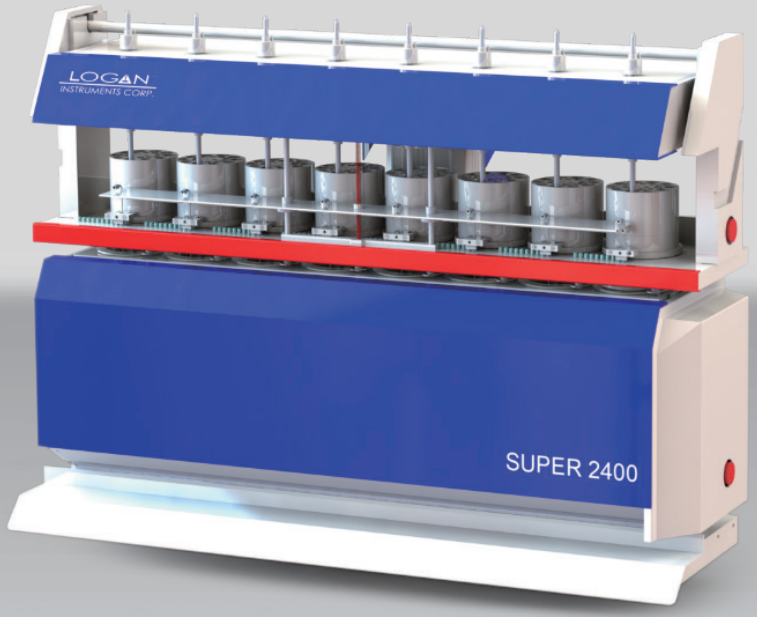
Oral & poster abstract submissions opens
March 1, 2023

eas.org

LOGAN
INSTRUMENTS CORP.

Logan Fully Automated Dissolution System

The Logan PELogan System 2400 is the only fully automated dissolution testing system that is designed and manufactured in the USA. Logan System 2400 can run 10 batches in accordance with USP 1 or 2 from media delivery to analysis unattended. All stages of the dissolution process are computer coordinated and carried out entirely without user intervention.



This system precisely delivers, preheats, and degasses media into 8 dry-heat vessels removing the added complication of a water bath. Each vessel has bottom and side cameras to record the dissolution for subsequent viewing and result verification. After the test is over, the vessels automatically empty the media, they are then sprayed, washed, and blow-dried, ready for the next 9 batches of test samples.

Logan System 2400 is equipped with two types of filter changers. The Super 2400 dissolution tester includes an automated filter tip changer for online UV analysis. An additional membrane filter changer is available for sample collection for offline HPLC analysis.

Online UV analysis

Filter changer for collection of samples for offline HPLC analysis

PVT 800

Logan PVT 800 toolset performs and records dissolution Performance Validation Testing (PVT) electronically, removing the uncertainties of manual recording. Logan PVT 800 toolset includes a temperature sensor, speed detector, vertical/horizontal, height, vibration, and centering gauges, etc. The PVT report is displayed on the PC screen, the data can be recorded in the hard drive and printed. With this advanced toolset, user intervention in the data-transferring process is eliminated. Logan PVT 800 toolset further improves compliance with FDA CFR 21 part 11.

19-C Schoolhouse Road Somrset, New Jersey 08873 USA 732 302 9888 infoDT@loganinstruments.com www.loganinstruments.com

Industry News

Copley's Appointment of New Applications Specialist, Clair Brooks, Highlights Commitment to Exemplary Customer Support.

Prioritising a stronger customer interface and enhanced application expertise as company grows at pace.

Nottingham, UK: Copley Scientific, a major provider of pharmaceutical testing systems with world-renowned expertise in the field of orally inhaled and nasal drug products (OINDPs), has appointed a new Applications Specialist to maintain a customer-centric focus as growth continues. Dr. Clair Brooks is a seasoned life sciences professional with a track record of delivering in-field support for a leading molecular diagnostics company. Her primary focus at Copley will be to help customers optimise equipment use - whatever challenges they face – ensuring they generate robust, reliable data and maximise their return on investment.

"The role of Applications Specialist is extremely important for us," said Mr. Jamie Clayton, Managing Director, Copley, "so filling it with such a great addition to our team makes this an exciting step. Copley's success is built on industry leading knowledge developed over decades by a dedicated team. Growth affords us the luxury of greater specialism, and the ability to add expertise and focus in areas of key importance. Understanding the evolving requirements of the pharmaceutical industry and providing our customers with the tools to meet them is paramount. This appointment will add to our existing capabilities to do that."

With an academic background in biochemistry and microbiology, Clair joins fresh from supporting the start-up and operation of heavily regulated testing labs. As an in-field Applications Specialist, her day-to-day work involved interfacing with expert scientists, helping to ensure regulatory compliance, and troubleshooting problems to tight timescales. This experience makes Clair perfectly placed to become the in-house expert on how best to use Copley products to support pharmaceutical development and manufacture. Key elements of her role include providing in-depth applications support across the complete Copley portfolio, delivering comprehensive user training – both onsite and at Copley HQ – and representing the needs of the customer in-house.

"We're committed to ensuring customers find Copley easy and rewarding to work with and I'm confident that Clair will play an important role in delivering on that commitment," continued Jamie. "I look forward to seeing her champion the customer perspective across our activities."

Mr. Jamie Clayton, Managing Director, welcomes Dr. Clair Brooks as Applications Specialist to Copley

Copley Signposts Growing Focus on Pharmaceutical Testing with Appointment of Dedicated Business Development Manager

Delivering enhanced, customer-centric service across the company's complete portfolio.

Nottingham, UK: Copley Scientific has appointed Mr. Imran Haneef as Business Development Manager, demonstrating its commitment to providing customers with high quality testing solutions for a wide range of pharmaceutical dosage forms. Haneef will focus exclusively on the solid dose, semi-solid, transdermal, and powder testing portfolio, which is renowned for its cost-efficiency, robust performance, and reliability. With his background in life science and laboratory equipment sales and a track record of building strong relationships with customers, he is an excellent addition to the growing Copley team.

"Copley is respected for its engineering expertise and compelling product range across many pharmaceutical dosage forms, so it's great to have the opportunity to bring someone on board to focus exclusively on this area of the business," said Mr. Matthew Fenn, Head of Business Development. "We're delighted to have attracted a candidate of Imran's calibre to the role. Copley is growing rapidly, and this success affords us multiple opportunities to enhance our customer offering. I'm confident that Imran will play an important role in helping us deliver our ambitious plans."

Mr. Haneef's academic background is in Pharmaceutical Science, including an MSc in Pharmaceutical Quality by Design, and he joins Copley from a leading lab instrumentation supplier with established expertise in life science testing. As a global Business Development Manager, he will focus on promoting awareness of Copley's comprehensive range of pharmaceutical testing equipment, providing guidance on the systems required to ensure compliance, and demonstrating the performance and optimal use of Copley solutions. Based in the UK, he will work with the company's established network of international distributors to connect with and deliver for customers across the globe.

"Whether you're investigating tablet dissolution, measuring critical quality attributes such as friability, or if you're testing semi-solids or suppositories, Copley has well-engineered solutions for highly productive and effective testing," said Fenn. "I'm looking forward to working with Imran to help customers choose the very best options for their lab."

Mr. Matthew Fenn, Head of Business Development welcomes Mr. Imran Haneef, Copley's new Business Development Manager - Pharmaceutical.

Logan Instruments' Release of Innovative Products

April 2023, Logan Instruments proudly launched the **Transdermal Diffusion Flow-Through Cell System**, which is available in 6, 8, 12, 16, and 24-cells configurations, offering scalability and flexibility to accommodate diverse research needs.

Key features that set the Logan Transdermal Diffusion Flow-Through Cell System apart:

- Integrated syringe pump ensures precise flow rates, surpassing traditional peristaltic pumps, and provides outstanding resistance to chemicals
- Dry heating system eliminates the need for water replacements, making the heating process more efficient and sustainable
- Innovative preheating design guarantees precise temperature control
- Adjustable flow rates range from 0.1–4 mL/min, with an accuracy of less than 1% error, enabling accurate and repeatable data collection
- Wide range of sample collection methods, suitable for studying drugs with diverse physical properties and formulation types
- Compliant with the latest USP <1724>, FDA 21 CFR Part 11, and cGMP/GLP regulations

Another new product that Logan launched in 2023: **AIR-1300**.

AIR-1300 is a fully automated shake and fire benchtop system that can test MDI, nasal spray, and nasal aerosol products with extreme precision and repeatability. Air-1300 provides total control over the testing techniques and guarantees accurate, controlled, and repeatable testing by eliminating manual inconsistencies.

- Continuous testing of up to 13 doses with high efficiency
- Built-in analytical balance enables automatic dose weighing
- Support for a wide variety of inhaler and nasal dosing test samples
- Compliant with USP <601>
- Temperature and relative humidity measurement functions
- User-friendly touchscreen display for user control

For inquiries from the U.S., please contact brian@loganinstruments.com.

For international inquiries, please contact joan@loganinstruments.com.

LOGAN INSTRUMENTS CORP.

AAPS PharmSci 360 Opening and Closing Plenary Speakers Announced

Registration for Oct. 22-25 Conference Now Open

Arlington, VA—AAPS is excited to announce the opening and closing plenary speakers for the PharmSci 360 conference (October 22-25, in Orlando, FL).

Thomas Hartung, MD, PhD, will deliver the opening plenary on Sunday, Oct. 22, 2023. He is currently the Doerenkamp-Zbinden-Chair for Evidence-based Toxicology in the Department of Environmental Health and Engineering at the Johns Hopkins Bloomberg School of Public Health and Whiting School of Engineering in Baltimore. He is Field Chief Editor of *Frontiers in Artificial Intelligence* and is the former Head of the European Commission's Center for the Validation of Alternative Methods (ECVAM).

Julie A. DeMartino, PhD, an independent consultant, will deliver the closing plenary on Wednesday, Oct. 25. She has been involved in drug discovery R&D with more than 3 decades of basic research and translational medicine experience. Following leadership roles at Merck & Co., Roche, and Merck KGaA, DeMartino founded her consulting firm, AcheneRx LLC, in 2021, to help pharma and biotech firms develop novel drug treatments for debilitating and progressive disease.

"I'm excited about the opening and closing plenary talks at this year's PharmSci 360," AAPS Executive Director Tina Morris, PhD, said. "Both talks will bookend a conference rich in innovation and full of the latest developments in pharmaceutical science. It will allow ample opportunities for attendees to discuss these advancements with colleagues from across pharma."

"We are very pleased to confirm the speakers for our opening and closing plenaries at PharmSci 360," AAPS 2023 PharmSci 360 Scientific Programming Committee Chair Otilia Koo said. "Drs. Hartung and DeMartino will speak to the latest developments in pharmaceutical sciences and provide their invaluable insights that attendees can use to progress both their research and career development, which are the important objectives of the conference."

Registration is now open for the 2023 PharmSci 360 conference: http://www.aaps.org/pharmsci/register.

American Association of Pharmaceutical Scientists (AAPS) is a 501(c)(3) non-profit association of more than 7,000 scientists and professionals employed in academia, industry, regulatory, and other research related to the pharmaceutical sciences worldwide. Its mission is to advance the capacity of pharmaceutical scientists to develop products and therapies that improve global health, which members pursue through four peer-reviewed journals and a variety of events in person and online. www.aaps.org

For more information:

Rebecca Stauffer

AAPS Communications Manager

staufferr@aaps.org

Simulations Plus Acquires Immunetrics to Expand its Immunology and Oncology Drug Development Capabilities

Acquisition increases breadth and depth of QSP expertise and range of therapeutic applications

Lancaster, CA-- Simulations Plus, Inc. (Nasdaq: SLP) ("Simulations Plus"), a leading provider of modeling and simulation software and services for pharmaceutical safety and efficacy, announced the acquisition of Immunetrics, Inc. ("Immunetrics"), a modeling and simulation company focused on accelerating drug development in oncology, immunology, and autoimmune diseases ¬– areas that are among the fastest growing therapeutics.

Under the terms of the Agreement, Simulations Plus agreed to pay the shareholders of Immunetrics cash consideration at closing in the amount of $15.5 million, including a $1.8 million hold-back, plus two future earn-out payments in the aggregate amount of up to $8 million based on the revenue performance of Immunetrics through December 31, 2024. Following the close of the transaction, Immunetrics will be a wholly owned subsidiary of Simulations Plus and will continue to operate under the Immunetrics name.

The acquisition strengthens the already-robust quantitative systems pharmacology (QSP) expertise at Simulations Plus and expands the range of therapeutic areas addressed by its software and services. QSP is a rapidly growing field of biomedical research that aims to model the mechanisms behind disease progression and quantify the pharmacokinetics (movement of drugs through the body) and pharmacodynamics (the body's biological response to drugs) of pharmaceuticals using mathematical computer models. QSP models are highly sophisticated and cost-intensive to build. With this acquisition, Simulations Plus is augmenting its established QSP offerings in a highly attractive field with additional proven models that can be rapidly deployed.

By adding Immunetrics' QSP services and software capabilities, Simulations Plus has increased the therapeutic areas addressed by its QSP models by more than 50% and introduced new areas of service to existing and potential clients. The acquisition is consistent with the company's disciplined strategy to grow a portfolio of complementary and innovative software platforms and services.

The acquisition of Immunetrics leverages Simulations Plus' existing infrastructure by expanding its therapeutic resources into largely underserved areas, including immunology and oncology.

"Today's announcement marks the beginning of a transformative offering in QSP for our clients," said Shawn O'Connor, Simulations Plus CEO. "Immunetrics brings proven QSP technology, a strong reputation in the market, and an incredibly talented team that we believe will provide us with a leading position in a rapidly growing field. Together, we intend to deliver valuable software solutions to help our clients provide optimized treatments to patients with speed and safety."

"We have been purposeful in our approach to company growth, investing in R&D and personnel, as well as making strategic acquisitions over the years. When we made the decision to expand into the QSP space in 2017, we acquired DILIsym Services and leveraged their expertise to expand into new therapeutic areas. Now, with the addition of Immunetrics, we will be able to provide support for an even greater range of therapeutic areas in a field with tremendous growth opportunity," O'Connor concluded.

Immunetrics CEO, Steven Chang added, "We are excited to join Simulations Plus and work together with our new colleagues to further expand and standardize the use of QSP in drug development. We complement

one another in areas of QSP applications and share a commitment to scientific rigor and positive client outcomes. I believe we are far greater together than the sum of our parts and will achieve significantly more as one unified organization."

The acquisition comes at a time when QSP use in the drug development process is becoming more prevalent.

"QSP is no longer an emerging field—12 years after the term was coined, it has become a critical component of drug development," noted Brett A. Howell, President of DILIsym Services, a division of Simulations Plus. "We are seeing it change the way companies run their drug discovery process, accelerating it while simultaneously reducing the likelihood of costly clinical trial failures down the line. The number of QSP-related FDA filings has also been on the rise, as the FDA and other regulatory agencies recognize the value of QSP modeling and predictions. We are excited to join forces with our new colleagues from Immunetrics and provide broader and deeper support to our clients."

After completion of the Immunetrics acquisition, Simulations Plus now has the capability to offer clients QSP modeling software and services solutions for 20 therapeutic areas:

- Acute myeloid leukemia (AML)
- Atopic dermatitis
- Cardiac wound healing and fibrosis
- Cardiovascular disease
- Complementary pathway diseases
- Drug-induced acute kidney injury
- Drug-induced liver injury from small molecules
- Gout
- Hypertension
- Idiopathic pulmonary fibrosis (IBF) and interstitial lung disease (ILD)
- Inflammatory bowel diseases (ulcerative colitis and Crohn's disease)
- Multiple myeloma (MM)
- Non-alcoholic fatty liver disease (NAFLD)
- Non-alcoholic steatohepatitis (NASH)
- Non-small cell lung cancer (NSCLC)
- Psoriatic arthritis/psoriasis
- Renal diseases and interplay with the cardiovascular system
- Rheumatoid arthritis (RA)
- Systematic lupus erythematosus
- Type 2 diabetes/metabolism

Many of Simulations Plus' existing and new QSP models are suited for applications in additional therapeutic areas with minor modification. The company will also continue developing new models in collaboration with clients and other partners.

Excel Partners, an investment bank with offices in New York and Los Angeles, acted as exclusive financial advisor to Simulations Plus in connection with this transaction. Procopio served as legal counsel to Simulations Plus in connection with this transaction. K&L Gates served as legal counsel to Immunetrics.

Simulations Plus Launches New Integrated Pulmonary Software and Services Package to Streamline Drug Development and Improve Patient Outcomes

Lancaster, CA -- Simulations Plus, Inc. (Nasdaq: SLP), a leading provider of modeling and simulation software and services for pharmaceutical safety and efficacy, announced the release of a new integrated pulmonary software and services package. This targeted package will support pharmaceutical companies by streamlining their pulmonary drug development processes, enabling them to make better-informed decisions and bring therapies to market faster.

The pulmonary package offered by Simulations Plus is founded on the industry leading GastroPlus® modeling and simulation platform, which can be used to predict localized exposure in the lungs. That localized exposure data is then utilized to inform the ILDsym™ or IPFsym™ platforms for efficacy predictions. As our client ventures launch and progress, Simulations Plus scientists will leverage their 40+ years of combined experience in pulmonary exposure and efficacy modeling to train in-house client experts on how to use the software and create models to accurately predict the exposure and efficacy of their pulmonary assets.

“Developing molecules intended to treat interstitial lung disease (ILD) and idiopathic pulmonary fibrosis (IPF) is a daunting challenge; they are serious conditions with no known cures,” explained Dr. Brett A. Howell, President of the DILIsym Services division at Simulations Plus. “We anticipate that the use of our targeted pulmonary package could lead to an acceleration in the development of treatments to slow or even halt progression of these diseases. By integrating the industry-leading platforms for PBPK/PCAT™, quantitative systems pharmacology (QSP), and the expertise of our scientists, along with the training of in-house scientists, we believe we can help our clients develop therapies that will improve and even extend the lives of patients worldwide.”

“Hiring and retaining skilled modelers is difficult, which is why many companies develop talent from within,” noted Vice President of Business Development Josh Fohey. “While we are always pleased to assist with our clients’ drug development programs as an extension of their team, we also want to empower them to engage with their models and data beyond what is typical with consulting relationships. As part of our Pulmonary Package, client teams benefit from a customized learning package using the tools in their program, implemented with support and guidance from our experts. Together, we can better understand lung absorption and efficacy and find new treatments for respiratory disease.”

Learn more about the newly released pulmonary package:
www.simulations-plus.com/breatheeasy

Verder Group Acquires ERWEKA

The Verder Group is proud to announce the successful acquisition of ERWEKA, a highly regarded German company renowned for its development and manufacturing of premium tablet testing equipment.

With this acquisition the Verder Scientific division further strengthens its position and accelerates the growth trajectory in targeted niche markets with high state-of-the-art equipment.

ERWEKA, founded in 1951, has a manufacturing and assembly facility in Langen, near Frankfurt, and sales offices in the US and Hong Kong. The company supplies dissolution and tablet testing equipment for pharmaceutical and life science companies, research and test laboratories, and universities all over the world. With its complementary technology to Verder Scientific, this acquisition is an opportunity to increase our market share.

Commenting on the acquisition, Andries Verder, CEO Verder Group, expressed enthusiasm for the opportunities it presents: "We are excited to welcome ERWEKA into the Verder family. Their strong market position and exceptional expertise perfectly complement the Verder Scientific existing portfolio. Together, we will unlock new possibilities, accelerate innovation, and create synergies that benefit our customers, employees, and stakeholders."

Claudia Müller, CEO ERWEKA, states: “Today marks an exciting milestone as our technology driven company joins forces with Verder Scientific. Together, we embark on a journey that not only guarantees a future-proof and robust foundation but also unveils unprecedented growth possibilities. Our shared vision is rooted in the belief that technology, when harnessed strategically, can transform lives and businesses alike."

ERWEKA will become an integral part of Verder´s Scientific Instruments Division, whose CEO, Dr. Jürgen Pankratz, stated: “We will take our time to deeply understand the customers needs and dynamics of this new business segment. I have no doubt that subsequently we will jointly derive a business plan that will make significant contributions to the future growth and success of ERWEKA.”

VERDER

Attend Eastern Analytical Symposium & Exposition: November 13-15, 2023

The Eastern Analytical Symposium and Exposition (EAS) is held annually to showcase leading-edge research and development in analytical chemistry and its allied sciences through the presentation of lectures, workshops, and short courses. An Exposition featuring the latest innovations in laboratory instrumentation and supplies related to these sciences is held concurrently with the symposium.

The 2023 Eastern Analytical Symposium (EAS) is set for November 13-15, with registration opening in mid-July. Our 2023 theme is 'Better Life with Analytical Chemistry'. As an Analytical Chemist, EAS provides multiple opportunities to hone your skills by attending a short course, workshop, technical presentation, seminar, or poster session. We are offering 30 short courses this year on a diverse array of analytical techniques and topics. We will also have several offerings geared toward students, such as career development workshops. In addition to exhibitors showcasing state-of-the-art instrumentation and technology, 2023 exhibitor offerings will include a technology tour, reception, mixer, Thermo Fischer Scientific seminar, and demonstration rooms hosted by Waters and Agilent Technologies. We are grateful to ALL our sponsors, and particularly thank our current Silver level sponsor – S-Matix and Waters – and our Bronze level sponsor – Bruker.

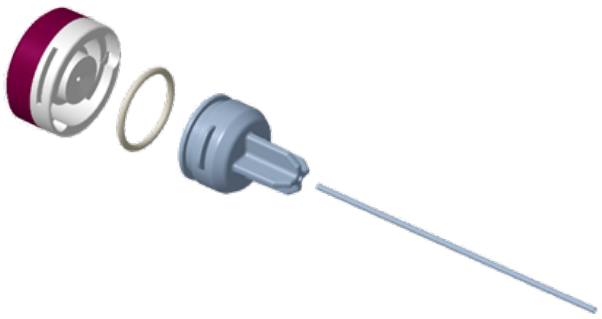
Our Monday Keynote Speaker, Dr. Vasilis Vasiliou (Susan Dwight Bliss Professor of epidemiology/Chair of environmental health sciences at the Yale School of Public Health), will speak on *Exposome and Human Disease: From Neurological Disorders to Diabetes and Cancer.* Tuesday's Breakfast Lecturer will be Dr. Frank Nichols (Professor of periodontology at UConn Health), presenting on *Fractionation and Characterization of Bacterial Complex Lipids Using Analytic Chemical and Mass Spectral Approaches*. The 2023 Plenary Lecture will be presented on Wednesday by Dr. Sibrina Collins (Executive Director of STEM Education for the College of Arts and Sciences at Lawrence Tech). Dr. Collins will speak on *Inclusive Stories in Chemistry: Celebrating Dr. Marie Maynard Daly*. Additionally, EAS is proud to sponsor six awards recognizing distinguished career achievements across analytical subdisciplines as well as our student research awards. We congratulate all 2023 awardees and encourage attendees to attend the award sessions.

EAS offers a diverse array of topics within the analytical sciences. There is something for everyone, and all within a friendly, convivial symposium. Visit our website for complete information and to register: www.EAS.org. Follow us on social media, and most of all, be sure to join us at the Crowne Plaza Princeton Conference Center, Plainsboro, NJ in November 2023 to discover how Life is Better with Analytical Chemistry.

Frank Romano
President, 2023
Eastern Analytical Symposium and Exposition

Distek Receives U.S. Patent for Innovative Dissolution Sample Probe Design

North Brunswick, NJ – Distek, Inc., a reputable leader in laboratory pharmaceutical instruments, is pleased to announce the award of U.S. Patent 11,454,570 for their G2 sampling probe designed for dissolution testing and related applications.



The G2 sampling probe, a two-piece design comprising a cap and base, securely twist-locks together to create a reliable and robust sampling system. Notably, the cap and base feature integrated media transfer regions that effectively reduce flow resistance. This design enhancement mitigates the risk of blockages or contamination during the dissolution testing process, ensuring accurate and dependable results.

"We are proud to receive this patent," said Jeff Brinker, President at Distek. "This patent highlights Distek's ongoing commitment to innovation and delivering advanced solutions to our valued customers."

To learn more about the G2 sampling probes and explore how Distek's solutions can enhance your laboratory operations, please contact Distek Customer Service at +1 732 422 7585, email info@distekinc.com, or visit www.distekinc.com.

ABOUT DISTEK, INC.

Distek engineers highly innovative, user-friendly instruments with advanced features. Our product range includes water bath and bathless dissolution systems, dissolution media preparation, in-situ fiber optic UV, bathless tablet disintegration and dissolution autosampling. Our bioprocessing solutions include the BIOne single-use bioreactor and the BIOne 1250 bioprocess control system for mammalian and microbial models.

Serving brand name, generic, and biosimilar drug manufacturers, CROs, CMOs, and more, Distek caters to a diverse market, including cultured foods, Nutraceuticals, agriculture, and government agencies. With ISO certification since 2002 and multiple patents, we ensure consistent service and continuous improvement.

Sean Gilmore
Distek, Inc.
121 North Center Drive
North Brunswick, NJ 08902
732.422.7585 | info@distekinc.com

Cipher

21 CFR Part 11 Instrument Control Software

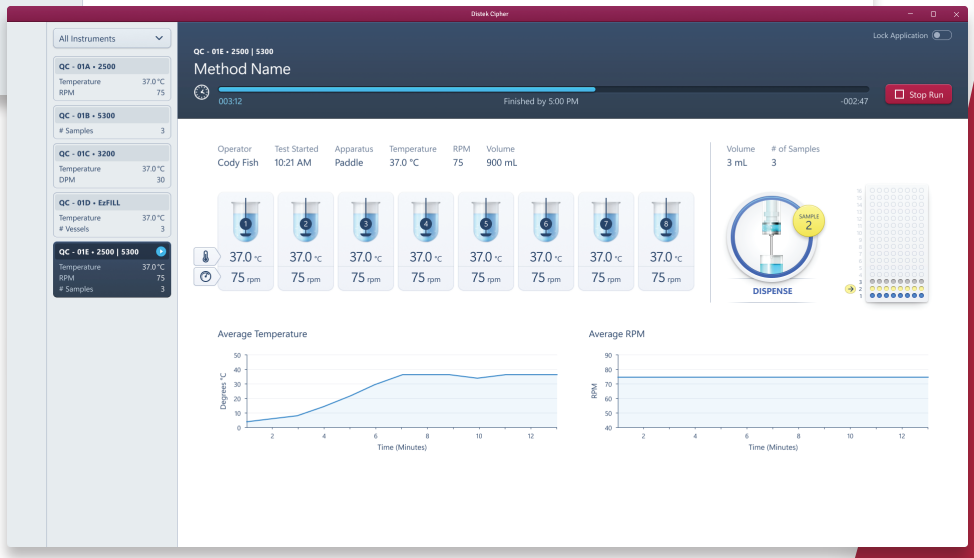
- Complete 21 CFR Part 11 and Data Integrity compliance for Distek dissolution, autosamplers, and disintegration instruments.
- Method Wizards simplify method creation.
- Remotely configure and monitor Distek instruments in real-time from your PC.
- Enable automatic export of record files for seamless integration with a LIMS package.

DISSOLUTION

FIBER OPTIC

MEDIA PREP

AUTOSAMPLER



Schedule your Free Cipher Demonstration

DISTEK

distekinc.com • info@distekinc.com

Agilent

Trusted Answers

Conquer Your Filtration Challenges

The Agilent NanoDis System provides formulation scientists with accurate release profiles of APIs. Users benefit from a complete, automated solution, including dissolution, filtration, and sampling, compliant with cGMP regulations.

For nano, and more. Equipped with hollow fiber filtration, the NanoDis System is particularly suitable for nanoparticle formulations. It can also be used for liposomes, parenterals, and any other products with filtration challenges requiring release-rate testing.

For speed-to-market. The automated, software-driven workflow helps achieve predictive dissolution profiles in less time, facilitating faster formulation development, validation, and time to market.

For more information about the NanoDis System, visit: www.agilent.com/chem/nanodis

DE57544302