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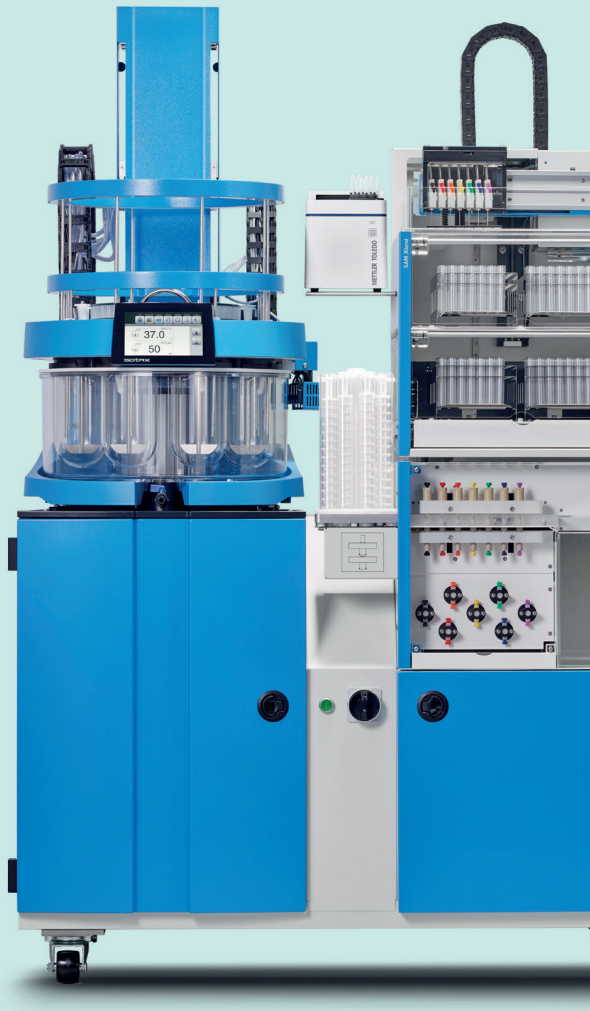

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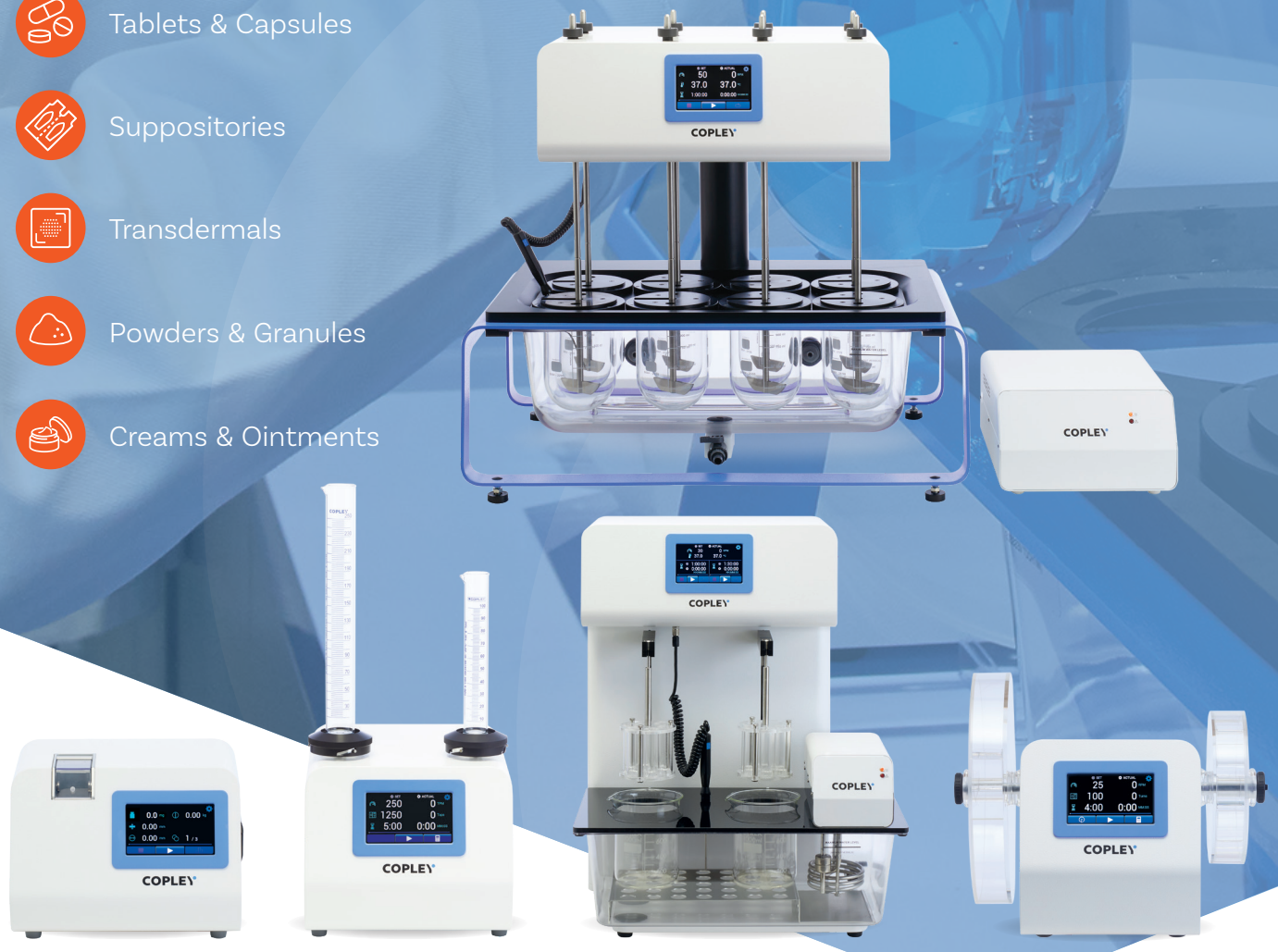

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Improved Melatonin Dissolution Properties: A Way Forward for Treating Children with Sleep Disorders

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ABSTRACT

Sleep problems, in particular the difficulty in initiating and maintaining sleep are important comorbidities in children and adolescents with attention deficit hyperactivity disorder (ADHD), accompanied by a range of negative consequences for both patients and their caregivers. Melatonin, a naturally occurring hormone that is important for coordinating the body's sleep-wake cycle, has been used to treat insomnia in children with ADHD. This study compares the dissolution properties of two melatonin tablets (1 and 5 mg Mellozzan and Melatonin AGB). Results showed that Mellozzan dissolved rapidly (90% within 5 minutes) in all pH levels tested, whereas Melatonin AGB dissolved slower (60% within 30 minutes). The fast dissolution properties of melatonin observed in Mellozzan indicates that this formulation is preferable for the treatment of children where the dissolution step is critical to reach the desired clinical effect.

KEYWORDS: Melatonin, dissolution properties, sleep problems, ADHD

INTRODUCTION

Melatonin is an endogenous hormone, secreted by the pineal gland, that regulates the circadian rhythm in mammals (*1*). In diurnal mammalian species, melatonin binds to receptors in the suprachiasmatic nucleus to diminish a wake-promoting signal from the circadian clock and thereby induce sleep (*2*). In parallel, melatonin also modulates the vast so-called "default mode network," a network active in daydreaming and wakeful rest to promote sleep-like changes in the brain (*3*, *4*). The physiology of melatonin secretion is illustrated in Figure 1.

A sleep disorder is a condition that impairs sleep quality and is a frequently overlooked medical disorder that affects individuals of all ages and interferes with physical, social, and mental function (*5*). Lifestyle and environmental factors contribute to sleep disorders, and there are short and long-term health consequences (*6*). Short-term consequences are dominated by psychological symptoms, e.g., affective disorders, cognitive, memory, and performance impairments, whereas the long-term consequences are mainly somatic and include hypertension, dyslipidemia, cardiovascular disease, weight-related issues, metabolic syndrome, type 2 diabetes mellitus, and colorectal cancer (*7*). Men with sleep disorders have also been shown to have an increased risk for all-cause mortality. Furthermore, the health care costs of sleep disorders in the United States represents approximately $94.9 billion (*8*).

Up to 50% of children will experience intermittent sleep-related problems; however, those with ADHD are more likely to develop clinically significant sleep disorders (*9*). According to some studies, as many as 50% of children with ADHD have a clinically relevant sleep disorder that can result in a variety of functional impairments (*10*). Sleep problems in children with ADHD have a major impact and negative consequences on children and caregivers, including (i) quality of life, (ii) impaired family functioning, and (iii) a decreased school attendance (*11*). Up to 12% of functional and social impairment variance in ADHD has been attributed to sleep problems rather than ADHD itself (*12*).

Administrating exogenous melatonin in humans improves sleep quality and reduces sleep onset, attenuates jet lag, has anti-inflammatory and anti-oxidative effects (*13*–*16*). Lately, there has been increased interest in medical applications of melatonin, especially related to circadian rhythm disturbance and sleep impairment, and several

*Corresponding author

products are now available in regulated pharmaceuticals markets as prescription drugs (*17, 18*). The purpose of treatment with melatonin is to reduce time to sleep, induce longer sleeping periods, and provide better functioning on the following day (*12, 19–22*). In this sense, it is important to consider the dissolution properties of the product to meet the desired formulation goal.

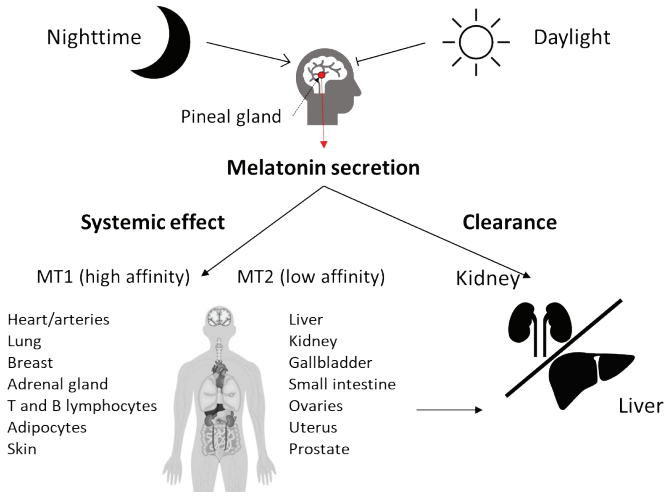


Figure 1. Melatonin secretion is regulated by daylight; Its secretion increases during sleep and decreases when eyes receive light from the sun. Activating MT1 (high affinity) and MT2 (low affinity) receptors melatonin has systemic effects and melatonin is subsequently cleared from the circulation by the liver and kidney.

For drugs administered orally, their bioavailability is influenced by several factors including the drug solubility and the dissolution rate. In particular, the release of drug from the formulation is frequently the rate-limiting step for gastrointestinal absorption of the active pharmaceutical ingredient (API) (*23*). During the formulation of a product, knowing the rate of metabolism and clearance of the API is crucial. This is particularly true for drugs such as melatonin that experience differences in their metabolism rate relative to the age of the patients. Melatonin, in prepubertal children, metabolizes faster than adults (*24*). Considering this, a better formulation of melatonin for the treatment of children is one that shows fast dissolving properties.

This study involved two drug products (Mellozzan and Melatonin AGB) containing melatonin as the API. The dissolution properties of the two products were tested with the aim of determining their suitability for the treatment of children with sleep disorders.

METHODS

Materials

All chemicals used were of analytical grade. Milli-Q water was used throughout the experiments. Mellozzan (EQL Pharma, Sweden) is N-acetyl-5-methoxy tryptamine ($C_{13}H_{16}N_2O_2$, 232.278 g/mol), with melatonin as the active ingredient. The formulation contains microcrystalline cellulose, pregelatinized starch, colloidal anhydrous silica, and magnesium stearate. Melatonin AGB (AGB Pharma, Sweden) formulation contains microcrystalline cellulose, calcium hydrogen phosphate dihydrate, and magnesium stearate. The reference for melatonin was the Certified Reference Material, Supelco, Lot no.: LRAC8057 (Bellefonte, PA, United States). The dissolution experiments were performed with 0.5, 1, 2, and 5 mg tablets of Mellozzan and 1 and 5 mg tablets of Melatonin AGB. The details of the products including manufacturer information are summarized in Table 1.

Table 1. The Samples and Their Properties

Description	Strength	Origin	Lot No.	Expiry Date	Analysis Date
Mellozzan tablet	1 mg	EQL Pharma	E0008E1	-	05-2021
Mellozzan tablet	5 mg	EQL Pharma	E0006E1	-	05-2021
Melatonin AGB tablet	1 mg	AGB	92045	05-2022	05-2021
Melatonin AGB tablet	5 mg	AGB	92043	03-2022	05-2021

Instrumentation

The dissolution tester was an apparatus 2, Sotax AT8x (article number 15180-1) acquired from BergmanLabora AB. The high-performance liquid chromatography (HPLC) platform used in the study was operated with a reversed phase separation column (Waters XBridge C18, 3.0 × 150 mm, 3.5 µm) using linear flow of the mobile phase (22% acetonitrile/78% 25 mM phosphate buffer, pH 3), with a flow rate of 0.75 mL/min and an injection volume of 30 µl. The samples were analyzed in an automated mode, and the autoinjector was kept at ambient temperature. The separation was performed at 40 °C and detected at a wavelength of 224 nm. The analysis cycle time was kept at 5.5 min.

Experiments

The experiments were executed by performing dissolution assays with several different dissolution media: NaCl, HCl pH 1.2 (chloride buffer), phosphate buffer pH 4.5, phosphate buffer pH 6.8, and Milli-Q water (n = 6 tablets per product). The dissolution media were prepared according to *European Pharmacopoeia* Chapter 5.17.1 Recommendations on Dissolution Testing (*25*). The dissolution medium volume was kept constant at 500 mL throughout the experiments with a temperature of 37.0 ± 0.5 °C and 50 rpm (paddle). The sampling for drug quantification was done after 5, 10, 15, 20, 25, and

30 minutes using a sampling volume of 5 mL and a 0.45-µm nylon filter. The quantification of drug content in the solution was determined by an HPLC analytical method.

Preparation of Standards

The respective stock standard solution was prepared by adding 5 mg melatonin standard to 100 mL volumetric flasks. The standard material was dissolved in Milli-Q water and diluted to volume. Next, the respective stock solution was diluted to five concentrations in the range of 0.5–19.5 µg/mL to create the standards for the calibration curve experiment. This range covers the concentration of all samples. For each dissolution media, the standards were diluted with the actual dissolution medium.

Data Analysis

The amount of drug in solution was estimated based on the concentration of the standards and the calibration curve. The actual results were reported as the percentage of the total drug content measured at each sampling time point. The values were plotted and analyzed in GraphPad version 9.3. A two-way ANOVA test with Bonferroni correction for multiple comparisons was applied to evaluate the influence of the drug content and the formulation of the products on the dissolution efficiency. For the dissolution comparison between formulations, a Mann-Whitney test was used to find significant differences based on the adjusted p-value for multiple comparisons.

RESULTS

The present study compared in vitro dissolution properties of tablets containing 1 and 5 mg of Mellozzan and Melatonin AGB at pH 1.2 and 6.8. The dissolution rate was calculated based on the concentration of the product in the solution over time. The results were based on the calibration curve with known concentrations of melatonin. The details of the products including manufacturer data are summarized in Table 1. Results of the dissolution tests are shown in Figures 2 and 3.

Melatonin AGB showed significantly slower rate of dissolution compared to Mellozzan in the two conditions evaluated. At the last time point (30 min), the amount of dissolved melatonin ranged between 45% and 60%. In contrast, more than 90% of Mellozzan was dissolved at the first time point (10 min), which was the case in both buffers. We did not observe significant differences in dissolution between 1 and 5 mg for Melatonin AGB or Mellozzan (Fig. 3), indicating that dose did not have an impact on dissolution of the API. The Mellozzan formulation allows the API to dissolve significantly faster when compared to the Melatonin AGB formulation at all time points (Fig. 3).

To perform a detailed characterization of the dissolution properties of Mellozzan, we further expanded both the content of drug as well as the dissolution condition. The concentration of dissolved drug was determined at five time points, ranging from 5–30 minutes, at 37 °C. For this analysis, four different strengths of Mellozzan tablets containing 0.5, 1, 2, and 5 mg of the API were studied. The dissolution assay was carried out in three buffers: chloride pH 1.2, phosphate pH 4.5, phosphate pH 6.8, and in Milli-Q water. We found that in general, regardless of the strength of the tablets and the dissolution conditions, the Mellozzan formulation allows for very quick release of the drug into the solution. After 5 minutes of exposure to the solution, over 90% of the API was released, which held true across all strengths of the formulation and experimental conditions assessed (Fig. 4). Our results

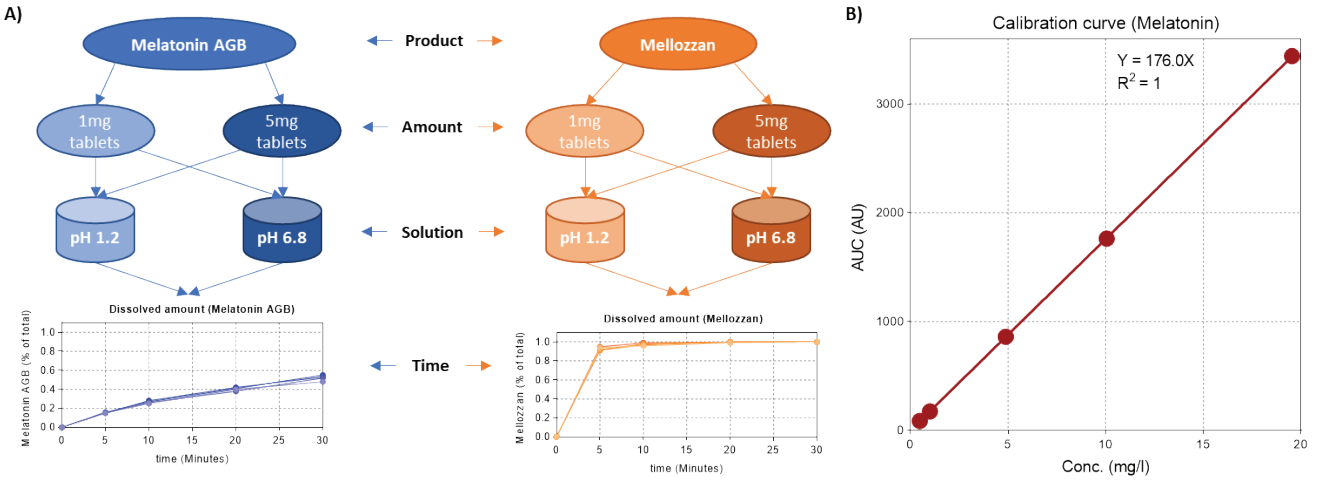
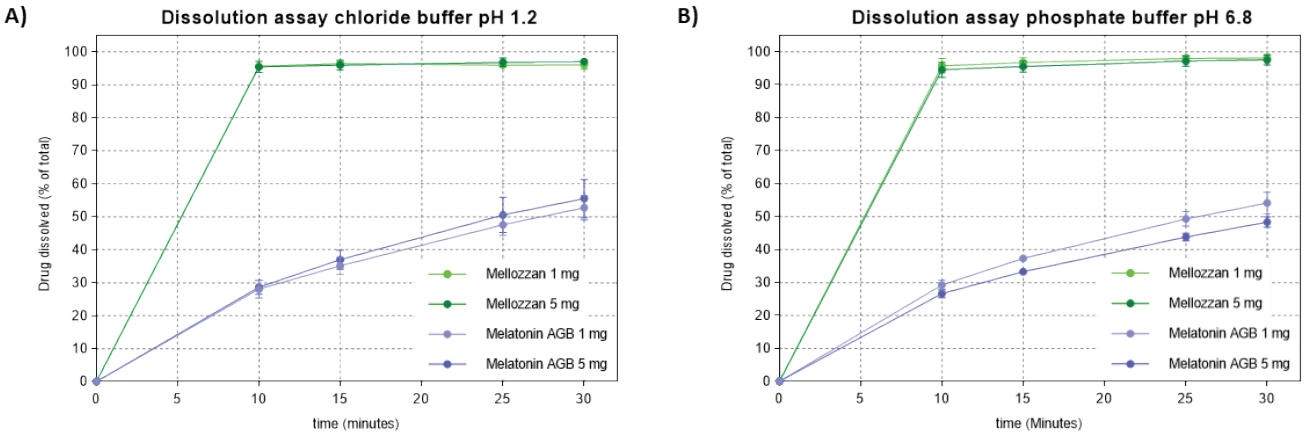


*Figure 2. Experimental design to assess the dissolution properties of Mellozzan and Melatonin AGB. (**A**) Tablets containing 1 or 5 mg of each product were submitted to a dissolution process in a pH 1.2 or pH 6.8 buffer. The cumulative amount of dissolved product was quantified every 5 mins until 30 mins after exposure. (**B**) Calibration curve used for quantification of the products.*



	10 min				15 min				25 min				30 min			
	Melatonin 1mg	Melatonin 5mg	Mellozzan 1mg	Mellozzan 5mg	Melatonin 1mg	Melatonin 5mg	Mellozzan 1mg	Mellozzan 5mg	Melatonin 1mg	Melatonin 5mg	Mellozzan 1mg	Mellozzan 5mg	Melatonin 1mg	Melatonin 5mg	Mellozzan 1mg	Mellozzan 5mg
Melatonin 1mg		ns	***	***		ns	**	**								
Melatonin 5 mg	ns		***	***	ns		*	*								
Mellozzan 1mg	****	****		ns	**	*		ns								
Mellozzan 5mg	****	*****	ns		**	*	ns									

	10 min				15 min				25 min				30 min			
	Melatonin 1mg	Melatonin 5mg	Mellozzan 1mg	Mellozzan 5mg	Melatonin 1mg	Melatonin 5mg	Mellozzan 1mg	Mellozzan 5mg	Melatonin 1mg	Melatonin 5mg	Mellozzan 1mg	Mellozzan 5mg	Melatonin 1mg	Melatonin 5mg	Mellozzan 1mg	Mellozzan 5mg
Melatonin 1mg		*	***	***		ns	**	**								
Melatonin 5 mg	ns		****	***	ns		****	****								
Mellozzan 1mg	****	****		ns	***	****		ns								
Mellozzan 5mg	****	***	ns		***	****	ns									

*Figure 3. Mellozzan dissolves significantly faster than melatonin in both low (**A**) and neutral (**B**) pH buffers.*
ns: not significant; asterisks (,**,***,****) indicate adjusted p-values (< 0.05, 0.01, 0.001, 0.0001, respectively).*

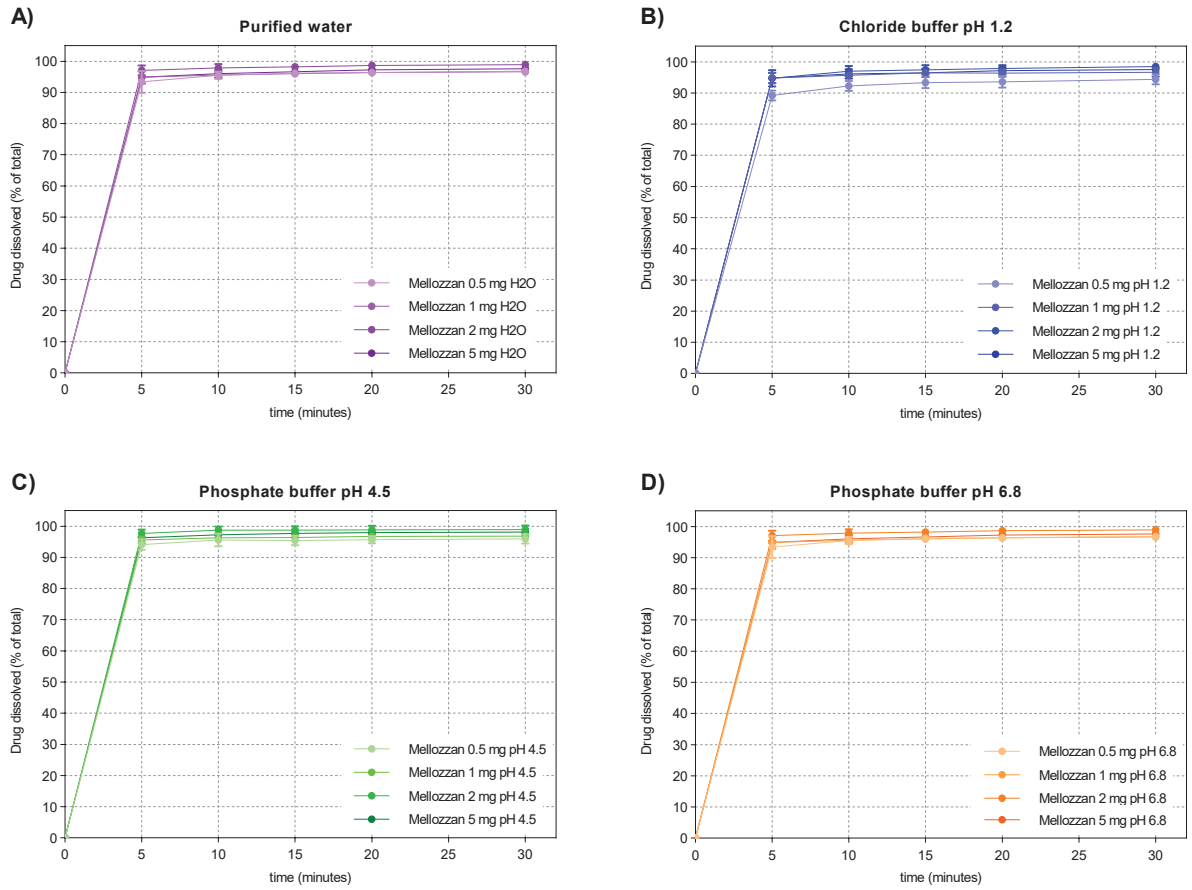


*Figure 4. Dissolution assay of Mellozzan tablets of different strengths in different conditions: (**A**) Milli-Q water, (**B**) chloride buffer pH 1.2, (**C**) phosphate buffer pH 4.5, (**D**) phosphate buffer pH 6.8.*

demonstrate that the formulation of Mellozzan has very similar dissolution properties at a wide range of pH from 1.2 to 6.8 including water (neutral).

DISCUSSION

Dissolution Characteristics

This study characterized the dissolution properties of Mellozzan in comparison with Melatonin AGB. We evaluated the influence of drug content, the formulation, and pH of the solution on dissolution of the API (melatonin). Mellozzan dissolved very fast (as soon as 5 minutes after exposure more than 90% of the drug in this formulation is in solution), regardless of the pH of the dissolution buffer. In contrast, less than 60% of Melatonin AGB is dissolved after 30 minutes of exposure to the solution. This is of major relevance for the bioavailability and pharmacokinetics of the drug within the body. The rapid dissolution properties of Mellozzan can accelerate absorption of the drug in the gastrointestinal tract, thereby resulting in faster onset of clinical effects, such as treating insomnia in children and adolescents with ADHD). Below these claims are examined in more pharmacokinetic detail.

Pharmacokinetics

Melatonin has a noticeable first pass metabolism, resulting in bioavailability of around 15% (*26*, *27*). This means that any oral dosage form of melatonin needs to be six times higher than that of any corresponding parenteral dosage form to reach the same concentration in plasma. In addition, melatonin is quickly absorbed into plasma from the gastrointestinal tract, with a $t_{1/2}$ absorption of 6 minutes (*28*, *29*). This has profound importance for the implications of our study. Quick absorption usually results in the mirroring of in vitro dissolution curves into plasma concentration curves, with a plasma clearance function moderating the curves shapes but not the onset characteristics.

It is expected that the rapidly dissolving Mellozzan product would have a faster onset of clinical effect than Melatonin AGB. From the dissolution curves and the $t_{1/2}$ absorption data, it can by hypothesized that the expected onset of clinical effect for Mellozzan, assuming appropriate strength, is 15–20 minutes, whereas the onset of clinical effect for Melatonin AGB, is 40–50 minutes.

Melatonin has a plasma half-life of around 45 minutes according to a first order kinetics (*27, 30*). This is regarded as a quick clearance and may have an impact on the minimum dosage for clinical effect based on the following logic. A slow release of melatonin into plasma will enable the clearance to act on the slowly rising plasma concentration for a long part of the half-life, thereby clearing a substantial amount of melatonin before it reaches the peak plasma concentration. Assuming that a specific threshold of plasma concentration is required to initiate sleep induction, it becomes evident that a formulation with a faster release into plasma (e.g., 90% within 5 min), as compared to its half-life, will only have a marginal impact on the peak plasma concentration. Consequently, the short half-life will only slightly affect the minimum tablet strength required. However, for a formulation with slow release into plasma (e.g., only 60% or less released after 30 mins), a half-life of 45 minutes will significantly attenuate the peak plasma concentration and therefore require a stronger dose. Therefore, we can conclude that the dosage to trigger sleep is higher for Melatonin AGB than for Mellozzan. This has important clinical implications for doctors who want to prescribe the most effective dose for children with ADHD and sleep disorders.

CONCLUSION

Here we provide data supporting the rapid dissolution properties of Mellozzan tablets compared to Melatonin AGB tablets. Characterization of the formulation revealed that Mellozzan, regardless of the strength (1 or 5 mg), dissolve more than 90% of API in the first 5 min of exposure to the solution. Clinically these findings strongly suggest faster absorption of melatonin in the gastrointestinal tract, a shorter time to reach a therapeutic plasma concentration, and shorter time for onset of action. This is particularly beneficial when treating insomnia in children and adolescents with ADHD.

FUNDING

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CONFLICTS OF INTEREST

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

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Dissolution Specifications for Ibuprofen Soft Gelatin Capsules: A Proposal

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ABSTRACT

The aim of the present study was to propose a *Q* dissolution value as a quality control test for 400-mg ibuprofen soft gel capsules using the dissolution recommendations included in the FDA dissolution database. Two commercial batches of the reference product and two batches of three generic products were selected. Dissolution profiles were determined by using apparatus 1 at 150 rpm with 900 mL of phosphate buffer pH 7.2 as dissolution media. To determine the probability of passing the USP dissolution test, the R program with Burdick codes were used. Quality control tests including rupture time were also performed. Results showed that a *Q* dissolution value of 80% at 20 minutes could be recommended as a quality control test. A moderate correlation was found between rupture time and the percentage dissolved at 10 minutes.

KEYWORDS: Ibuprofen, soft gelatin capsules, release profiles, rupture test, dissolution

INTRODUCTION

Ibuprofen, the first member of propionic acid derivatives introduced in 1969, is the most frequently prescribed non-steroid anti-inflammatory drug (NSAID) because of its prominent analgesic and antipyretic role (*1*). Ibuprofen is highly soluble in 1-octanol, with a pKa value of 4.9 (*2*). The Ibuprofen biowaiver monograph describes it as Biopharmaceutical Classification System (BCS) class II active pharmaceutical ingredient (API), having high permeability and pH-dependent solubility with minimum solubility at pH 2 (*3*). The drug is supplied as tablets, oral suspension, capsules, and soft gelatin capsules, which is the preferred dosage form among adults due to its increased absorption rate, thereby reducing time for the expected effect (*4*). Several factors can influence release of the API from soft gelatin capsules, including physical properties of the gelatin shell, physical and chemical properties of the fill material, and moisture exchange between the shell and fill material (*5*, *6*). Therefore, dissolution is an important test for drug product performance of this dosage form.

Currently there are dissolution requirements available for ibuprofen tablets and oral suspension (*7*, *8*). Although the first liquid gel ibuprofen capsule was approved by the US FDA in 1995, there is little information available about the dissolution characteristics of this dosage form. In the FDA dissolution method database, the recommendations for ibuprofen and ibuprofen potassium soft gelatin capsules are to use apparatus 1, at 150 rpm, and 50 mM phosphate buffer pH 7.2 as dissolution medium (*9*).

Considering that the dissolution test is an important tool to describe the performance characteristics of oral dosage forms and to ensure the batch-to-batch quality, the main objective of the present study was to evaluate the dissolution profiles of 400-mg ibuprofen soft gelatin formulations from the Mexican market and to propose a *Q* dissolution quality control acceptance criteria specification for this dosage form.

METHODS

Chemicals

Ibuprofen pharmaceutical secondary standard (purity 99.7%) was obtained from Sigma Aldrich, USA. Analytical grade monobasic sodium phosphate, sodium hydroxide, potassium hydroxide, anhydrous ethanol, phosphoric acid, and methanol were acquired from J.T.Baker. Water was obtained from a Milli-Q Reference (Millipore-Merck) system.

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Products

Products were selected according to their commercial availability. Two commercial batches of the Mexican reference product (Actron) and two commercial batches of three generic products containing 400-mg ibuprofen soft gelatin capsules were evaluated. All products were commercially available in Mexico and were randomly encoded as: R1 and R2 (reference product), A1, A2, B1, B2, C1, and C2 (generic products).

Quality Attributes

Physical attributes such as shape and color were carefully recorded for all the products.

Assay and uniformity of dosage units were evaluated as follows.

For assay sample preparation, 10 intact capsules were individually weighed to obtain their gross weights, taking care to preserve the identity of each capsule. Then the capsules were cut with a sharp open blade, the content was removed, and the emptied capsules were washed with methanol, allowing the solvent to evaporate at room temperature. The content of the 10 capsules was mixed and ibuprofen was determined by using United States Pharmacopoeia (USP) 43 method for ibuprofen tablets (*7*). The assay was performed by reverse-phase high-performance liquid chromatography (HPLC). The system used was a Shimadzu HPLC, which consisted of a dual plunger pump (LC-10 ATVP), a UV-Vis detector (SPD-10AVP) equipped with system controller (CBM-20A, UFLC), and an autoinjector (SIL-10A).

To assess uniformity of dosage units, the same sample preparation procedure used for assay was followed. Individual shells were weighted, and the net content of ibuprofen was calculated from the weight of content removed for the individual capsules and the result of the assay.

Rupture Test

A rupture test was carried out in accordance with the procedure described in *USP* General Chapter <2040> for the evaluation of soft gel capsules (*10*). The test was performed using six dosage units.

Preparation of Buffer Solution

Phosphate buffer pH 7.2 was prepared as dissolution medium according to *USP* requirements (*11*).

Dissolution Studies

Dissolution studies were conducted using USP apparatus 1 (Vankel 7000) at 150 rpm and 900 mL of phosphate buffer pH 7.2 as dissolution medium at 37 °C. Twelve dosage units were evaluated for each product. Samples were taken at 5, 10, 15, 20, 30, and 45 min without medium replacement. In all cases, 5-mL samples were removed. Samples were filtered through a 35-µm full flow filter (Agilent Technologies), diluted with the dissolution medium, and analyzed using a previously validated spectrophotometric method with dual beam UV at 222 nm.

The method was linear from 4–20 µg/mL. The coefficients of variation for intra-day and inter-day measurements ranged from 0.5–2.4% and 1.3–2.8%, respectively. The percentage of relative error values did not exceed 1.1%, indicating that the method employed was precise and accurate. Furthermore, the method was selective because no interferences were found between the active drug, the excipients, or the color capsule shell of the products studied.

Data Analysis

Once the dissolution performance was evaluated, the probability of passing the USP dissolution test (Q) was estimated using the simulation approach proposed by Burdick et al. (*12*). The program codes written for the R environment were obtained from the book's website. The simulations were performed using R statistical software version 4.1.3. The codes allowed us to calculate the probability of each sample to pass the dissolution test in the two main stages and overall. The software also generated a heat map of probability data for the different batches studied. The correlation between rupture time and percentage of drug dissolved was calculated using the same software.

RESULTS AND DISCUSSION

Products and Quality Attributes

Table 1 shows the quality attributes of the products studied. Although differences in shape and color were observed between products, results from the assay test were satisfactory (93.9–101.4%). Additionally, all products met the acceptance criteria of uniformity of dosage units ($L1 \leq 15$).

In 2007, USP introduced the rupture test as a performance test for dietary supplements contained in soft-shell capsules. Although it is not a requirement for drug products, we conducted this test because it is a rapid and simple way for qualifying the film strength of soft gelatin capsules. Table 1 shows that rupture time was similar between batches and between the products R, A, and C, but batches of product B had the slowest rupture time and the highest variability within products.

Table 1. Physicochemical Characteristics of Ibuprofen Soft Gelatin Products

Product	Shape	Color	Assay, %	Uniformity of dosage units, % L1 ≤ 15	Mean rupture time, min (range)
R1	Oval	Yellow	93.9	9.3	5.03 (3–6.5)
R2	Oval	Yellow	98.5	3.0	5.05 (4–6)
A1	Oblong	Red	97.3	4.9	6.23 (5.3–7.3)
A2	Oblong	Red	97.8	5.2	7.25 (3.5–10.2)
B1	Oblong	Purple	94.8	8.4	11.05 (8.2–14)
B2	Oblong	Purple	94.3	8.4	13.37 (10.3–17.4)
C1	Oblong	Pale Yellow	101.4	4.0	5.16 (3.3–8.3)
C2	Oblong	Pale Yellow	99.1	2.3	6.02 (3.4–9.4)

Dissolution Study

To date, there are no reports about the dissolution behavior of 400 mg ibuprofen soft gel capsules. The experimental conditions selected for the study were those proposed by the FDA; however, we included the sampling time at 15 min to determine if the products could meet the specification for very rapidly dissolving products. Figure 1 shows the mean dissolution profiles obtained. The dissolution method was able to differentiate between the products. Products R, A, and C met criteria for very rapid dissolution (i.e., 85% dissolved at 15 min), and both batches of product B met criteria for rapid dissolution (i.e., 85% dissolved at 30 min). The slow initial ascending phase in the dissolution profile for both batches of product B could be associated to the long rupture times, because a moderate correlation (r = 0.7031) was found between rupture time and the percentage dissolved at 10 min.

Specification Settings

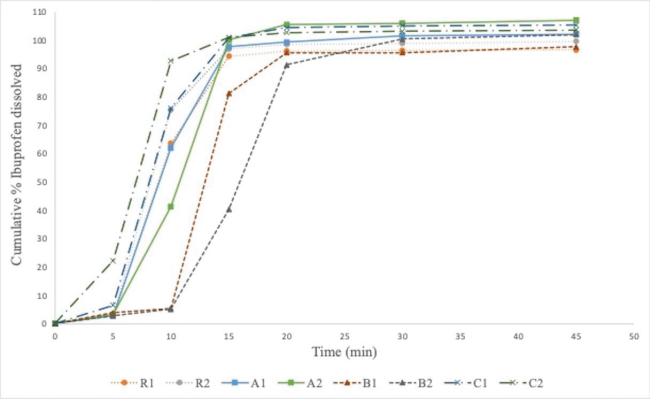

Figure 1. Mean dissolution profiles of different commercial products containing ibuprofen in soft gelatin capsules (400 mg) (n = 12).

Considering that ibuprofen soft gel capsules are generally more preferred by the consumers and that the dissolution profile is one of the critical quality attributes of a drug product, we propose a dissolution specification limit, defined by Q as a mean value at a given time point, that allows discrimination between acceptable and non-acceptable batches. Figure 2 shows the dissolution profile of each dosage unit evaluated from the different products and batches studied (n = 12 observations) using the FDA dissolution method. Differences were found in the percentage of API dissolved at 15 and 20 min. For 400-mg ibuprofen soft gel capsules, the Federal Commission for Protection Against Sanitary Risks (COFEPRIS) from Mexico requires a bioequivalence study for marketing approval. Because the products studied are commercially available, the 15-min time point could be overdiscriminating, so the 20-min time point was chosen.

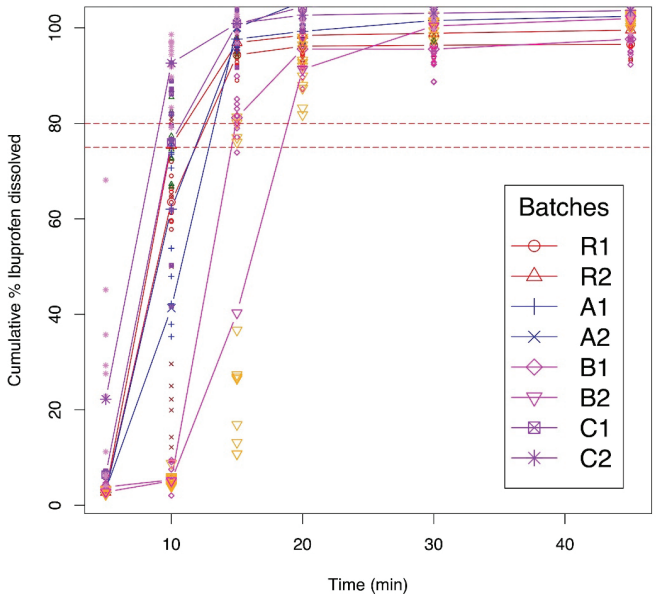

Figure 2. Dissolution profile of each dosage unit evaluated from the different products and batches of 400-mg ibuprofen soft gelatin capsules (n = 12).

To set the Q acceptance criteria specification, values of 75% and 80% were selected. As shown in Table 2, all the individual dissolution percentage values were above 80%, therefore a value of Q = 80% at 20 min is an adequate specification setting.

Considering the small number of batches evaluated and that the data showed a normal distribution, Burdick et al. R program codes (which use Monte Carlo techniques) were used to simulate data for Q. Simulations were performed with 10,000 random values of the mean and standard deviation of the batches studied (Table 2). The results

showed that most batches would pass in stage 1, except one batch from B product (46% of probability), which would be passing in stage 2 with a 100% of probability. The results indicate that the manufacturing process for product B (e.g., rupture time) could be improved to pass the USP stage 1 test.

Table 2. Probability to Pass USP Dissolution Test (Q = 80% at 20 min)

Product	Ibuprofen (%) Dissolved at 20 min Mean (SD) (n = 6)	Probability (%) to Pass Stage 1	Probability (%) to Pass Stage 1 or 2	Probability (%) to Pass Overall
R1	93.9 (0.90)	100	100	100
R2	96.9 (0.63)	100	100	100
A1	101.2 (1.14)	100	100	100
A2	108.2 (1.27)	100	100	100
B1	98.6 (1.90)	100	100	100
B2	91.0 (5.11)	45.7	100	100
C1	103.1 (1.55)	100	100	100
C2	102.5 (2.16)	100	100	100

CONCLUSIONS

The FDA-recommended dissolution method was able to differentiate between multiple brands of ibuprofen soft gelatin capsules. The rupture time might be an indicator of the variability of drug release for this dosage form. A *Q* dissolution value of 80% at 20 minutes could be recommended as a quality control test.

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CONFLICT OF INTEREST

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

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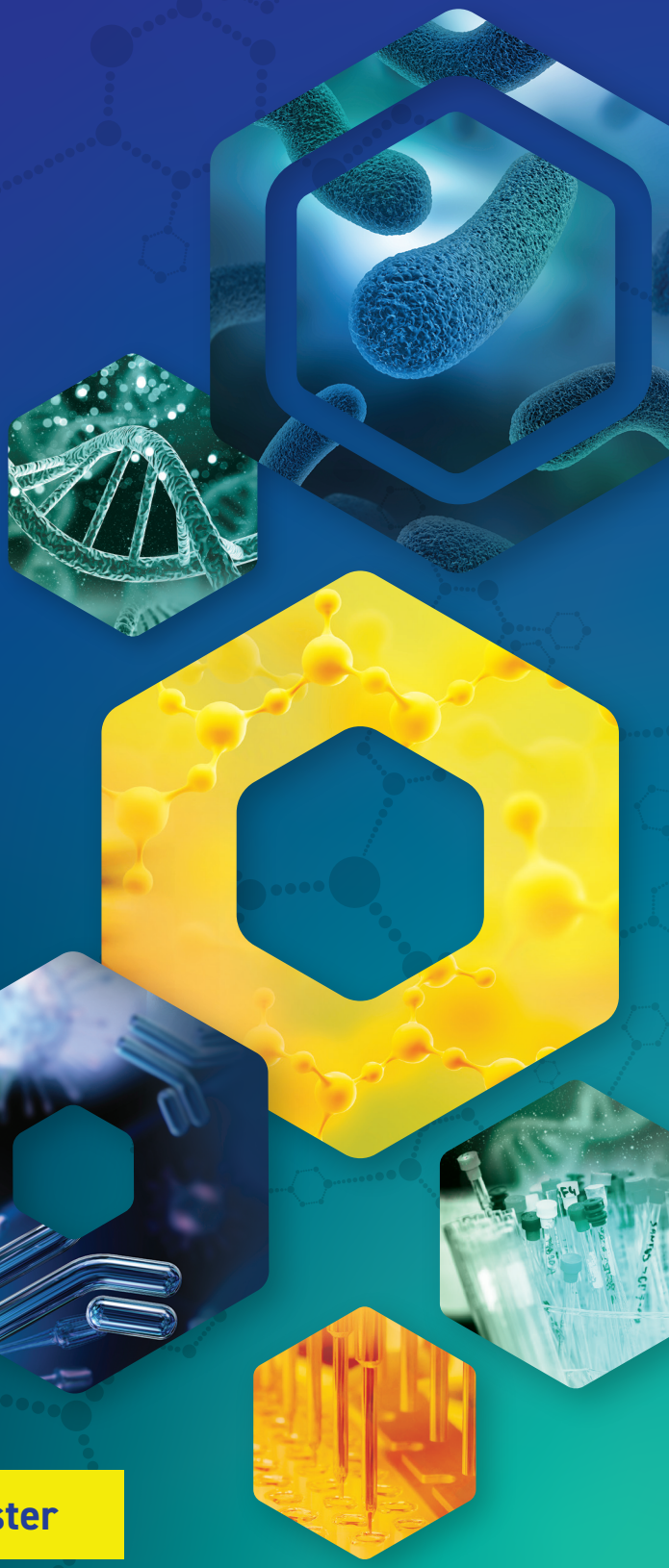

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Using a Laser Monitoring Technique for Dissolution and Thermodynamic Study of Celecoxib in 2-Propanol and Propylene Glycol Mixtures

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ABSTRACT

In the current work, a laser monitoring technique was used to study dissolution and solubility of celecoxib (CBX) in 2-propanol and propylene glycol mixtures at temperatures of 293.2–313.2 K. The solubility data were fitted to mathematical models, i.e., the van't Hoff model, the mixture surface model, the Jouyban-Acree and Jouyban-Acree-van't Hoff equations, and the modified Wilson model. Model accuracy was evaluated by mean relative deviation (MRD%) for back-calculated solubility values. The thermodynamic behavior of CBX dissolution was evaluated according to the van't Hoff and Gibbs equations. CBX exhibited maximum solubility in 2-propanol with a mass fraction of 0.8 at all temperatures. CBX dissolution was identified as an endothermic and enthalpy-driven process, which was more favorable in mixtures with high drug solubilizing capacity. The various models described solubility data from the laser monitoring technique adequately, and the studied cosolvent mixtures have the potential to be used in analytical pharmaceutical development or as intermediate bulk solutions for CBX products.

KEYWORDS: Solubility, celecoxib, mathematical models, thermodynamics, dissolution

INTRODUCTION

Celecoxib (CXB, Fig. 1) is a nonsteroidal anti-inflammatory drug (NSAID) that is prescribed to ease the symptoms of osteoarthritis and rheumatoid arthritis. In comparison to other NSAIDs, CXB shows better efficacy in these pathophysiological states (*1*). The anti-inflammatory, analgesic, and antipyretic activities of CXB are based on a selective banner for cyclooxygenase-2 (COX-2), which has a role in biosynthesis of prostaglandin (*2*). According to the Biopharmaceutics Classification System, CXB belongs to class 2, with high permeability and low solubility (*3*).

In different stages of drug discovery and development, equilibrium solubility is an important property and represents critical knowledge in pre-formulation, preparation of liquid pharmaceutical dosage forms, purification, and/or extraction (*4*). Different formulation techniques were used for the solubilization of CXB including solid dispersions, mesoporous formulations, cyclodextrin inclusion complex, microencapsulation, micellar formulation, nanoemulsion formulation, polymeric nanoparticles, co-crystal, hydrotropy, and cosolvency (*5–13*). Cosolvency is a feasible and reliable technique for solubilization of a drug compound exhibiting low aqueous solubility. Previously reported cosolvency systems for CXB include: NMP (N-methyl-2-pyrrolidone) and water; 2-propanol and water; ethanol and water; 1-propanol and water; choline chloride (ChCl)/ethylene glycol, ChCl/glucose, ChCl/maltose, or ChCl/urea and water; PEG (polyethylene glycol) 200, 400, or

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600 and water; PEG 200, 400, or 600 and ethanol; and PG (propylene glycol) and ethanol. However, solubility of CXB in 2-propanol and PG has not been reported. Both of these solvents are commonly used in the pharmaceutical industry.

The aims of the present study are (1) solubility determination of CXB in mixtures of 2-propanol and PG; (2) data fitting to selected cosolvency equations; and (3) investigation of the thermodynamic behavior for dissolution of CXB.

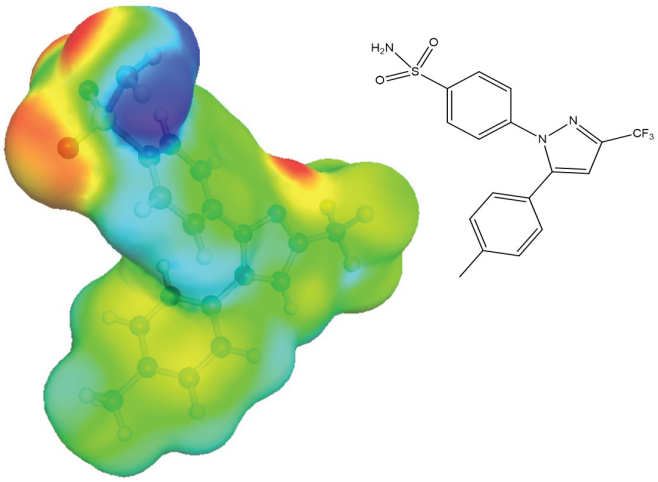

Figure 1. Molecular structure of CXB and sigma surface of most stable coformer as calculated from density functional theory (DFT) based on triple-zeta valence polarized basis set (TZVP) level of theory (BIOVIA COSMOquick database v.2020, Dassault Systèmes Germany GmbH). CXB: celecoxib; PG: propylene glycol.

METHODS

Materials

Raw CXB powder (0.990, Arastoo Pharmaceutical Company, Iran), PG (Scharlau Chemie, Spain), and 2-propanol (Merck, Germany) were used materials for the preparation of the mixed solvents.

Solubility Determination

We used a custom automated smart system equipped with a laser monitoring technique for the determination of CXB solubility in 2-proppanol and PG mixtures. The system adds powder to the solubility vessel using a mechanical arm, and a laser probe is used for particle monitoring. The solubility of CXB in a binary system has been investigated and reported using this method (*14*).

For the current study, 120 g solvent or mixed solvents were prepared and transferred into the dissolution vessel. The temperature was set at the desired value (293.2–313.2 K) and the setup was turned on. After an initial scope of the solution to check its purity, CXB powder was dispersed into the vessel using a robotic arm. A magnetic stirrer was used for the solution while monitoring with the laser probe. The addition of CXB powder continued until the mixture became saturated, at which point a green light on the instrument indicated the end of the experiment. Solubility was computed using the weight of powder added to the dissolution vessel.

Data Analysis

The solubility data measured for CXB were correlated to mathematical models and equations including: van't Hoff; mixture response surface (MRS); Jouyban-Acree; Jouyban-Acree-van't Hoff; and modified Wilson's. The details of these models and equations are mentioned in our previous publications (*15*, *16*).

After data fitting, the mean relative deviation (MRD%) of the back-calculated value was computed using the following equation to investigate the model's accuracy.

$$\mathrm{MRD\%} = \frac{100}{N}\sum\left(\frac{|\text{Calculated Value} - \text{Observed Value}|}{\text{Observed Value}}\right) \quad \text{Eq. (1)}$$

where N is the number of data points. MRD% facilitates the comparison between datasets or models with different scales due to normalizing the data by dividing the variance to the observed values. Prior work suggests that MRD may be the best error criterion (*17*).

Thermodynamic Studies

The enthalpy, entropy, and Gibbs free energy change as the apparent thermodynamic parameters were computed according to the van't Hoff and Gibbs equations. T_{hm} is temperature of the mean harmonic, which is computed from the following equation:

$$T_{hm} = n / \sum_{i=1}^{n}(1/T) \quad \text{Eq. (2)}$$

where n is the number of temperatures (*18*). The intercept and slope of the curve of ln x against $(1/T - 1/T_{hm})$ were used for computing $\Delta G°$ and $\Delta H°$ of procedure, and Gibbs equation was employed to calculate $\Delta S°$. In addition to thermodynamic parameters, the portion of entropy (ζ_{TS}) and enthalpy (ζ_{H}) to $\Delta G°$ were also computed (*19*).

RESULTS AND DISCUSSION

Solubility

Experimental data generated for CXB in 2-propanol and PG mixtures at different temperatures along with standard deviation are given in Table 1. CXB shows maximum solubility in 2-propanol with a mass fraction of 0.8 at all temperatures. Furthermore, in any given solvent composition, solubility was positively related to temperature. A comparison between CXB solubility values obtained in the current study for neat 2-propanol

Table 1. Experimental Mole Fraction Solubility Data Obtained for CXB in 2-propanol + PG Mixtures at Different Temperatures

w_i[a]	293.2 K	298.2 K	303.2 K	308.2 K	313.2 K
0.00	2.19 × (± 0.33) 10^{-3}	3.86 × (± 0.54) 10^{-3}	6.36 × (± 0.56) 10^{-3}	8.19 × (± 0.81) 10^{-3}	1.15 × (± 0.02) 10^{-2}
0.10	3.47 × (± 0.56) 10^{-3}	5.53 × (± 0.09) 10^{-3}	8.34 × (± 0.84) 10^{-3}	9.74 × (± 0.13) 10^{-3}	1.30 × (± 0.08) 10^{-2}
0.30	4.24 × (± 0.26) 10^{-3}	6.20 × (± 0.76) 10^{-3}	8.97 × (± 0.76) 10^{-3}	1.16 × (± 0.23) 10^{-2}	1.45 × (± 0.25) 10^{-2}
0.50	5.04 × (± 0.16) 10^{-3}	8.47 × (± 0.79) 10^{-3}	1.07 × (± 0.15) 10^{-3}	1.31 × (± 0.26) 10^{-2}	1.59 × (± 0.27) 10^{-2}
0.70	7.47× (± 0.77) 10^{-3}	1.09 × (± 0.09) 10^{-3}	1.37 × (± 0.10) 10^{-3}	1.56 × (± 0.17) 10^{-2}	1.75 × (± 0.22) 10^{-2}
0.80	7.55 × (± 0.88) 10^{-3}	1.14 × (± 0.09) 10^{-3}	1.40 × (± 0.04) 10^{-3}	1.69 × (± 0.08) 10^{-2}	1.86 × (± 0.32) 10^{-2}
0.90	7.08 × (± 0.42) 10^{-3}	9.26 × (± 0.70) 10^{-3}	1.13 × (± 0.14) 10^{-3}	1.36 × (± 0.16) 10^{-2}	1.66 × (± 0.17) 10^{-2}
1.00	5.95 × (± 0.53) 10^{-3}	8.23 × (± 0.28) 10^{-3}	1.06 × (± 0.17) 10^{-3}	1.28 × (± 0.20) 10^{-2}	1.51 × (± 0.09) 10^{-2}

[a]w_i is mass fraction of 2-propanol in 2-propanol + PG mixtures in the absence of CXB.
CXB: celecoxib; PG: propylene glycol.

(x = 8.23 × 10^{-3}) with a reported value (x = 9.02 × 10^{-3}) in the literature showed very good agreement considering typical experimental variation by the purity of the compound and analytical methodology used (*20*). Solubility of CXB in other solutions was not reported for comparison.

Solubility of CXB has been investigated in various mixtures of cosolvent + water including: NMP + water; 2-propanol + water; ethanol + water; 1-propanol + water; ChCl/ethylene glycol, ChCl /glucose, ChCl/maltose, ChCl/urea + water; PEGs + water; PEGs + ethanol; PG + ethanol; and the present system (2-propanol + PG). The solubility profiles are given in Figure 2, which shows that most systems possess the same trend for CXB solubility, with a maximum amount in neat solvent (*1*). However, the solubility profile of CXB in PEG 200 + ethanol and in 2-propanol + PG displayed a maximum mass fraction (w_i) of 0.8–0.9. A comparison between the studied systems for CXB solubility demonstrated that both PEG 600 + ethanol and 2-propanol + PG had an excellent solubilization effect on CXB, which is a poorly soluble drug.

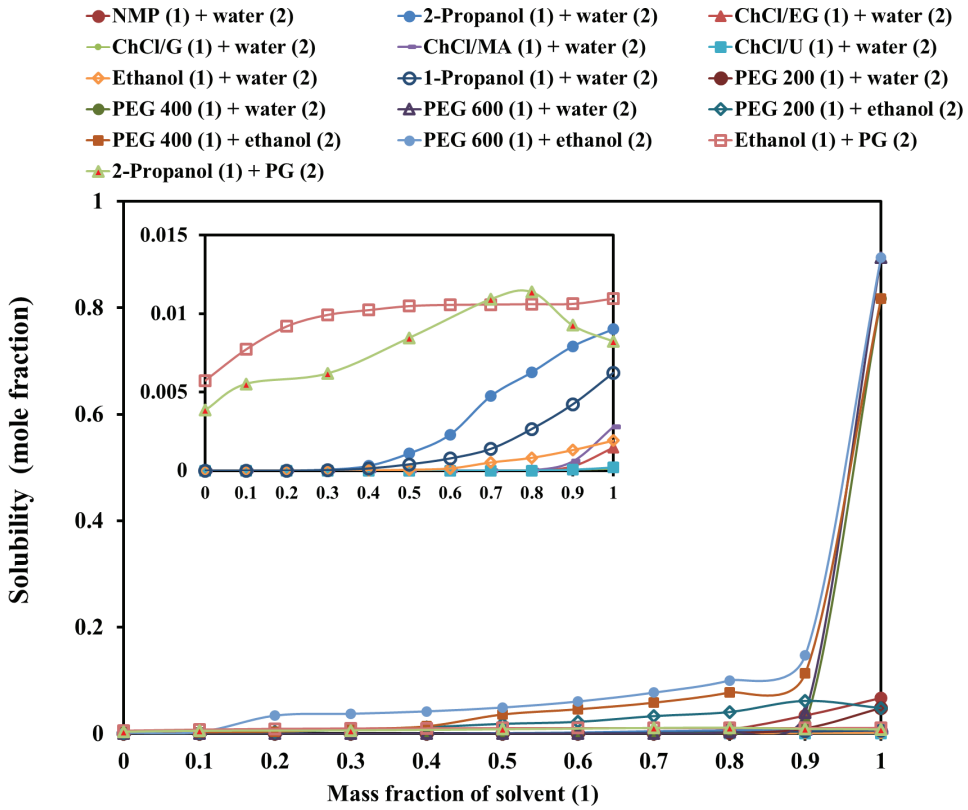

Figure 2. Comparison of CXB mole fraction solubility profiles in different reported systems at 298.2 K.
indicates data from the current study (2-propanol + PG mixture); (1) indicates first solvent that mass fraction is reported based on this solution; (2) indicates second solvent.
CXB: celecoxib; NMP: N-methyl-2-pyrrolidone; ChCl: choline chloride; EG DES: ethylene glycol deep eutectic solvent; PEG: polyethylene glycol; PG: propylene glycol

The solubilization efficacy of each system was computed using σ and ω parameters, which were computed using the equations reported in Ref. (*21*). The ω and σ values are equal when maximum solubility is in the neat cosolvent. These parameters were calculated for CXB in the above-mentioned mixtures, and the results are summarized in Table 2. The high solubilization power based on the solubilization factor of ω was for PEG 400 + water, demonstrating the high capability of this cosolvent for solubilization of CXB.

Table 2. Comparison of Solubilization Powers of Various Cosolvents Studied for CXB

Solvent Mixtures	Solubilization efficacy (σ)	Updated version of solubilization efficacy (ω)
NMP + water	5.92	5.92
2-Propanol + water	5.05	5.05
ChCl/EG DES + water	4.26	4.26
ChCl/glucose DES + water	3.15	3.15
ChCl/maltose DES + water	4.54	4.54
ChCl/urea DES + water	3.41	3.41
Ethanol + water	4.72	4.72
1-Propanol + water	4.97	4.97
PEG 200 + water	6.13	6.13
PEG 400 + water	7.36	7.36
PEG 600 + water	7.39	7.39
PEG 200 + Ethanol	1.40	1.68
PEG 400 + Ethanol	2.63	2.63
PEG 600 + Ethanol	2.67	2.67
Ethanol + PG	0.28	0.28
2-Propanol + PG	0.33	0.33

CXB: celecoxib; NMP: N-methyl-2-pyrrolidone; ChCl: choline chloride; EG DES: ethylene glycol deep eutectic solvent; PEG: polyethylene glycol; PG: propylene glycol

Mathematical Modeling

Solubility is dependent on both temperature and solvent composition. Thus, the models used for cosolvency systems are a function of temperature or solvent composition or both.

The van't Hoff is a simple model for the representation of solubility data as a function of temperature. Therefore, it needs an individual equation for each solvent composition. The model coefficients for each equation along with MRD% are given in Table 3. The overall MRD% is low (5.5%), which confirms the model accuracy for solubility prediction.

The MRS is a linear model that relates the solubility data to solvent composition. Therefore, it needs an individual equation for each investigated temperature. The model coefficients for each equation along with MRD% are given in Table 4. The overall MRD% for this model is 2.7%.

Table 3. van't Hoff Model Parameters and Corresponding MRD% for Back-Calculated CXB Solubility Data in 2-Propanol + PG Mixtures

w_i[a]	A	B	MRD%
0.00	-4242.727	9.396	3.7
0.10	-3839.795	8.169	1.5
0.30	-4053.756	9.038	7.2
0.50	-3803.638	8.169	6.6
0.70	-5040.844	12.019	7.4
0.80	-5678.728	13.949	3.8
0.90	-5908.488	14.573	6.7
1.0	-7493.098	19.525	7.0
		Overall MRD%	**5.5**

[a]w_i *is mass fraction of 2-propanol in 2-propanol + PG mixtures in the absence of CXB.*
CXB: celecoxib; MRD: mean relative deviation; PG: propylene glycol.

Table 4. MRS Model Constants at Investigated Temperatures and MRD% for Back-Calculated CXB Solubility Data in 2-Propanol + PG Mixtures

Temperature (K)	β_1	β_2	β_3	β_4	β_5	MRD%
293.2	-6.155	-5.153	0[a]	0[a]	2.871	3.6
298.2	-5.628	-4.851	0[a]	0[a]	2.808	3.0
303.2	-5.113	-4.593	0[a]	0[a]	2.106	3.5
308.2	-4.872	-4.398	0[a]	0[a]	1.921	2.1
313.2	-4.509	-4.199	0[a]	0[a]	1.303	1.2
					Overall MRD%	**2.7**

[a]*Not statistically significant ($p > 0.05$).*
MRS: mixture response surface; CXB: celecoxib; MRD: mean relative deviation; PG: propylene glycol.

The modified Wilson model may be employed as a non-linear model for data correlation at various temperatures. Again, individual equations are needed for each investigated temperature. The model coefficients for each equation along with MRD% are given in Table 5. The overall MRD% for this model was 2.3%.

Using several models can be a problematic for solubility prediction. In the current study, for example, one must use eight equations for solubility prediction with the van't Hoff model, and five equations using MRS and the modified Wilson models. The Jouyban-Acree and Jouyban-Acree-van't Hoff equations relate the solubility to both temperature and solvent composition. Thus, they need just one regression step and obtain one equation for all data. The model coefficients for each equation along with MRD% are given in Table 6. The overall MRD% was 6.7% for Jouyban-Acree and 8.9% for the Jouyban-Acree-van't Hoff.

Table 5. Modified Wilson Model Parameters at Investigated Temperatures and MRD% for Back-Calculated CXB Solubility Data In 2-Propanol + PG Mixtures

Temperature (K)	λ_{12}	λ_{21}	MRD%
293.2	1.437	1.252	3.2
298.2	1.323	1.406	3.0
303.2	1.211	1.374	3.2
308.2	1.152	1.416	1.6
313.2	0.999	1.470	0.6
		Overall MRD%	**2.3**

CXB: celecoxib; MRD: mean relative deviation; PG: propylene glycol.

Table 6. Parameters Calculated for the Jouyban-Acree and Jouyban-Acree-van't Hoff Models and MRD% for Back-Calculated CXB Solubility Data in 2-Propanol + PG Mixtures

Jouyban-Acree Model Parameters	
J_0	617.798
J_1	0[a]
J_2	0[a]
MRD	6.7%
Jouyban-Acree-van't Hoff Model Parameters	
A_1	19.525
B_1	-7493.098
A_2	9.396
B_2	-4242.727
J_0	618.032
J_1	0[a]
J_2	0[a]
MRD	8.9%

[a]*Not statistically significant ($p > 0.05$).*
CXB: celecoxib; MRD: mean relative deviation, PG: propylene glycol.

Using one equation for correlation or prediction is the main advantage for a cosolvency model, which can be helpful in the pharmaceutical industry. Another advantage of these models is using a minimum number of data points for model training. These data points are solubility data in mono-solvents at the minimum and maximum investigated temperatures and solutions with mass fractions (wi) of 0.7, 0.5, and 0.3 at 298.2 K. After training, the MRD% for predicted values are 5.9%, 5.2%, 10.3%, 16.1%, and 26.8% for 293.2, 298.2, 303.2, 308.2, and 313.2 K, respectively (overall MRD is 12.8%).

Thermodynamic Studies

The apparent thermodynamic parameters including $\Delta H°$, $\Delta G°$, and $\Delta S°$ of CXB dissolution are given in Table 7. All parameters are positive, with the maximum (62.17 kJ.mol^{-1}) and minimum (31.66 kJ.mol^{-1}) for w_1 = 0.0 and w_1 = 0.7 for $\Delta H°$, respectively, the maximum (161.89 J.mol^{-1}) and minimum (68.06 J.mol^{-1}) for w_1 = 0.0 and w_1 = 0.7 for $\Delta S°$, respectively, and a minimum value of 10.94 kJ.mol^{-1} for w_1 = 0.8 for $\Delta G°$. CXB dissolution in 2-propanol and PG is an endothermic process and more favorable in a mixture with high capability for CXB solubilization. $\zeta_H > \zeta_{TS}$ was seen in all mixtures, demonstrating enthalpy is the main contributor of $\Delta G°$ in the dissolution process.

Based on thermodynamic parameters, the enthalpy-entropy compensation curve was plotted for investigation of the involved mechanism in the dissolution process (Fig. 3). CXB shows a trend mainly with a positive slope, indicating an enthalpy-driven mechanism for the cosolvent action that could be attributed to better drug solvation.

The enthalpy of solution reflects the nature of the intermolecular interactions and its variation results from the contribution of several kinds of interactions, endoergic cavity formation and exoergic solute-solvent interactions (*22*). The enthalpy of cavity formation is endothermic because work must be done against the cohesive forces of the solvent to accommodate the solute. This unfavorable contribution should decrease as the solubility parameter of the medium becomes more like that of the solute. Solute-solvent interactions are

Table 7. Apparent Thermodynamic Parameters for Dissolution Behavior of CXB in 2-Propanol + PG Mixtures at T_{hm} = 303.0 K

w_i[a]	$\Delta G°$ (kJ.mol^{-1})	$\Delta H°$ (kJ.mol–1)	$\Delta S°$ (J.mol–1.K–1)	$T\Delta S°$ (kJ.mol–1)	ζ_H	ζ_{TS}
0.00	13.11	62.17	161.89	49.05	0.559	0.441
0.10	12.41	49.07	120.96	36.65	0.572	0.428
0.30	12.07	47.32	116.32	35.24	0.573	0.427
0.50	11.63	41.93	100.01	30.30	0.581	0.419
0.70	11.04	31.66	68.06	20.62	0.606	0.394
0.20	10.94	33.72	75.18	22.78	0.597	0.403
0.90	11.34	31.97	68.07	20.62	0.608	0.392
1.00	11.60	35.17	77.79	23.57	0.599	0.401

[a]*w_i is mass fraction of 2-propanol in 2-propanol + PG mixtures in the absence of CXB.*
CXB: celecoxib, PG: propylene glycol.

exothermic and result mainly from van der Waals and Lewis acid-base interactions. The exothermic heat of mixing values suggests that solute-solvent interactions overcome the energetically unfavorable cavity term and are responsible for favorable free energy changes.

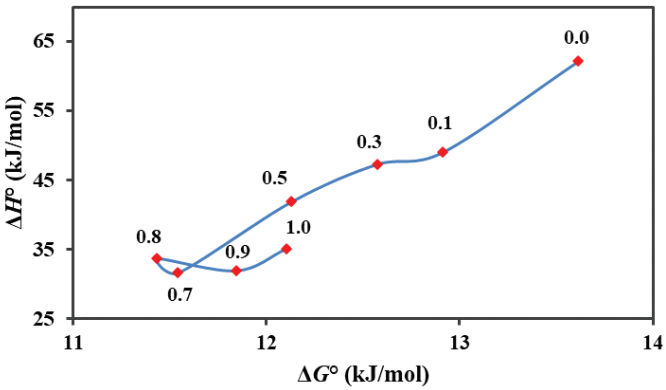

Figure 3. Enthalpy-entropy compensation plot for CXB in 2-propanol + PG mixtures at T_{hm} = 303.0 K. Red data points represent mass fraction of 2-propanol in the 2-propanol + PG mixtures in the absence of CXB. CXB: celecoxib; PG: propylene glycol.

CONCLUSIONS

A laser monitoring technique was used to study dissolution and solubility of CBX in 2-propanol and propylene glycol mixtures at temperatures of 293.2–313.2 K. CBX exhibited maximum solubility in 2-propanol and PG mixtures with a 2-propanol mass fraction of 0.8. CBX dissolution was identified as an endothermic and enthalpy-driven process. The various models described solubility data from the laser monitoring technique adequately, and the studied cosolvent mixtures have the potential to be used in analytical pharmaceutical development or as intermediate bulk solutions for CBX products.

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CONFLICT OF INTEREST

The authors declare no conflict of interest related to this article.

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Biorelevant Dissolution Testing of Numerically Optimized Multiparticulate Drug Delivery Systems of Gliclazide

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ABSTRACT

Gliclazide (GLZ) is an ampholyte with pH-dependent solubility in the gastrointestinal pH range. Although the effects of different pH values on GLZ release have been thoroughly investigated in compendial dissolution media, the effects of gastrointestinal fluid components and pH are not well known. Multiple response optimization was carried out employing two optimization criteria to obtain different release profiles (optimized alginate-gelatin beads, OP-1 and OP-2). Thermograms indicated polymorph formation (OP-1) and changes in GLZ crystallinity (OP-2). Fourier transform infrared (FT-IR)-spectra confirmed GLZ chemical stability. GLZ release in gradient compendial and biorelevant media was studied employing two dissolution methodologies using fed state simulated gastric and intestinal fluid (FeSSGF and FeSSIF, respectively). A validated HPLC/UV method for GLZ analysis in biorelevant media was developed. OP-1 and OP-2 showed low relative error between the actual and predicted values. In the gradient biorelevant media, OP-1 showed faster GLZ release than OP-2. In the gradient compendial media, OP-1 showed slower GLZ release in pH 1.2 and faster release in pH 7.4 than OP-2. Generally, both formulations showed slower GLZ release in biorelevant compared to compendial media. SEM images of OP-1 showed tiny pores on the bead surface after GLZ release in biorelevant media. Meanwhile, thin polymer layers were diffused around the beads (OP-1 and OP-2) after GLZ release in compendial media. In conclusion, GLZ release was mainly affected by pH rather than media components. A cost-effective biorelevant dissolution methodology was proposed.

KEYWORDS: Gliclazide, biorelevant media, numerical optimization, gradient conditions, cost-effective methodology, dissolution

INTRODUCTION

Biorelevant dissolution tests enable understanding of how a drug is predicted to perform after administration. The test can be utilized during formulation development to predict the dissolution and bioavailability of many drugs (*1*). For some drugs, dissolution tests may be used to establish an in-vitro correlation for evaluating the in-vivo performance. In addition, they can predict the effect of food on the bioavailability of many drugs, especially poorly soluble ones (*2*, *3*). Compendial dissolution media are typically utilized for quality control tests. However, compendial media do not enable predicting the in vivo performance of poorly soluble compounds, as the composition of those media may not represent the physiological state of the gastrointestinal (GI) tract at the time of drug administration (i.e., fed or fasted condition) (*1*).

Simulating GI conditions with biorelevant media is performed in many laboratories; however, biorelevant media are expensive due to their complexity, and they should be freshly prepared directly before conducting the dissolution test, which limits widespread use (*4*, *5*). The International Pharmaceutical Federation (FIP) published two biorelevant media: fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF). FeSSIF contains bile salt and lecithin, with pH, buffer capacity, and osmolality of the intestine (*5*).

*Corresponding author

The conventional dissolution media contains synthetic surfactants that form micelles, whereas FeSSIF and FaSSIF contain natural surfactants that form more complex lipid aggregates (*3*). In these media, several properties are taken into consideration such as pH and bile salt concentration (*6*). For poorly soluble drugs, bile salts and phospholipids may significantly affect the drug dissolution and transport in the small intestine. There is a growing interest in the standardization of biorelevant dissolution methodology. Moreover, different studies have utilized pharmacokinetic modeling with biorelevant dissolution testing for the prediction of the in vivo behavior of many drugs (*7–10*).

For modified-release (MR) dosage forms, dissolution is a critical quality attribute. Drug release from these formulations should follow a predefined delivery pattern. MR dosage forms are exposed to changing conditions as they move through the GI tract, which can affect the drug release. Thus, it is necessary to establish drug release test conditions in a way that these effects can be observed and predicted using a series of media in one experiment (*11*). In a gradient dissolution test, the release profiles can be studied using the same settings with varying pH conditions to detect drug release changes that might occur by changing pH as the dosage form moves through the GI tract (*12*).

Gliclazide (GLZ) is a second-generation sulphonylurea used for the management of type II diabetes mellitus (*13*, *14*). It is a white crystalline powder, relatively insoluble in water (*15*). GLZ belongs to BCS class II drugs (low solubility and high permeability drugs), hence GLZ dissolution is the rate-limiting step for its absorption (*16–18*). It is a hydrophobic drug, a weak acid (pKa = 5.8), and it exhibits a pH-dependent solubility (*15*, *18–20*).

The aim of the current study was to investigate the GLZ release rate from two different numerically optimized multiparticulate drug delivery systems in both compendial and biorelevant media. The optimization criteria were considered to obtain different GLZ release patterns in different pH values. The study also focused on validating GLZ quantification methodology and establishing a cost-effective dissolution methodology in biorelevant media using gradient conditions.

METHODS

GLZ powder was donated from Sigma Pharmaceutical Industries, Menoufia, Egypt. For the preparation of alginate-gelatin (AL-GL) beads, high viscosity sodium alginate and gelatin (Bovine-B) from Sigma Aldrich (USA) and 50% w/w glutaraldehyde and anhydrous calcium chloride from ADWIC (Egypt) were used. HPLC-grade acetonitrile, ethyl acetate, and methanol from TEDIA (USA) were used. Hydrochloric acid (HCl) 30–34% (El-Nasr Pharmaceutical Chemicals Co., Egypt), potassium di-hydrogen phosphate (ADWIC), and sodium hydroxide pellets (Laboratory Rasayan, India) were used for the preparation of compendial media. Sodium chloride extra pure (NaCl) (Laboratory Rasayan, S. D. Fine-Chem Ltd., India), sodium acetate trihydrate (ADWIC), acetic acid 96% (ADWIC), taurocholic acid sodium salt (Aldrich Chemicals, USA), lecithin (≥ 97% for biochemistry, Roth, Germany), full cream UHT-milk (Juhayna, Egypt) were used to prepare the biorelevant media. Milli-Q purified water (Millipore Corp., Billerica, MA, USA) was used.

Multiple Response Optimization and Preparation of Alginate-Gelatin (AL-GL) Beads

Multiple response optimization was carried out based on a previous study; the optimized AL-GL beads were prepared according to that same study (*21*). Alginate and gelatin were dissolved in water (1:40 w/w ratio of polymer to distilled water). GLZ powder was quantitatively transferred to AL-GL solution while stirring, and the formed suspension was dropped on curing solutions using a peristaltic pump (falling distance was 7.5 cm, 3.5-mm tube [Rainin Dynamax, USA]). The curing solutions consisted of different concentrations of glutaraldehyde (GA, X3) in 0.2 M CaCl2 solution (w/v) kept at 5 ± 0.5 °C in a temperature-controlled circulator water bath (F20-VC, Julabo, Germany). The formed beads were kept in the curing solution while stirred for 30 min, then washed with distilled water and left to dry until reaching a constant weight. Blank beads (drug-free) were also prepared using the same method. The composition of the optimized AL-GL beads is summarized in Table 1.

Evaluation of the Optimized Beads

GLZ Loading and Incorporation Efficiency

For each formulation, accurately weighed beads corresponding to a theoretical weight of 20 mg of GLZ were ground to a powder and shaken in 250 mL of phosphate buffer (pH 7.4) for 24 h at 37 °C ± 0.5 (shaking water bath, Lab-Line, USA). Each sample was filtered through a 0.45-µm filter, diluted, and analyzed spectrophotometrically at 225 nm (Beckman, DU-650, USA). GLZ loading and incorporation efficiency (IE) were calculated according to the following equations (*22*):

GLZ loading % = (Drug weight in beads / weight of beads) x 100;

IE % = (Actual amount of drug in beads/ theoretical amount of drug in beads) x 100.

Table 1. Composition and Characteristics of OP-1 and OP-2

			OP-1 (*21*)	OP-2
Optimization criteria		Y_1: IE	Maximized	Maximized
		Y_2: (*Q* 0.5 h)	5% ≤ Y_2 ≤ 20%	20% ≤ Y_2 ≤ 30%
		Y_3: (*Q* 2 h)	15% ≤ Y_3 ≤ 25%	30% ≤ Y_3 ≤ 40%
		Y_4: (*Q* 4 h)	60% ≤ Y_4 ≤ 70%	50% ≤ Y_4 ≤ 60%
Desirability			1	1
Studied factors		X_1: GLZ%	17.94%	19.04%
		X_2: AL:GL	1:1	1:1
		X_3: GA%	0.1%	10.63%
Predicted responses		Y_1: IE	82.78%	70.14%
		Y_2: (*Q* 0.5 h)	7.86%	22.38%
		Y_3: (*Q* 2 h)	21.32%	33.51%
		Y_4: (*Q* 4 h)	68.46%	57.93%
Actual responses (mean ± SD, *n* = 3)		Y_1: IE	81.69 ± 3.98	70.15 ± 0.64
		Y_2: (*Q* 0.5 h)	7.98 ± 0.02	22.22 ± 0.62
		Y_3: (*Q* 2 h)	20.98 ± 0.17	35.94 ± 1.60
		Y_4: (*Q* 4 h)	67.43 ± 2.76	61.3 ± 2.15
Relative error		Y_1: IE	1.32%	-0.01%
		Y_2: (*Q* 0.5 h)	-1.53%	0.71%
		Y_3: (*Q* 2 h)	1.59%	-1.28%
		Y_4: (*Q* 4 h)	1.5%	-5.81%
Regression coefficient (r^2) of kinetic release models	**Biorelevant media**	Zero-order	0.9964	0.9822
		First-order	0.9568	0.9705
		Higuchi	0.9727	0.9299
		Hixson & Crowell	0.9817	0.9757
	Compendial media	Zero-order	0.9502	0.9875
		First-order	0.7102	0.9780
		Higuchi	0.8750	0.9474
		Hixson & Crowell	0.7033	0.9664

AL: alginate; IE: incorporation efficiency; GA: glutaraldehyde; GL: gelatin; GLZ: gliclazide; Q: drug release; X: factor; Y: response.

Differential Scanning Calorimetry (DSC)

The thermal behavior was investigated by DSC (DSC-50, Shimadzu, Japan) to evaluate the state of GLZ in different tested samples. Samples (5 mg) were weighed into aluminum pans (heated in a nitrogen atmosphere), using an empty pan as a reference. The thermal analysis was carried out using a heating ramp from 25–350 °C at a 10 °C per minute scale-up rate. A nitrogen purge (25 mL/min) was maintained.

Fourier Transform Infrared Analysis

The tested samples were ground and mixed thoroughly with potassium bromide (1:5 ratio of sample to KBr). The powder was compressed at a pressure of 5 tons for 5 min in a hydraulic press to form KBr disks. Scans were obtained at a resolution of 4 cm^{-1} (FT-IR-6100 spectrometer, Jasco, Japan) from 400–4000 cm^{-1}.

GLZ Release Studies

Dissolution Test in Compendial Media

The dissolution test in compendial media was carried out using USP apparatus 1 (rotating basket) (AT8-XTEND, Sotax, Switzerland). The dissolution medium was 900 mL of filtered and degassed 0.1 N HCl for 2 h, followed by 900 mL of phosphate buffer at pH 7.4, maintained at 100 rpm and 37.0 ± 0.5 °C. Samples were collected at specified time points (0.5-h intervals for up to 7 h), filtered through a 0.45-µm filter, replaced with fresh dissolution medium, and analyzed for GLZ content with a UV-visible spectrophotometer (Beckman, DU-650, USA) at 225 nm against the corresponding blank solution (*22*).

Preparation of Biorelevant Media

The composition of FeSSGF was previously described by Jantratid et al. (*23*). It consisted of 50% ultra-heat treated milk (UHT milk) used to simulate the fed gastric conditions

added to the blank simulated gastric medium (Table 2) (*23*). FeSSIF was previously reported by Klein (*24*). It consisted of bile salt (sodium taurocholic acid) and lecithin dissolved in a blank simulated intestinal medium (Table 2). Most of the components were simply dissolved in Milli-Q water except for lecithin, which required ultrasonication (Sonics, USA) to completely dissolve. Both FeSSIF and FeSSGF were freshly prepared for each experiment. The selected simulated colonic fluid (SCoF) was reported by Fotaki et al. (*1*). Acetate buffer was used to adjust the desired pH (5.8) and buffer capacity (Table 2).

Table 2. Composition of Biorelevant Media Used to Simulate Fed State Condition in Gastrointestinal Tract

Component	FeSSGF* (*23*)	FeSSIF (*24*)	SCoF (*1*)
Sodium taurocholate	-	15 mM	-
Lecithin	-	3.75 mM	-
Acetic acid	17.12 mM	8.65 g	170 mM
Sodium acetate	29.75 mM	-	-
Sodium chloride	237.02 mM	11.874 g	-
Sodium hydroxide	-	4.04 g	157 mM
Deionized water (qs add)	1L	1L	1L
pH	5	5	5.8

**Blank medium mixed with UHT milk (1:1).*
Dash (-) indicates not applicable.
FeSSGF: fed state simulated gastric fluid; FeSSIF: fed state simulated intestinal fluid; SCoF: simulated colonic fluid.

Modification and Validation of HPLC/UV Method for GLZ Quantification

For the determination of GLZ in FeSSGF and FeSSIF, several HPLC methods were investigated. The selected HPLC method was mainly guided by previously published methods (*22*, *25*).

Preparation of Standard Solutions

Each calibration standard was prepared by adding a calculated volume of suitable GLZ standard solution to 100 µL of drug-free medium (either FeSSGF or FeSSIF). The calibration standard concentrations ranged from 0.1–30 µg/mL GLZ. The internal standard (glyburide) was used in a concentration of 2.5 µg/mL.

Preparation of Samples

GLZ extracting solvent (ethyl acetate) was added to the calibration standard or dissolution sample. The solvent layer (containing GLZ) was separated and evaporated under a vacuum. The dried calibration standards and dissolution samples were then reconstituted with 150 µL of mobile phase directly before injection.

Chromatographic Conditions

The mobile phase was a mixture of filtered and degassed deionized water and acetonitrile (45:55, Millipore vacuum filtration system with membrane filter, 0.45 µm) pumped at a flow rate of 1 mL/min with isocratic elution. The sample run time was 6 minutes. The UV detection wavelength was 230 nm. HPLC apparatus consists of: Waters 600 E Multi Solvent Delivery System Controller equipped with Rheodyne injector P/N 7725i, and Waters 2487 Dual λ Absorbance Detector coupled to Millennium 32 computer program. Column (Lichrosorb RP-18, 10 µm, 250 x 4.6 mm i.d., Merck, Germany) was kept at room temperature, protected by a guard column (Perisorb 30-40, Merck).

Dissolution Tests in Biorelevant Media

Two different dissolution test methods were investigated: USP apparatus 1 (rotating basket) and shaking water bath, a cost-effective alternative method.

USP Apparatus 1

The first dissolution medium was 900 mL of FeSSGF for 2 h, followed by 900 mL of FeSSIF for 3.5 h, followed by 900 mL of SCoF for 8 h. Each medium was maintained at 100 rpm and 37.0 ± 0.5 °C. The baskets were loaded with a weight of beads corresponding to 60 mg of GLZ. Samples were collected at specified time points (every 0.5 h for the first 6 h then every 1 h until 14 h), filtered through a 0.45-µm filter, replaced with fresh dissolution medium, and analyzed for GLZ content.

Shaking Water Bath

A shaking water bath was maintained at 37.0 ± 0.5 °C and 100 rpm. Glass stoppered 50-mL conical flasks were filled with 25 mL of each dissolution medium (2.78% of the official volume and the weight of beads was adjusted using the same factor) as follows. First, FeSSGF was added for 2 h, followed by FeSSIF for 3.5 h, followed by SCoF for 8 h. Samples were collected at specified time points (every 0.5 h for the first 6 h then every 1 h until 14 h), filtered through 0.45-µm filter, and replaced with fresh dissolution medium.

For analytical purposes, the same tests were carried out using blank beads (drug-free) and samples were collected at the same time intervals. The collected samples were analyzed for GLZ content using the validated HPLC/UV method (for FeSSGF and FeSSIF samples) and UV/spectrophotometric method at 225 nm (for SCoF samples).

Mathematical Comparison of GLZ Release Profiles

Release profiles of GLZ were compared using the fit factors f_1 and f_2 (*26*). A difference factor, f_1, between 0 and 15 ensures a minor difference between two products, and a similarity factor, ƒ2, between 50 and 100 ensures similar dissolution profiles (*26*). Dissolution efficiency (DE) was also calculated from the area under the dissolution

curve at time (t), measured using the trapezoidal rule, and expressed as a percentage of the area of the rectangle described by 100% dissolution at the same time (*26*).

Fitting to different kinetic release models was also evaluated. Zero-order release model, First-order release model, Higuchi square root of time model, and Hixson–Crowell cube root model were assessed (*21*).

Scanning Electron Microscopic (SEM) Analysis

SEM (Quanta GEF, Netherlands) imaging before and after GLZ release was performed and analyzed.

RESULTS AND DISCUSSION

Multiple Response Optimization and Evaluation of the Optimized Formulations

Multiple response optimization was carried out based on previously published work (*21*). A three-factor three-level face-centered design (FCD) was implemented where GLZ%, AL:GL ratio, and glutaraldehyde (GA)% were the studied factors (X_1, X_2, and X_3, respectively). The studied responses were GLZ IE and release (Q) after 0.5 h, 2 h, and 4 h (Y_1: IE, Y_2: Q 0.5 h, Y_3: Q 2 h, and Y_4: Q 4 h, respectively). After a comprehensive evaluation of the regression models, two different optimization criteria were employed (OP-1 and OP-2) (Table 1). Noticing that increasing GA% resulted in faster GLZ release in the acidic medium (pH 1.2) and a slower release in phosphate buffer (pH 7.4) in addition to its significant negative effect on IE, the numerical optimization was utilized to obtain different suggested solutions (formulations) with the highest desirability. The objective of the optimization criteria was to obtain two formulations; one of them enables faster drug release in 0.1 N HCl pH 1.2 (OP-2) and the other enables faster drug release in phosphate buffer pH 7.4 (OP-1) (*21*).

In the current study, the optimized formulation (OP-2) was prepared based on numerical optimization (*27*). The optimization criteria for OP-2 were to maximize GLZ IE (Y_1), while GLZ release responses were specified with constraints ($20\% \leq Y_2 \leq 30\%$, $30\% \leq Y_3 \leq 40\%$, $50\% \leq Y_4 \leq 60\%$). The selected optimized formulations showed a desirability of 1. The relative error values for OP-1 and OP-2 were low, confirming the validity of the model (*28*, *29*) (Table 1).

Characterization of the Optimized Beads

Both OP-1 and OP-2 beads showed white color that changed to either yellow color (OP-1) or yellowish-brown color (OP-2) after drying. It was observed that the darker beads' color is linked to the higher concentration of GA (*21*). OP-1 and OP-2 beads were spherical before drying; however, due to the mild stickiness of OP-1 beads, their shape was less regular than OP-2 after drying. This could be attributed to incomplete cross-linking of gelatin as a result of using lower GA concentration (*21*). This effect was reflected in the coefficient of variation (CV% = 15.2 and 6.3 for OP-1 and OP-2, respectively).

Thermograms

DSC studies were carried out for pure GLZ, pure polymers, blank beads, and GLZ-loaded beads (Fig. 1A). DSC-thermogram of blank OP-2 beads (drug-free) showed a broad endothermic peak at 91.54 °C and disappearance of the exothermic peak of alginate as a result of its crosslinking and/or interaction between alginate and gelatin. This thermal behavior was also observed in blank OP-1 beads (*21*). Thermogram of GLZ-loaded OP-2 beads showed disappearance of a GLZ endothermic peak, which might indicate a change in the crystallinity of GLZ (*30*). Loss of crystallinity might enhance the drug dissolution; however, this effect was not observed in the current study. This could be attributed to the delay of GLZ wetting by crosslinked polymers, hence slowing GLZ release (*21*). In contrast, the thermogram of GLZ-loaded OP-1 beads showed endothermic peaks at 152.78 °C and 163.07 °C. This indicates formation of GLZ polymorphs (*21*).

FTIR Analysis

FTIR analysis was carried out for pure GLZ, pure polymers, blank beads, and GLZ-loaded beads (Fig. 1B). Both blank OP-1 and blank OP-2 beads (drug-free) showed amide carbonyl stretch peaks at 1629.55 cm^{-1} that indicated amide bond formation between the amino group of gelatin and the carboxylic group of gelatin or alginate (*31*, *32*). The FTIR spectra of GLZ-loaded OP-1 and OP-2 beads showed asymmetric sulfonyl (S=O) stretching peak at 1348 cm^{-1} and symmetric sulfonyl (S=O) stretching peak at 1162.87 cm^{-1}. The amide carbonyl stretch peak at 1631.48 cm-1 was also observed. The observed peaks are characteristic for GLZ, so no chemical change was indicated.

Selection Criteria of Biorelevant Media

The selected biorelevant medium simulating the fed state gastric condition is composed of a mixture of blank gastric medium and UHT milk in a ratio of 1:1 as previously proposed (*23*) (Table 2). FeSSGF should simulated the physicochemical properties of a standard meal while being experimentally practical (*24*, *33*). Standardized homogenized cows' whole milk is similar to a standard breakfast meal with regard to the ratio of the components, pH, and physicochemical properties (*24*). As the stability of fresh milk at 37 °C is challenging, UHT milk was used. The brand and quality of milk were standardized to avoid variation in its composition (*24*).

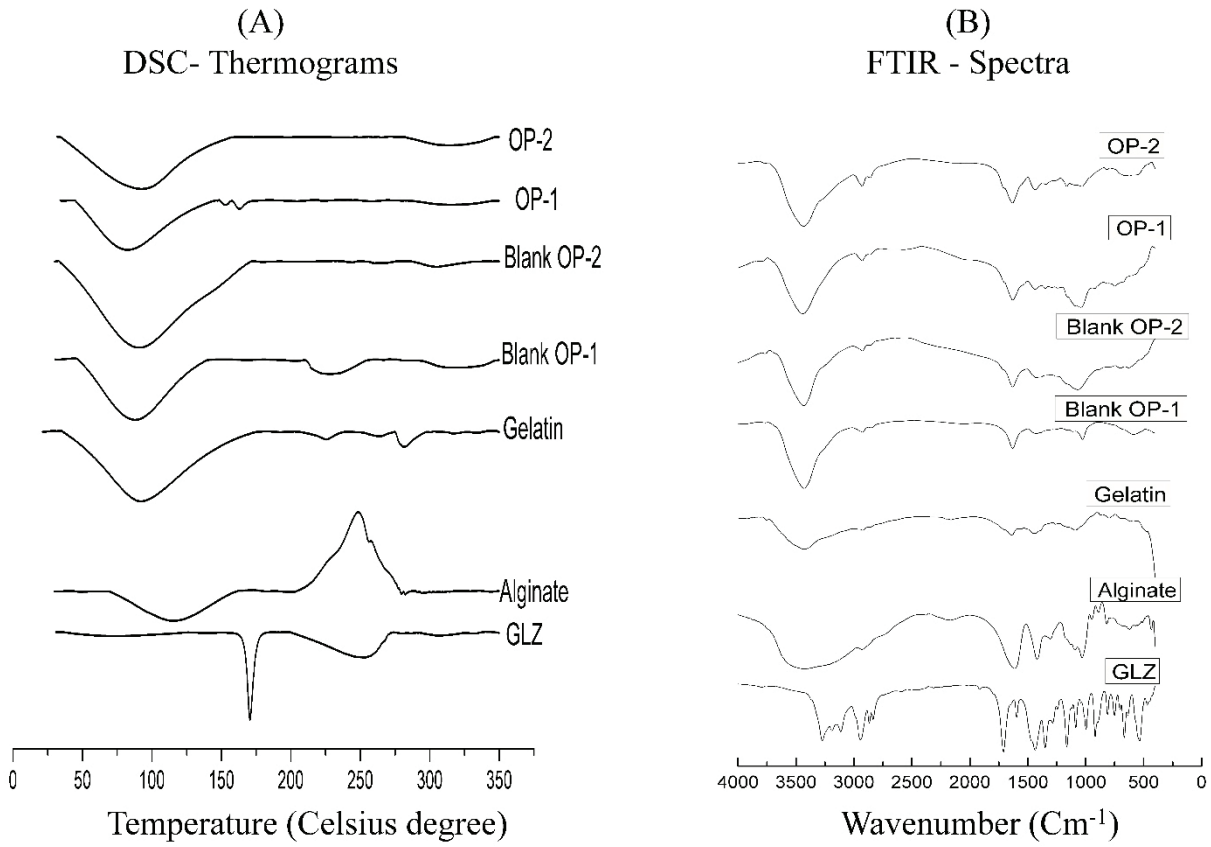

Figure 1. Physicochemical characterization of gliclazide (GLZ) optimized formulations (OP-1 and OP-2): DSC-thermograms (A) and FTIR spectra (B) of GLZ, sodium alginate (high viscosity), gelatin (Bovine-B), GLZ-loaded, and blank optimized beads. DSC: differential scanning calorimetry; FTIR: Fourier transform infrared.

The selected biorelevant medium simulating the intestinal conditions in the fed state (Table 2) was as proposed by Klein (*24*). It contained molar ratios of sodium taurocholate and lecithin that simulate the in-vivo ones. The composition of the intestinal fluid is also dependent on the type of the ingested meal, though, to a lower extent than the gastric fluid. Food-induced bile secretion increases bile salt levels in the small intestine between 8 and 20 mM up to 40 mM (*33*, *34*). The ratio between bile salts and phospholipids depends on the phospholipid concentration present in food. It is reported to range from 2:1 to 5:1 (*33*, *35*). FeSSIF composition was first proposed by Dressman et al. (*36*). It simulates the intestinal fluid regarding bile salt, phospholipids in addition to pH, osmolarity, and buffer capacity (*33*). The FeSSIF proposed by Dressman et al. included acetic acid and potassium chloride for buffer capacity and osmolarity adjustment instead of acetic acid, sodium acetate, and sodium chloride in the medium proposed by Klein (*24*). Generally, the preparation methods of biorelevant media require emulsification in a chlorinated solvent or may involve sequential addition. The preparation method in this study was simple and does not require the addition of a chlorinated solvent.

The selected SCoF medium composition was proposed by Fotaki et al. (*37*) (Table 2). Generally, the development of SCoF is mainly dependent on pH and short-chain fatty acid concentrations. Acetate buffer was used to adjust the desired pH (5.8) and buffer capacity (*1*).

Residence Time in the Gastrointestinal Tract

In this study, FeSSGF was used for 2 hours, then replaced with FeSSIF for 3.5 hours, which was finally replaced with SCoF for 8 hours. Dimensions of the dosage form drastically affect the residence time. Moreover, gastric emptying is faster in the fasted state than in the fed state causing the dosage form to reach the higher pH regions of the intestine rapidly. The gastric residence time is usually shorter for multiparticulate systems than for single-unit systems (*11*, *38–40*). However, the spherical shape makes it easier for beads to reach the colon and retain in the ascending colon. This supports the longer duration of action of beads (*41*). Jantratid et al. (*11*) suggested the residence times of pellets in different regions of the GI tract based on the literature data and applied them to diclofenac sodium MR pellet biorelevant dissolution tests (*11*). The proposed residence times of pellets were utilized in the current study.

Quantification of GLZ in the Biorelevant Media

The complexity of the composition of the biorelevant media necessitates the validation of drug analytical methods to ensure obtaining reliable and reproducible results. It is difficult to establish an analytical method to accurately quantify a drug in milk-based media (FeSSGF) as the drug may distribute into different phases of milk. Jantratid et al. used the 'infinity point' approach (*11*). The limitation of this approach is that it does not give enough data about the dissolution rate in the FeSSGF as it estimates the total amount of drug dissolved at the end of this phase. For accurate quantification of GLZ in biorelevant media using the gradient conditions, the analytical method was modified and validated.

HPLC/UV Method Validation for Quantification of GLZ in FeSSGF and FeSSIF

The HPLC chromatograms revealed that GLZ was eluted at 4.3 minutes and the internal standard (IS) was eluted at 5.5 minutes. No interfering peaks were detected neither from the biorelevant media nor the blank beads. This indicates good resolution and selectivity (Fig. 2).

Two HPLC calibration curves at 230 nm were constructed by plotting GLZ/glyburide peak area ratio against GLZ concentrations in the ranges of 0.5–5 µg/mL and 5–60 µg/mL for each biorelevant medium. Linear relationships were established between GLZ standard concentrations and (GLZ/glyburide) peak areas ratio.

In FeSSGF, the coefficients of determination (r^2) were found to be 0.9986 and 0.9988 for the calibration curves of the lower concentrations and the higher concentrations, respectively. The response factors (procedural constant) were found to have mean values of 0.39 ± 0.03 and 0.42 ± 0.043 for the lower concentrations and the higher concentrations, respectively, with a relative standard deviation (RSD) less than 10%.

In FeSSIF, the r^2 values were 0.9937 and 0.9968 for the calibration curves of the lower concentrations and the higher concentrations, respectively. The response factors (procedural constant) were found to have mean values of 0.216 ± 0.032 and 0.33 ± 0.042 for the lower concentrations and the higher concentrations, respectively, with RSD less than 15%.

Three replicates of the samples spiked with different amounts of GLZ (covering the range of 0.5–60 µg/mL) were analyzed. The accuracy was measured as the mean percentage recovery. The recovery ranged from 90–109% for the standard concentrations in FeSSGF and from 90–105% for the standard concentrations in FeSSIF.

The analytical precision was determined by the CV% of the peak area ratios, which ranged from 4.2–12.21% for the standard concentrations in FeSSGF and from 2.3–11.28% for the standard concentrations in FeSSIF.

Comparative Dissolution of GLZ in Biorelevant Media

In the current study, two different dissolution methods were investigated. The main objective was to establish a reliable cost-effective methodology and to utilize the flexibility and reproducibility of the drug release data that can be obtained from the multiparticulate drug delivery systems. As GLZ is administered with food (*42*, *43*), it is

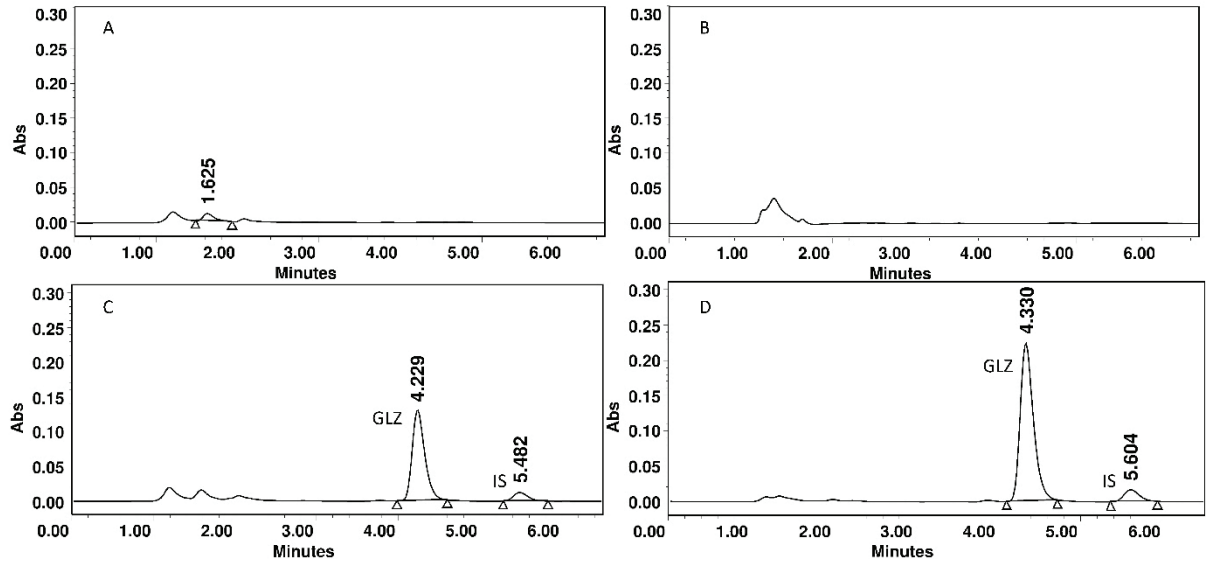

Figure 2. High-performance liquid ultraviolet chromatograms of blank FeSSGF (A), blank FeSSIF (B), GLZ with IS in FeSSGF (C), and GLZ with IS in FeSSIF (D). FeSSGF: fed state simulated gastric fluid; FeSSIF: fed state simulated intestinal fluid; GLZ: gliclazide; IS: internal standard; Abs: absorbance.

important to study its dissolution in the simulated fed state using gradient conditions.

The excellent weight-dose proportionality of the multiparticulate drug delivery systems enabled the reduction of biorelevant media volume to 25 mL instead of 900 mL (official volume). Figure 3A shows that the optimized AL-GL beads showed similar reproducible GLZ release patterns using USP apparatus 1 and the shaking water bath (f_2 = 72 and 79, f_1= 9 and 11 for OP-1 and OP-2, respectively). Both OP-1 and OP-2 showed slower release rates of GLZ in biorelevant media compared to the compendial media (Fig. 3). This was attributed to the physicochemical properties of GLZ, which is a weak acid with pH-dependent solubility (*15*, *18*). It is an ampholyte with a pH-dependent solubility in the GI pH range (*19*). Although its solubility is higher in the alkaline media, it has a reasonable solubility value in pH (1.2) that is comparable to its solubility in pH (7.4). However, GLZ shows very low solubility in the pH range of 2.5–6.5. Skripnik et al. studied GLZ release from MR GLZ tablets (market products) in different dissolution conditions (*44*). They used a ready-made biorelevant medium prepared as half-FaSSIF (fasted-state simulated intestinal fluid). In their study, complete drug release was achieved with apparatus 2 at 100 rpm, phosphate buffer (pH 6.8), during 24 h. However, half FaSSIF (pH 6.8), showed similar results to those obtained with phosphate buffer pH 6.8. Patel et al. also studied the effect of the bile salts, lecithin, and surfactants content in the ready-made FeSSIF powder (prepared in phosphate buffer pH 7.4) GLZ release (*45*). They concluded that the dissolution of GLZ was not affected by the content of biorelevant media while it might be affected by the simulated pH value itself, which was not studied (*45*, *46*). In the current study, the pH of biorelevant media was adjusted to simulate the fed-state conditions. The results indicated a pronounced effect of pH on GLZ release in both biorelevant and compendial media.

OP-1 showed faster GLZ release in all biorelevant media (FeSSGF, FeSSIF, and SCoF) compared to OP-2 (DE = 43.63% and 24.32%, respectively). About 83% of GLZ was released from OP-1 after 14 hours compared to 50% from OP-2 (Fig. 3A). On the other hand, GLZ release from OP-1 was slower than OP-2 in 0.1 N HCl (pH 1.2, DE 2h = 10.19% and 26.73%, respectively), and it was faster in pH 7.4 (Fig. 3B). This release pattern was accurately predicted based on the previously implemented design of experiments and response surface methodology (*21*).

Both OP-1 and OP-2 showed a zero-order GLZ release pattern in the compendial and biorelevant media (Table 1). Zero-order release refers to systems where the drug release rate does not depend on the concentration (*47*). These results suggested that the reservoir system of AL-GL beads was not affected by the biorelevant media while slower release patterns were obtained.

This is the first study that investigates GLZ release from a multiparticulate drug delivery system in fed-state

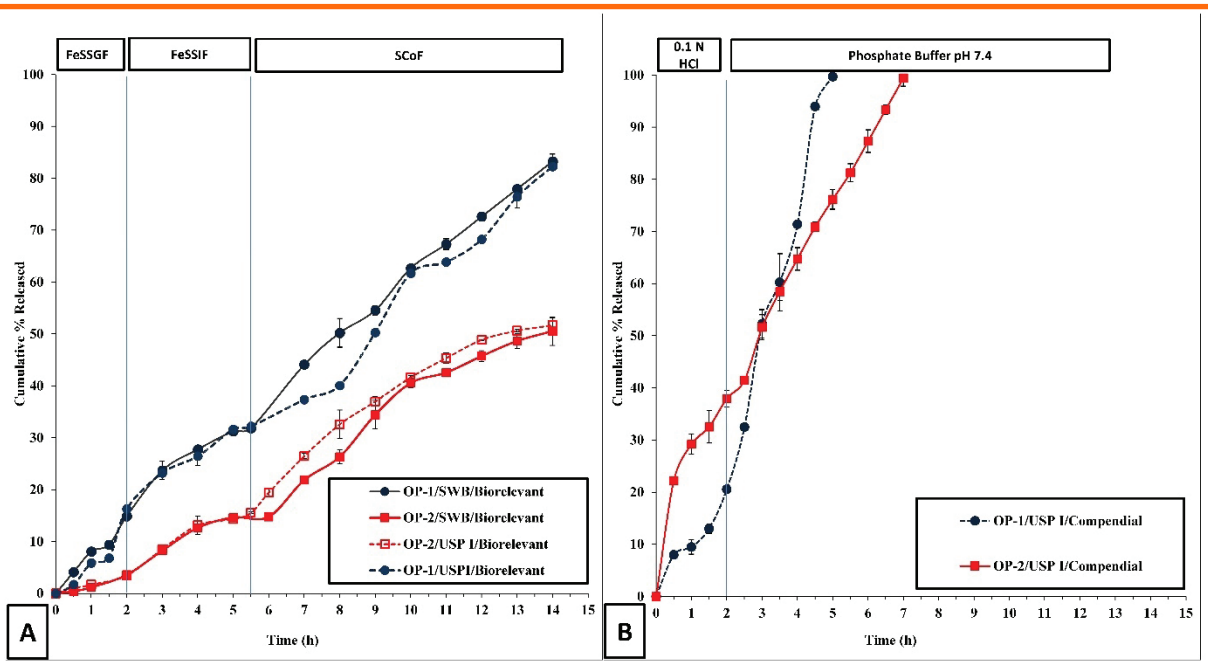

*Figure 3. Cumulative release of gliclazide (%) from optimized formulations (OP-1 and OP-2) in biorelevant media employing USP I apparatus and shaking water bath (SWB) (**A**) and in compendial media (**B**). FeSSGF: fed state simulated gastric fluid; FeSSIF: fed state simulated intestinal fluid; SCoF: simulated colonic fluid; HCl: hydrochloric acid.*

biorelevant media applying gradient conditions. The cost-effective methodology was mainly dependent on the excellent weight dose proportionality of the multiparticulate drug delivery systems that enabled the reduction of the biorelevant media volume to 25 ml instead of 900ml. Furthermore, the biorelevant media were prepared from their components and the preparation method was simple and does not require the addition of a chlorinated solvent as frequently carried out.

Scanning Electron Microscope (SEM)

Figure 4 shows OP-1 and OP-2 beads integrity (X 100) and surface topography (X 3000) before and after GLZ release. For OP-1 beads, a thin layer of crosslinked polymers diffused around the bead after GLZ release in compendial media suggests a change in the crosslinked polymers, and no pores were observed on the surface (Fig. 4B). After GLZ release in biorelevant media from OP-1 beads, no change was observed in the integrity of beads but there were numerous tiny pores on the surface (Fig. 4C); these pores were suspected to be responsible for GLZ release out of the beads. OP-2 beads integrity was drastically affected after GLZ release in compendial media. A widely diffused thin layer of the polymers was observed, and no pores were observed on the surface (Fig. 4B). After GLZ release in biorelevant media from OP-2 beads, no change was observed in the integrity of the bead, but the surface showed peeling of thin layers or flakes of the crosslinked polymers (Fig. 4C). The transverse sections of OP-1 and OP-2 beads showed no drug particles retained after GLZ release in compendial media (Figs. 4D and 4E). However, some drug particles were observed in the core of OP-1 and OP-2 beads after GLZ release in the biorelevant media (Figs. 4F and 4G); this was attributed to the slower drug release rate obtained in biorelevant media.

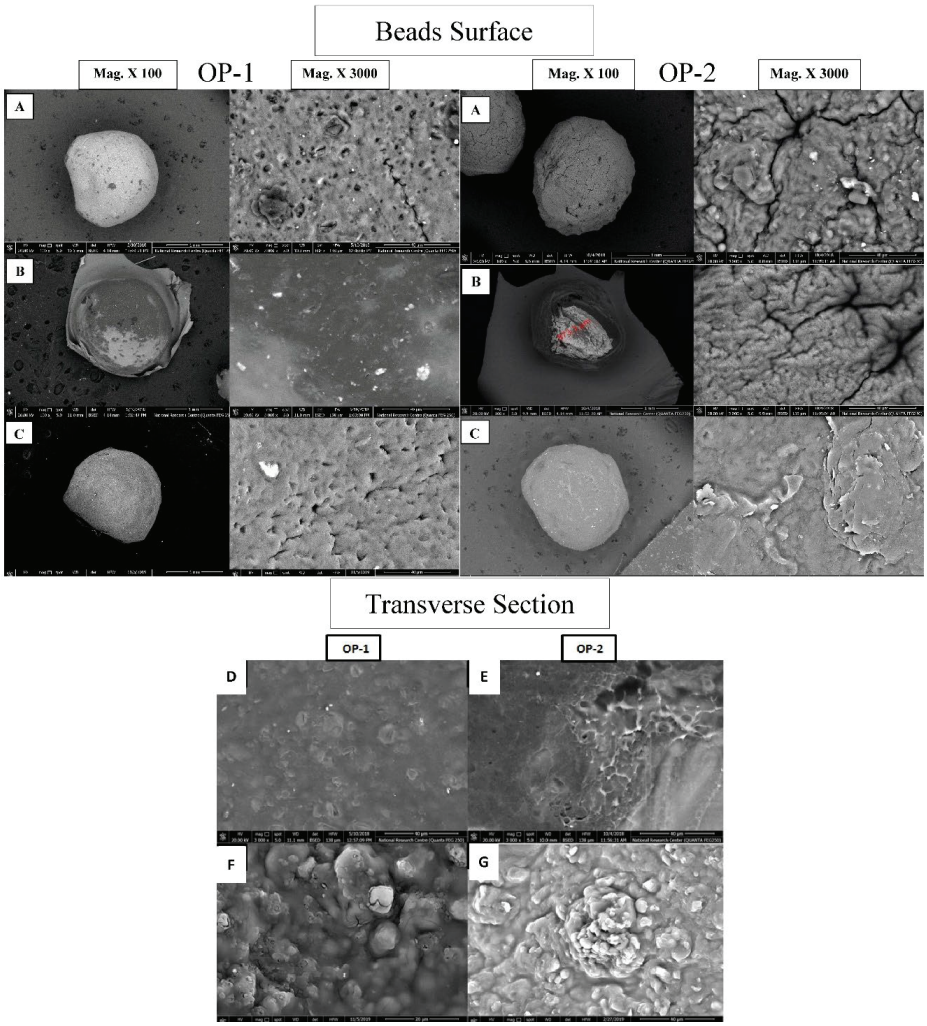

*Figure 4. Scanning electron microscope images of the optimized formulations (OP-1 and OP-2 beads) before GLZ release (**A**), bead surface after GLZ release in compendial media and biorelevant media (**B** and **C**, respectively), and transverse section of beads after GLZ release in compendial media (**D** and **E**) and biorelevant media (**F** and **G**).*

CONCLUSION

The current study indicated the pronounced effect of pH on GLZ release in both biorelevant and compendial media. This effect was reflected on the bead integrity and surface topography. GLZ release from AL-GL beads in biorelevant media was slower than its release in compendial media. The optimized formulation, OP-1, showed faster GLZ release than OP-2 in biorelevant media and phosphate buffer pH 7.4.

This is the first study that investigates GLZ release from a multiparticulate drug delivery system in fed-state biorelevant media applying gradient conditions. In addition, the methodology was much more cost-effective compared to the ready-made media in terms of the cost of components and ease of preparation.

CONFLICT OF INTEREST

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

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Report on the Virtual Workshop: A Quest for Biowaiver, Including Next Generation Dissolution Characterization and Modeling

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INTRODUCTION

The virtual workshop, "A Quest for Biowaiver, Including Next Generation Dissolution Characterization and Modelling," was held on November 16–17th, 2022, via the MS Teams platform. The conference was co-sponsored by Jagiellonian University Medical College (JUMC) in Cracow, Poland and the American Association of Pharmaceutical Scientists (AAPS). The workshop was chaired by Vivian Gray (AAPS) and Prof Aleksander Mendyk (JUMC), with the support of the co-chairs Prof Nikoletta Fotaki (AAPS), Prof Jie Shen (AAPS), and Dr Jakub Szlęk (JUMC).

The main workshop themes included regulatory aspects, best practices on dissolution testing, and next-generation dissolution modeling. The objectives of the meetings were to provide participants with practical knowledge they can apply to their current work, as well as new concepts that will improve and broaden their experience. During the workshop, participants learned best practices for developing discriminative dissolution methods and expanded their knowledge of drug product characterization. In addition, they were introduced to new modeling concepts to support dissolution specifications. With a virtual format, the workshop attracted participants from all over the world.

The workshop included four sessions that were dedicated to specific questions related to dissolution studies. Each session was followed by a discussion between the panelists and participants. The topics of the various workshop sessions were as follows:

- Session 1: Regulatory Aspects and Expectations
- Session 2: Basics and Best Practices on Dissolution Testing
- Session 3: Next Generation Characterization for Dissolution Testing
- Session 4: Modeling and Artificial Intelligence Approaches

A total of 278 individuals registered for the virtual workshops. Most of the registered participants indicated industry (71%) and academia (24%) as their affiliation (Fig. 1). The largest number of participants signed up for the workshop from the United States, Poland, and India (Fig. 2).

On the first day of the conference, 198 participants joined the meeting. On the second day, 128 attendees joined the event, giving a total of 326 participants in the 2-day live workshop.

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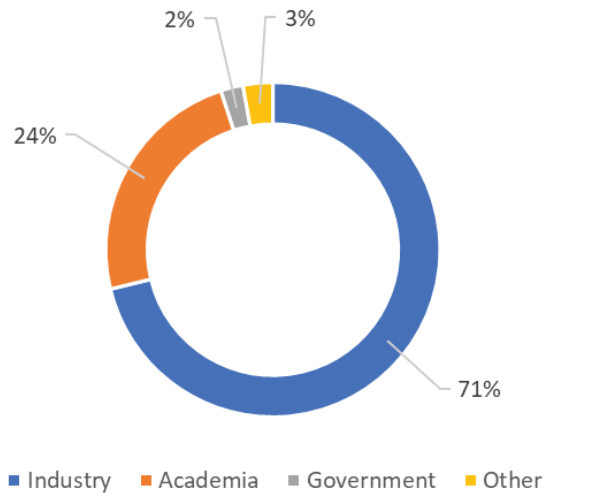

Figure 1. Number of registered workshop participants by institution type.

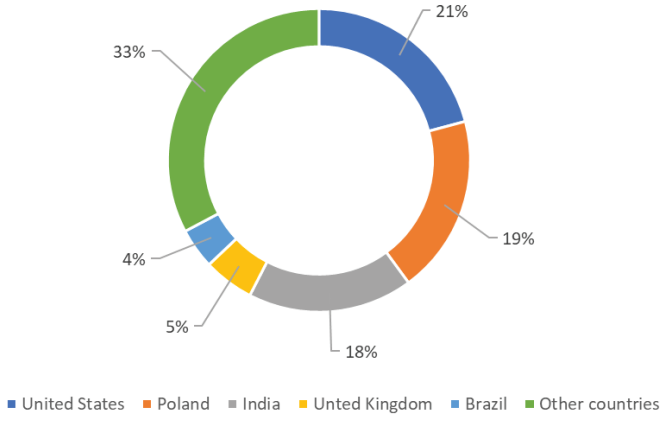

Figure 2. Number of registered workshop participants by country.

SESSION 1: REGULATORY ASPECTS AND EXPECTATIONS

The virtual workshops opened with an introduction given by Vivian Gray and Prof Aleksander Mendyk, who outlined the agenda and goals of the meeting. The first session, "Regulatory Aspects and Expectations," was moderated by Prof Aleksander Mendyk. The aim of the session was to review the biopharmaceutics classification system (BCS)-based biowaivers and to discuss the regulatory aspects of dissolution testing from both the US FDA and European perspectives. The first talk entitled, "Biopharmaceutics Classification System-Based Biowaivers ICH M9," was given by Dr James Mann and Dr Xavier Pepin.

The BCS, which classifies molecules based on their solubility and permeability, was first published by Amidon et al. back in 1995 and led the US FDA to publish the first guidance on a BCS-based biowaiver in 2000 (*1*). The biowaiver concept was extended to other territories and adopted by the EU in 2010. The result of this staggered uptake of BCS-based biowaivers led to a lack of harmonization between territories, which proved challenging for pharmaceutical companies to navigate. There was lack of harmonization around whether BCS class 1 and 3 were both accepted or just BCS 1; dissolution apparatus, hydrodynamic conditions, and dissolution medium volume were some of the issues. In addition, some ICH countries like Japan did not formally recognize BCS-based guidelines. This was seen as an area ripe for ICH harmonisation and in 2019 after much discussion among member companies and the pharmaceutical industry, the harmonized guideline on BCS-based biowaivers was published in the form of ICH M9 (*2*).

The ICH M9 harmonization process focused on four main areas: solubility, permeability, excipients, and dissolution (*2*). For solubility, the main debate was around whether solubility should be classified based on highest strength or highest dose. The final guidance classifies based on highest single therapeutic dose but with some allowances to study strength if additional data are provided. The guidance also allows alternative methods for solubility classification based on the apparent full dissolution in 250 mL medium, which can be useful for amorphous drugs or salts of free moieties. Permeability classification is ideally based on human data using absolute bioavailability data. A high permeability would be granted if the bioavailability ≥ 85% or if the sum of urine parent, Phase 1 oxidative and Phase 2 conjugative metabolites, and faecal Phase 1 oxidative and Phase 2 conjugative metabolites exceeds 85% of the administered dose. In vitro assessment against approved high permeability references using Caco-2 cell lines can also help determine the drug high permeability. In addition, unless absolute bioavailability is used for determination of high permeability, the drug should be demonstrated to be stable in the gastrointestinal (GI) tract. For excipients, decision trees on allowed differences between test and reference were provided with more stringent criteria for BCS 3 drugs. For dissolution, the major discussion points were whether to include water in the medium and to allow 75 rpm paddle speed for apparatus 2. In the final guidance, water was not included, and 75 rpm is not specifically included, but scientifically justified approaches can be used if coning or high variability is observed. Overall, ICH M9 is welcomed by industry and is a great stride forward; however, the global acceptability needs to be achieved, particularly in the circumstances where flexibility and scientific justification are allowed.

The next talk was given by Dr Margareth R. C. Marques

(United States Pharmacopeia) concerning "Performance Tests in the U.S. Pharmacopeia."

Dr Marques presented an overview of the USP general chapters related to drug product performance tests. The scope of the following chapters was discussed: <1092> The Dissolution Procedure – Development and Validation; <701> Disintegration, and <711> Dissolution, both harmonized with the *European Pharmacopoeia* and *Japanese Pharmacopoeia*; <1094> Capsules – Dissolution Testing and Related Quality Attributes; <1711> Oral Dosage Forms – Performance Tests. Also, she presented the general chapters related to products applied to the skin: <3> Topical and Transdermal Drug Products – Product Quality Tests; <724> Drug release; and the major revision made to <1724> Semisolid Drug Products – Performance Tests to align with the new FDA guidances related to products applied to the skin. The chapter <1236> Solubility Measurements was also discussed. This chapter contains the composition of some simulated biological fluids, both for human and veterinary applications, that can be used to assess product performance during formulation development. She summarized the activities of the USP Expert Panel on New Advancements on Product Performance Testing, which has already published several papers on the performance tests of dosage forms other than tablets and capsules (*3*–*6*). Note: The proposals for any revisions to the *USP–NF* are published in *Pharmacopeial Forum*, available free of charge at www.uspnf.com for a period of 90 days for public comments.

The closing lecture of the first session was given by Prof Aleksander Mendyk, who spoke on "Dissolution Method Development from European perspective."

Prof Mendyk focused on the comparisons of pathways of dissolution method development and synergies between US and Europe. He emphasized on the tendency to harmonize various regulations both in Pharmacopoeias (*USP* vs. *Ph.Eur*) and scientific guidelines. However, some discrepancies are still pending, i.e., f_2 calculation, yet ICH is another example of a successful consensus reached under the umbrella of the M9 guideline described by Dr James Mann and Dr Xavier Pepin.

The first part of the workshop ended with a question and answer session with attendees and speakers.

SESSION 2: BASICS AND BEST PRACTICES ON DISSOLUTION TESTING

The second session of the workshop, "Basics and Best Practices on Dissolution Testing," was moderated by Prof Jie Shen. The main themes were the challenges of developing a discriminatory dissolution method, the influence of post-approval changes on dissolution testing, and the implementation of a statistical approach to generic development. The first speaker of the session was Vivian Gray (*Dissolution Technologies*), during which she gave a talk entitled, "Challenges When Developing a Discriminatory Dissolution Method."

Vivian began with defining "discriminatory" method and why it is necessary, reiterating that discriminating methods can contribute to specifications that can distinguish between bioequivalent and bioinequivalent batches. She explored the necessary characteristics of a discriminatory method and gave resource material that provided regulatory and industry expectations. The primary references were the EMA Reflections paper and *USP* chapter <1092> The Dissolution Procedure: Development and Validation; she also provided two literature references of interest (*7*–*9*).

An outline was provided on how to develop a discriminatory method. The first step is to identify those critical quality attributes (CQA) related to the drug substance, drug formulation, and drug product manufacturing process. She gave examples in each category. The second step is to identify which of these attributes affect the in vivo release. The third step is to manufacture drug product that reflects the upper and lower limits (± 20%) of that variable, ideally about two or three variations for each category (drug, drug formulation, manufacturing process). Fourthly, run these variation products, preferably one variable at a time versus the target product. Lastly, compare the dissolution profiles and determine if there are significant differences among the variables and the target. Hopefully, there will be at least two or three variables that the method can pick up differences. If not, then go to a backup method that is possibly more complex and may not achieve sink conditions. She concluded with in addition to a discriminatory method there should be an in vivo linkage element to the in vitro method data.

Next, Dr Andreas Abend gave a talk on "Current Challenges of Dissolution Testing in Support of Postapproval Changes for Oral Drugs."

Dissolution testing is widely used in the pharmaceutical industry to gain insight into bioperformance of drugs when in vivo drug substance release is a prerequisite of drug absorption and/or distribution of the drug to the site of action. Different in vitro methods aimed to mimic the physiological environment the drug may encounter after administration are usually applied

during drug product development to screen formulation candidates and in support of biopharmaceutics risk assessment. These methods are often performed under conditions that are not deemed appropriate for routine product quality assessment (*10*). Once formulation and manufacturing conditions relevant for late-stage clinical trials have been identified, the development of quantitative analytical methods and acceptance criteria (i.e., product specifications) begins (*10*). At this stage, a dissolution method that can be routinely operated in a quality control (QC) lab is validated according to applicable guidance (e.g., ICH Q2, USP, etc.) (*11*). One of the key challenges of late-stage drug development and product lifecycle management is the assessment of manufacturing changes on product quality. In general, health authorities classify deliberate manufacturing changes as minor, moderate, and major depending on their potential impact on in vivo performance of the drug. The US FDA issued several guidance documents for industry in the 1990s to clarify the expected dissolution tests required to support manufacturing changes for immediate and modified release solid oral products and on dissolution method development (*12–14*). In addition, for IR drugs, global harmonized guidance on how to apply for biowaivers based on the BCS is now implemented by health authorities that are members of ICH (*1*, *2*). In the context of product lifecycle management ICH M9 can be applied to BCS 1 drugs under certain circumstances for major manufacturing changes which may otherwise require in vivo bioequivalence (BE) studies. BE studies may also be waived under certain conditions for over-encapsulated drugs used in blinded clinical trials or to demonstrate BE of lower strength in case BE was already demonstrated at a higher strength (*15*).

The assessment of moderate manufacturing changes on in vivo performance is typically based on comparisons of dissolution profiles of drug product made according to the new manufacturing process (the "test product") and the existing, typically regulatory approved, process conditions (the "reference product"). For biowaiver applications following ICH M9, the test and reference products are usually a new formulation made under representative manufacturing conditions versus a reference listed drug (i.e., drug product already approved) (*2*). In some cases, dissolution profiles comparisons are made by using the approved QC dissolution method, whereas in other cases (e.g., level 2 formulation changes, BCS-based biowaivers, etc.) dissolution testing is performed in various aqueous media under conditions described in applicable pharmacopeias and guidance.

Although many superior mathematical models to test for dissolution profile similarity exist, the dissolution similarity factor (f_2) proposed by Flanner and Moore is widely used in the industry and by regulatory agencies to assess similarity (*16–18*). Regardless of the mathematical approach that is either expected by regulators or – in case health authorities are open to alternative approaches – has been chosen by the applicant, a decision on similarity and thus in vivo impact can only be made with confidence if differences in the rate and extent of drug released in vitro measured by the applied dissolution method(s) are indicative of differences in the rate and extent of drug release in vivo, which subsequently indicate differences in systemic exposure (i.e., confirming BE) (*19*, *20*).

Dissolution testing performed under multiple pH conditions or the approved QC method, which may or may not contain surfactants, is not a priori indicative of unacceptable in vivo performance unless these methods are clinically relevant (*21*). Once a clinically relevant dissolution method (CRDM) has been developed and validated, this method should be used to assess the impact of manufacturing changes as opposed to any dissolution methods with unknown clinical relevance (*22*). A clinically relevant dissolution specification (CRDS) can be established via traditional bracketing approaches or in silico. In addition, one can develop upper and lower ranges of dissolution profiles within which products exhibiting dissolution profiles falling inside these ranges ("safe space") are deemed equivalent to the reference product (*19*, *23*, *24*). Therefore, companies should invest in the development of CRDS and safe spaces especially for IR drugs containing poorly soluble drug substances. To develop an appropriate dissolution method where rate and extent of drug release are limited by drug substance solubility, surfactants are required to achieve complete drug release within 60 minutes. However, justification of appropriate surfactant levels or agitation conditions are always challenging unless a link to in vivo data is available.

Scientists in industry are encouraged to define the dissolution similarity assessment test conditions, test materials, mathematical hypothesis, mathematical method, and acceptance criteria based on dissolution performance experience from reference material made under the approved conditions as well as pilot batches made under the anticipated new manufacturing conditions prior to any dissolution profile assessment, regardless of whether CRDS and safe space are in place or not. This is especially important to avoid unexpected results (failure to demonstrate similarity, unexpected variability) or "cherry picking" mathematical models that

may give more favorable results. Likewise, this should avoid the temptation to use readily available software and apply a variety of mathematical models until the desired result is obtained. When it comes to good science, understanding drug substance, formulation, and process variables that impact the in vitro rate and extent of drug dissolved are critical to relate to in vivo performance. This does not necessarily imply that all dissolution specifications or methods require developing CRDS and safe space – the decision not to link in vitro and in vivo data should be based on rigorous risk biopharmaceutics risk assessment and overall product lifecycle management considerations.

The last talk entitled, "A Statistical Approach on Generic Development" was given by Prof Aleksander Mendyk.

Prof Mendyk introduced regulatory framework of ICH Q6A and Q6B, detailing product specifications in qualitative manner and presenting an evolution of requirements for development towards quantitative inferences as per ICH Q8(R2). He presented an empirical approach using ANOVA for selection of crucial critical process parameters and more sophisticated computational tools, i.e., rule-based artificial intelligence systems (Cubist). As for the latter he highlighted flexibility, interpretability, and simplicity of this tool to be used for design space selection in a quantitative and multidimensional manner.

The meeting ended with a question and answer session, which also closed the first day of the workshop.

SESSION 3: NEXT GENERATION CHARACTERIZATION FOR DISSOLUTION TESTING

The second day of the virtual workshop began with a third session moderated by Prof Nikoletta Fotaki entitled, "Next Generation Characterization for Dissolution Testing." The session addressed concerns related to visualization of transport in pharmaceutical systems, biopredictive testing, and novel approaches on dissolution methods for microsystems. The first talk was given by Prof Przemysław Dorożyński and covered "Drug Dissolution in a Snapshot - Visualization of Mass Transport in Pharmaceutical Systems."

Elucidation of drug dissolution mechanisms is a highly demanding task. Drug release mechanisms cannot be explained simply based on the drug release results. Only a comprehensive approach to the issues will help understand the drug release mechanism. Such an approach requires the coupling of drug release testing with other methods, e.g., with non-destructive imaging methods, i.e., magnetic resonance imaging (MRI,) micro-computed tomography (micro-CT), and supporting techniques, such as nuclear magnetic resonance relaxometry (NMR) performed in situ during dosage form incubation in dissolution media.

In the presentation, the practical and scientific aspects of the application of imaging studies concomitantly with drug dissolution were discussed. Characterizing the internal structure of a drug delivery system via imaging may be a powerful tool in the development of a generic drug product. It enables identification of the optimal drug manufacturing methodology, but it could also be used to analyze the potential behavior of drug delivery systems in the GI tract, which could be a risk mitigation factor prior to BE studies (*25, 26*). MRI can also be applied as a tool for elucidating the dissolution profile features (i.e., kinetics, kinetics changes, and variability) (*27*). Imaging techniques, in conjunction with other methods, were recently used to investigate mass transport phenomena within polymeric matrix systems (*28*).

The next speaker, Dr. habil. Grzegorz Garbacz, spoke on "Biopredictive Testing as a Tool Supporting Rational Development of Oral Medicines."

Bio-predictive studies have a significant role in the R&D cycle of oral drugs, from API studies through formulation, preparation, and initiation of clinical trials to product manufacturing. The three most important factors affecting release of API from a solid dosage form or drug delivery performance in the human GI tract are pH, temperature, and pressure (mechanical agitation). All these factors vary significantly depending on the particular section of the GI tract and the prandial state. Both fasted and fed conditions were recently investigated using a telemetric capsule Smartpill™ capable of continuous monitoring of pH, temperature, and motility forces (*29*).

Based on knowledge of the specific physiology of the digestive system, bio-predictive studies can be considered as an extension of pharmacopoeial dissolution tests. However, to conduct representative bio-predictive characterization of oral drugs, simple and straightforward tools are necessary. These devices should simulate dynamic fluctuations of pH, motility, temperature, and volume changes of the GI tract. In addition, they are intended to deliver data that are suitable for the simulation of absorption and pharmacokinetic (PK) modeling. One such tool is pHysio-grad® (Physiolution). The apparatus is a fully automated, dynamic system developed for the simulation of physiological pH gradients characteristic for the small intestine and colon. The system utilizes

a hydrogen carbonate buffer in which pH reduction is achieved by injecting carbon dioxide into the system. In contrast, to raise the pH of the medium, air or inert gas is introduced into the system, which displaces the carbon dioxide. The apparatus has several types of configurations allowing, among others, the use of liquid titrates and gases or measurements in small volumes. Another tool used for biopredictive studies is the Advanced Modular Platform (Physiolution). The multifunctional design of the apparatus allows the combination of USP apparatus type 1 and 2 functionalities with Stress Test Device, transfer models, and pH controller. The forces acting on the drug form in the GI tract are simulated by the device through a balloon placed in the drug chamber, which exerts pressure on the test product under pumping and deflating. Another apparatus, which can be used to test IR formulations is PhysioCell (Physiolution). This novel flow-through device is divided into three compartments, by which it reflects realistic pH, flow rate, and mechanical stresses impacting the drug formulation during GI tract transfer.

In summary, the cutting-edge biopredictive methods developed by Physiolution enable realistic simulation of the GI tract and support the rational, physiology-driven development of oral medicines. Moreover, applying biopredictive methods can shorten the time and decrease market development cost as well as reduce the risk of clinical trials and therapy failure.

The final talk of the session was given by Prof Nikoletta Fotaki on "Novel Approaches on Dissolution Methods for Microsystems; Case Study: Liposomes."

First, Prof Fotaki discussed why there is a need for a discriminatory test for liposomes. FDA guidelines only state that a validated release test should be performed for liposomes with a suitable release medium and with suitable agitation. She described the current state of the in vitro release testing of liposomes. The release medium is selected according to the solubility, stability, and ease of drug assay. A surfactant or an organic solvent can be added to increase the drugs' solubility or to accelerate its release and should have a physiological pH (7.4) and osmolality (275-300 mOsm/L); currently, the most commonly used is PBS. Next, Prof Fotaki discussed points to consider for the release medium, emphasizing the importance of proteins, as they would have an effect on drug solubility/release from formulation. Regarding the dissolution testing apparatus and operational conditions, the current guidelines include sample dialysis as well as separate and continuous flow methods. She gave a perspective on the points to consider, including the need to simulate the hydrodynamics in the bloodstream, the concurrent circulation of liposomes and released drug, and the need for an in vitro test to facilitate dispersion of moving particles. A detailed case study on the development of in vitro release studies for liposomal formulations was described, where the effect of buffer, synthetic surfactant, protein, and hydrodynamics were presented. Afterwards, she presented the development of clinically relevant in vitro test conditions. The final part of her presentation related to the use of PBPK modeling to identify in vivo predictive release tests for parenteral liposomal formulations. She concluded her presentation by noting the importance of understanding the factors affecting drug release from liposomes by composition of medium and simulation of hydrodynamics at the site where drug will be released from formulation.

The session ended with a series of questions and answers.

SESSION 4: MODELING AND ARTIFICIAL INTELLIGENCE APPROACHES

The fourth session of the workshop, "Modeling and Artificial Intelligence Approaches," was moderated by Vivian Gray. Presentations included use of artificial intelligence in in vitro-in vivo correlation (IVIVC), physiologically based biopharmaceutics modelling (PBBK), and biopredictive dissolution. The first talk, "IVIVC Based on Artificial Intelligence," was given by Prof Aleksander Mendyk.

Prof Mendyk began by reviewing a classic case of a level A IVIVC performed with direct implementation of the FDA guideline to be inefficient in this specific case. He then introduced an AI-based tool called a symbolic regression (SR), working under principles of genetic programming (GP). As an open source system, HeuristicLabs was challenged with the data from the case study and showed excellent improvement of both internal and external predictability of IVIVC. As the structure of the resulting IVIVC model is extremely complicated and the data setup positions it between level A and multiple level C models, this approach is still experimental and therefore not to be applied on a regular daily basis. At the end of his presentation, Prof Mendyk described his regression in vitro in vivo relationship (RIVIVR) package capable of handling the case study data in an automated manner with superior predictability, but under the heuristic principles of empirical model development and thus difficult to validate under the principles of regulated environment. His last remark emphasized data quality, which is crucial to empirical modeling like the one presented in his talk.

The following presentation was given by Dr Sandra Suarez-Sharp, entitled "The Application of PBBM in Support of Formulation, Manufacturing, and Controls Changes via Safe Space Biowaivers."

Demonstration of BE of a drug product following major changes in the formulation, manufacturing, and controls (CMC changes) plays an important role in drug product development and lifecycle management. Regulatory agencies have published several guidance documents to decrease the regulatory burden (via biowaivers) following CMC changes (*2*, *30*, *31*). The safe space framework offers an integrated approach to biowaivers, encompassing both the conventional and mechanistic approaches in the construction of in vitro-in vivo relationships (IVIVRs) or IVIVCs (*24*). Recently, the US FDA published a guidance on the "Use of Physiologically Based Pharmacokinetic Analyses — Biopharmaceutics Applications for Oral Drug Product Development, Manufacturing Changes, and Controls" (also known as the PBBM guidance) for the purpose of waiving not only BE studies based on building a safe space but to also aid in biopharmaceutics risk assessment and setting clinically relevant drug product specifications (*32*).

Selection of the safe space approach depends on the type and amount of data available, and it is likely that a safe space built based on the mechanistic (PBBM) approach will result in wider manufacturing and regulatory flexibility than one based on conventional approaches. One advantage of the PBBM-safe space approach is that it is not confined to building IVIVCs, increasing the likelihood of gaining regulatory flexibility. Precisely, PBBM facilitates the establishment of the essential in vitro-in vivo link by delineating a mechanistic understanding of the in vivo drug release and its interaction with the physiology. This level of understanding results in the construction of IVIVRs, offering a simpler and feasible path to biowaivers, especially for immediate release drug products for which the rate of success of IVIVCs is rather low. Safe space pillars are the IVIVC and IVIVR, thus, the safe space approach is governed by IVIVC/IVIVR principles. As such, for regulatory decision making, at least two release rates with corresponding Cp-time profiles are needed for its establishment. However, to support high risk CMC changes, at least three formulation variants should be used in its construction. For generic drug products, in addition to building the safe space around the target formulation, the Reference Listed Drug (RLD) should also be included. It should be noted that from the regulatory perspective, extrapolation outside the knowledge space for high-risk dosage forms, e.g., extended-release formulations and BCS class 2 or 4 compounds, is not recommended. During drug product development, however, the need for extrapolation is expected and constitutes a plausible and proven path for successful formulation selection.

In summary, safe space construction via the PBBM approach has the potential to expand the manufacturing and regulatory flexibility delineated under several regulatory frameworks such as BCS, IVIVC, and similarity testing.

The third and final lecture of the workshop was given by Prof Sebastian Polak, "3D Printing Combined with Biopredictive Dissolution and PBPK/PD Modeling for the Personalized Therapy Optimization - Are We There Yet?"

During his presentation, Prof Sebastian Polak discussed the potential of model-steered 3D printing combined with biopredictive dissolution and physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling for the need of personalized therapy optimization. Model-informed precision dosing (MIPD) is a concept suggesting utilization of model-based prediction methods for optimizing treatment benefit-to-harm balance based on individual characteristics of the patient, disease, treatment, and other factors. Theoretical workflow consisting of several elements – PBPK/PD models, 3D printed tablets with the model-proposed dose, information range and flow, and the place of a real patient was presented. The discussed example was based on the Parkinson's disease, which is a multisystem neurodegenerative condition that manifests itself through motor and non-motor symptoms including tremor, bradykinesia (slowing of motion and difficulty in initiating movement), and rigidity. This disease requires precise and variable therapy, which could potentially be supported by MIPD, but there are several obstacles inhibiting implementation. These include 3D printing method standardization, high throughput quality control dissolution testing, and others (*33*, *34*).

This last presentation was followed by a question-and-answer session.

The workshop ended with the closing remarks given by Prof Aleksander Mendyk. He thanked the speakers for the time and effort they put into their presentations, as well as the audience for attending the meeting and participating actively in the question and answer sessions. He also stressed the importance of exchanging ideas between academia and industry, which can positively influence cooperation between the two communities. Finally, Prof Mendyk expressed hope for other virtual meetings in the

future, which proved to be a great tool for exchanging experiences among participants and experts from around the world.

The 2-day virtual workshop was well received by the participants, who addressed the organizers with positive feedback after the conference.

CONFLICT OF INTERESTS

The authors have no disclosures related to this article.

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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 We are adapting a sampling system to our dissolution equipment that can be connected to a sampling robot. With this new system the probe remains inside the vessel during the entire test. We have more than 80 products that will be tested using this new system. What evaluations or qualifications do we need to perform in this situation?

A The suitability of this new system must be demonstrated for each one of the products. Drug adsorption, leachables, extractables, and the possible interference of having the sampling probe resident inside the vessel must be evaluated for each one of the products that are going to be analyzed with this system. It is possible that this system will not be suitable for some of the products. All these evaluations must be documented.

Q We are running a dissolution test of hypromellose capsules with 0.01 N HCl, pH 2.0, at 37 °C, and the dissolution is not complete in 15 minutes. We would like to know if we can add enzymes as stated in USP general chapter <711> Dissolution.

A No, the addition of enzymes as stated in <711> Dissolution is not likely to solve the issue. The enzymes described in <711> are proteases and are intended to be added to the dissolution medium when there is evidence of cross-linking in gelatin capsules. Since hypromellose is not a protein, it is a cellulose derivative, capsules made of this material may naturally take longer to dissolve. You may need to evaluate different dissolution times and agitation speeds in the dissolution test validation procedure.

Q For the preparation of simulated gastric fluid, can we use a different pepsin activity?

A Yes, you can use pepsin with any activity, you just need to weigh an amount to provide an activity of about the middle range of 800–2500 units per 1000 mL of dissolution media.

Q Should the linearity range for the validation of a dissolution method include the lowest point in the dissolution profile?

A The linearity range for the validation of dissolution method should include all points in the dissolution profile. The validated range should include the lowest expected level up to and including the upper limit of uniformity of dosage units.

Q Why was a new Prednisone tablet reference standard released?

A The release of the new USP Dissolution Performance Verification Standard – Prednisone RS is a part of USP's commitment to continuous enhancement of our products and services. The introduction of this new reference standard and the associated revisions to General Chapter <711> Dissolution are being recommended based on feedback from various USP stakeholders.

Q What is the difference between the new reference standard USP Dissolution Performance Verification Standard – Prednisone RS (catalog #1222818) and the current reference standard USP Prednisone Tablets RS (catalog #1559505)?

A Based on internal USP studies that have been performed, the new reference standard is considered more sensitive to operational and mechanical variables of instrument setup, less sensitive to media degassing, and more reproducible. The packaging configuration has also been changed. Each blister pack of six tablets is packaged in an aluminum sachet to provide additional protection against moisture.

Q Will the USP Prednisone Tablets RS be discontinued?

A Yes, the current reference standard USP Prednisone Tablets RS will be discontinued on or about April 28, 2023 in anticipation of the associated revisions to General Chapter <711> Dissolution becoming official on May 1, 2023.

Q Can I still use the current reference standard (USP Prednisone Tablets RS catalog #1559505) for PVT after the official date of the revised documentary standard?

A No, USP Prednisone Tablets RS (catalog #1559505) cannot be used to meet the requirements of apparatus suitability in General Chapter <711> Dissolution where the use of USP Dissolution Performance Verification Standard – Prednisone RS is specified.

Q Will USP provide a guidance documents and resources like the Dissolution Toolkit to help with mechanical calibration and PVT using the new reference standard?

A Yes, the updated guidance document "USP Guideline on Procedures for Mechanical Calibration and Performance Verification Test Apparatus 1 and Apparatus 2" is currently available on the PVT landing page https://www.usp.org/small-molecules/pvt and under the Compendial Tools section on the USP website at https://www.usp.org/resources/compendial-tools

Q If the new USP Dissolution Performance Verification Standard – Prednisone RS tablet has decreased sensitivity to degassing, then is degassing the media still required?

A Yes. The collaborative studies were conducted requiring the USP method for degassing media, so degassing media is still required to successfully complete the performance verification test.

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

Calendar of Events

May 23–24, 2023

Drug Dissolution in Oral Drug Absorption, Sponsored by M-CERSI

Location: University of Maryland School of Pharmacy, Pharmacy Hall, Baltimore, MD, USA
Registration will close May 12 at noon ET. www.pharmacy.umaryland.edu/dissolution2023

May 25, 2023

Dissolution Discussion Group Quarterly Online Meeting—Looking Ahead: The dissolution Lab of the future

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

June 5–July 28, 2023

University+ PBPK Summer Camp (Academic Affiliation Required for Registration)

Location: Online
Registration: https://www.simulations-plus.com/events/university-pbpk-summer-camp/

June 27, 2023

Population Simulation and Virtual Bioequivalence

Location: A Coruña, Spain
Registration: https://www.simulations-plus.com/events/population-simulation-virtual-bioequivalence-a-coruna-spain/

July 11, 2023

European Complimentary Introduction to GastroPlus® Workshop

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

July 24–28, 2023

Controlled Release Society 2023 Annual Meeting

Location: Las Vegas, NV, USA
For information, visit http://www.controlledreleasesociety.org/meetings/annual

July 27, 2023

Dissolution Discussion Group Quarterly Online Meeting—Go with Your Gut: A Biorelevant Dissolution Media Discussion

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

August 29–31, 2023

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/

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

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

On Demand Events

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/

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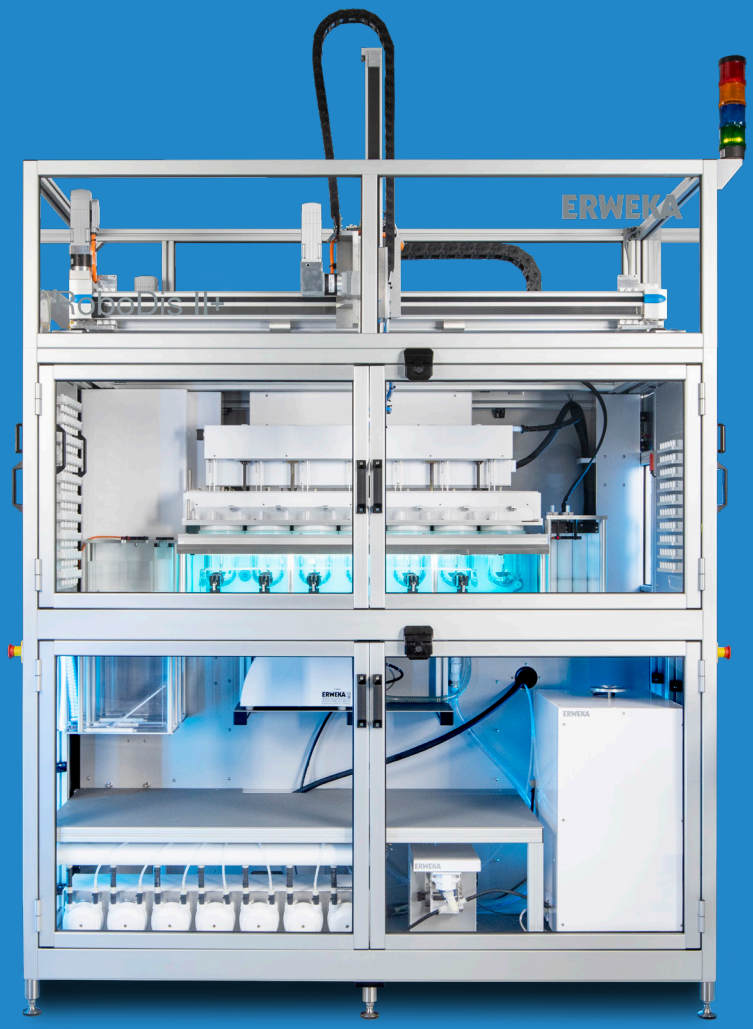

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Application of PERMETRO in Bioequivalence and IVIVC

The Logan PERMETRO™ system consists of a proprietary dynamic dissolution-permeation apparatus and novel artificial intestinal membrane. PERMETRO was developed to overcome the limitations and bottlenecks of traditional IVIVC permeation testing by improving prediction of particle engineering, reformulation effects, and bioavailability enhancement.

PERMETRO System Applications:

- OSD R&D and QC to assist formulation screening
- Provide data support before in vivo BE experiments
- Generic vs. original comparison studies
- IVIVC and IVIVC model development
- Gastrointestinal absorption of fasted/meal medication
- GIT absorption differences of CR formulations in different modes of administration, e.g., water, alcohol

Current dissolution tests do not monitor drug permeation. Typical pre-BE tests require a lot of resources, and there are ethical issues. To overcome these problems, PERMETRO studies the dissolution curves and permeability comparison of generic vs. original drugs. It can predict and increase the success rate of BE experiments and reduce the requirement for in vivo studies.

The integrated PERMETRO system includes:

- Logan dynamic permeation cells
- PermeaPad™ - Logan's novel artificial intestinal membrane
- A unique dual-pump design that integrates the dissolution sampling pump and permeation pump
- Automatic collection of permeation test samples at all time points
- Dry heating for convenient cleaning and support for online HPLC analysis
- Online HPLC analysis

1. Dissolution-Permeation Studies

PERMETRO makes simultaneous in vitro dissolution and permeability testing possible and more representative of the in vivo state. The knowledge gained saves valuable research time and expense in vivo testing. Drug permeation can be tested completely in vitro.

The in vitro bioequivalence prediction system can use artificial intestinal membranes to simulate the release and absorption process of drugs from the stomach to the intestine. The device is compatible with USP methods 1-7, and the backend can be used with UV or HPLC analysis systems.

2. Branded vs. Generic BE Studies

Although the dissolution profiles of generic and reference APIs may be the same, the dissolution rates of excipients that affect permeability can be different. Excipients such as SLS can change the permeability of the gastrointestinal tract, so the absorption rates of the drugs are different, which may easily lead to BE failure.

For more information about PERMETRO, contact infoDT@loganinstruments.com

Simulations Plus and Global Agrochemicals Leader to Collaborate on Machine Learning Models

Data sharing partnership will expand chemical coverage space and improve model performance in support of new approach methodologies to ensure product safety

LANCASTER, CA: Simulations Plus, Inc. (Nasdaq: SLP), a leading provider of modeling and simulation software and services for pharmaceutical safety and efficacy, announced it has entered a new collaboration with a large agrochemicals company to extend the industry's top-rated machine learning model for the prediction of ionization constants (pK_a) in the ADMET Predictor® platform.

The team at Simulations Plus will use the partner company's proprietary measurements, drawn from its vast internal databases, to build and refine its predictive model that can accurately predict pKa values of various chemical compounds. Additionally, Simulations Plus will create and evaluate new algorithms and techniques to further enhance the predictive capabilities of the model.

Dr. Robert Fraczkiewicz, Research Fellow and Project Lead, said: "The importance of this new partnership cannot be overstated, as the outcomes will help improve the accuracy of predictions and greatly expand the chemical coverage space that can be accurately analyzed. In turn, this should help drive next generation safety assessment strategies using ADMET Predictor and GastroPlus®. This is especially important for the chemicals, cosmetics, and consumer goods industries, as global regulations have restricted the use of animal testing. Data sharing collaborations between organizations are becoming increasingly valuable in the advancement of machine learning and its applications, and our team is dedicated to providing our partners with reliable, secure, and efficient models and workflows that help them succeed."

"The utilization of ADMET Predictor within 'non-pharmaceutical' markets has been growing, and this collaboration with one of the most innovative companies in this space should help accelerate its adoption," added Dr. Eric Jamois, Senior Director for Key Accounts and Strategic Alliances. "By combining our advanced property prediction technologies with the data and expertise of our partner, we can achieve more accurate and reliable results. This will help to ensure new chemical products are developed with the highest level of safety. Improvements made to ADMET Predictor will be made available for all clients to apply to their research activities. Simulations Plus continues to invite future collaborations which benefit all user groups and, most importantly, the global communities that we serve."

Simulations Plus Enters New Strategic Collaboration to Discover Anticancer Therapies Through Its AI-Driven Drug Design Technology

Drug discovery services partnership with Sino-American Cancer Foundation focuses on the development of actionable hits against the MTHFD2 target

LANCASTER, CA: Simulations Plus, Inc. (Nasdaq: SLP), a leading provider of modeling and simulation software and services for pharmaceutical safety and efficacy, announced that it established a strategic research collaboration with the Sino-American Cancer Foundation (SACF). This collaboration will leverage Simulations Plus' staff and Artificial Intelligence-driven Drug Design (AIDD) technology in the ADMET Predictor® software platform to support the discovery and design of novel inhibitors of methylenetetrahydrofolate dehydrogenase 2 (MTHFD2), an emerging cancer target.

Per the terms of the collaboration, Simulations Plus will develop quantitative structure-activity relationship (QSAR) models for efficacy against MTHFD2, using information from SACF as well as academic and patent literature. The biologists and computational and medicinal chemists in the Early Drug Discovery Services team at Simulations Plus will work with the researchers at SACF to define the multi-objective parameters against which the lead molecule(s) will be optimized. The new AIDD Module in ADMET Predictor® will then be employed to generate libraries of virtual compounds that are optimized for potency and other chosen parameters. The teams will select promising candidates for synthesis and testing, and ensuing rounds of QSAR model building and AIDD optimization will be performed until the milestone criteria in the collaboration agreement are achieved.

"We value the trust and confidence SACF has in our team and AI technology to complement and accelerate conventional drug design and lead optimization processes," said Dr. Jeremy Jones, Principal Scientist at Simulations Plus and project lead. "By combining their drug discovery expertise with our algorithmic and data science know-how, we are confident we will successfully support their hit-to-lead target development activities."

As part of this agreement, SACF will provide upfront funding to Simulations Plus to design a set number of compounds for efficacy against MTHFD2 which will be exclusive to SACF. Subsequent milestone payments will be made as key research and development goals are met.

"SACF has had an active interest in AI for de novo design for some time, and we have found a trusted partner in Simulations Plus to help us integrate this into our research," added Dr. Frank Luh, CEO of SACF. "Scientists from the two organizations will work side-by-side to combine SACF's data with all that Simulations Plus offers to identify the next generation of compounds that could help in the treatment of cancer."

John DiBella, SLP Division President, said, "The Early Drug Discovery Services offering at Simulations Plus is tailor made for this type of partnership, where our team of experts, including computational, medicinal, and cheminformatics specialists, provide end-to-end AI-driven drug discovery and optimization support to complement the SACF team. Simulations Plus continues to invite future collaborations which benefit organizations and, most importantly, the global communities we serve."

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Save time. Instant feedback helps the user investigate aberrant results or abnormalities at an early stage and reduce errors.

For more information about the Agilent 280-DS, visit:
www.agilent.com/chem/280-DS

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