dx.doi.org/10.14227/DT300223P66

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

Jeovanis Gil[1], Johan Malm[1], and György Marko-Varga[2*]
[1]*Section for Clinical Chemistry, Department of Translational Medicine, Lund University, Skåne University Hospital Malmö, Malmö, Sweden.*
[2]*Clinical Protein Science & Imaging, Biomedical Centre, Department of Biomedical Engineering, Lund University, Lund, Sweden.*

email: gyorgy.marko-varga@bme.lth.se

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.

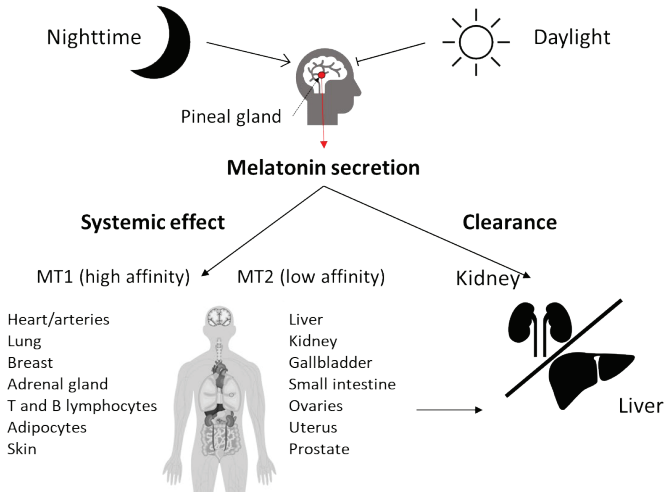


Figure 1. Melatonin secretion is regulated by daylight; Its secretion increases during sleep and decreases when eyes receive light from the sun. Activating MT1 (high affinity) and MT2 (low affinity) receptors melatonin has systemic effects and melatonin is subsequently cleared from the circulation by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

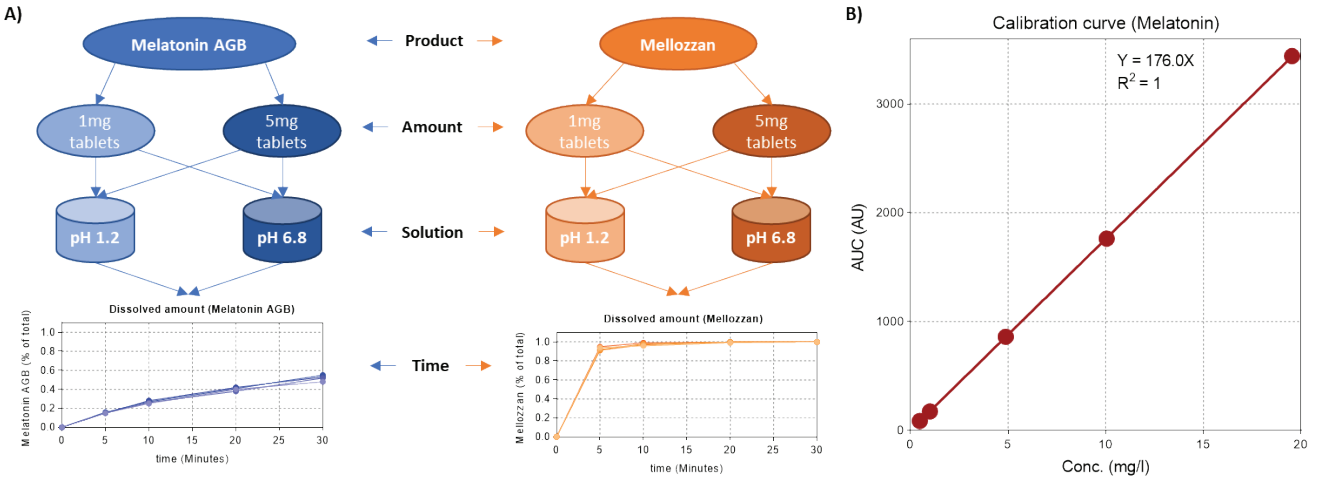
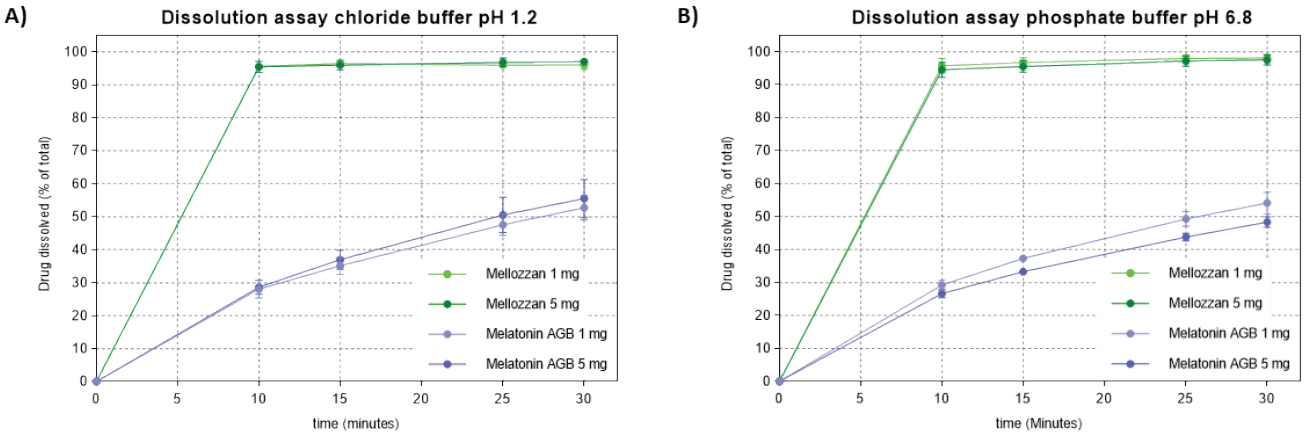


*Figure 2. Experimental design to assess the dissolution properties of Mellozzan and Melatonin AGB. (**A**) Tablets containing 1 or 5 mg of each product were submitted to a dissolution process in a pH 1.2 or pH 6.8 buffer. The cumulative amount of dissolved product was quantified every 5 mins until 30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

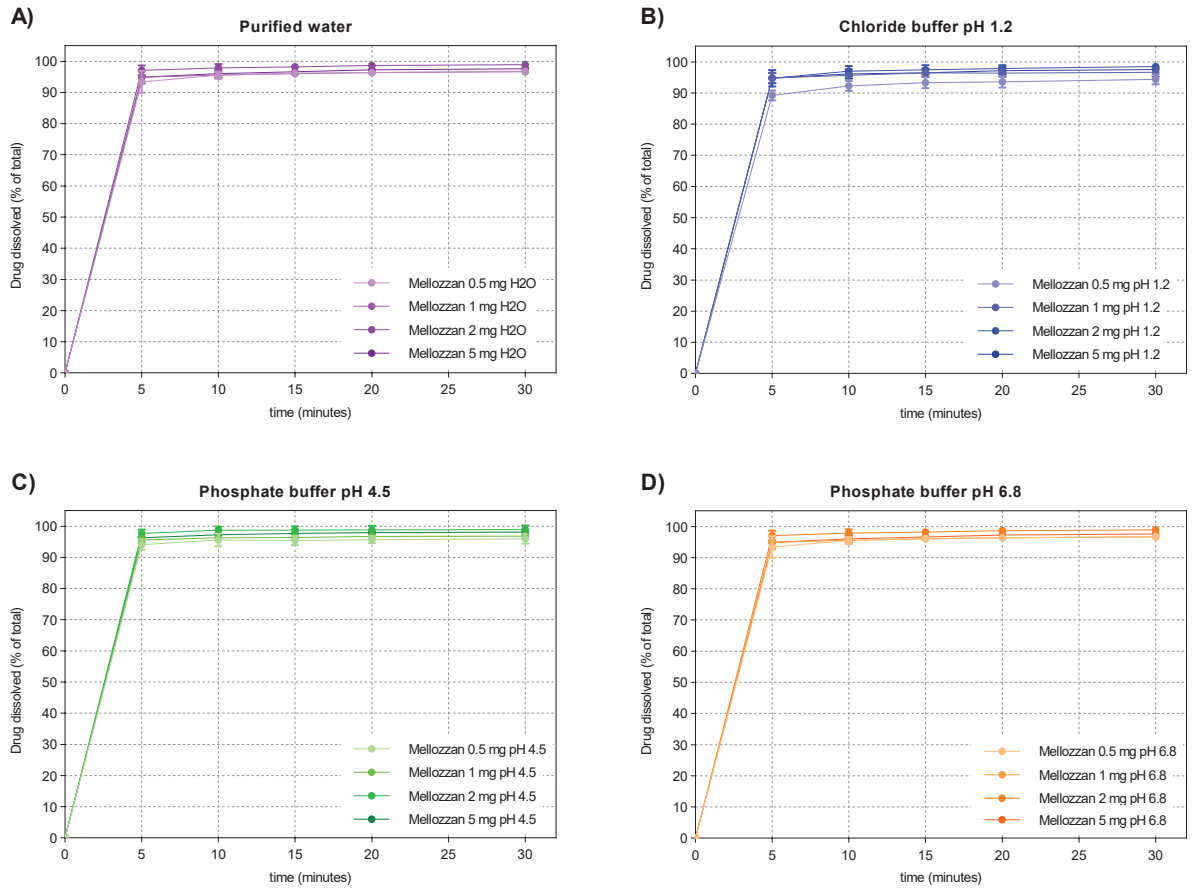


*Figure 4. Dissolution assay of Mellozzan tablets of different strengths in different conditions: (**A**) Milli-Q water, (**B**) chloride buffer pH 1.2, (**C**) phosphate buffer pH 4.5, (**D**) phosphate buffer pH 6.8.*

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

This study was supported by EQL Pharma AB, Lund, Sweden.

CONFLICTS OF INTEREST

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

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