Chapter

Per Os Administered Modified-Release Solid Formulations of Melatonin: A Review of the Latest Developments Including the Design of Experiments (DoE) Approach

Angeliki Siamidi and Yannis Dotsikas

Abstract

The pineal hormone melatonin (MLT) is a derivative of the amino acid *L*-tryptophan and controls the circadian diurnal rhythm and the seasonal biorhythm. Exogenous administration is aimed at alleviating sleep-related dysfunctions and jet lag, as it decreases sleep-onset latency, increases total sleep time and improves overall sleep quality. Besides these indications, MLT has been shown to have other actions, such as antioxidant, immune enhancement and anticancer. It has also been shown to be useful against cardiovascular, neurological and psychiatric diseases. In the context of this work, a review of the related literature on the modified release of MLT from its *per os* administered formulations is presented, including the utilization of the design of experiments (DoE) for the selection of the optimal composition of melatonin formulations. The chapter offers an account of the recent advantages on MLT's solid dosage forms suitable for treating sleep disorders, referring either to its onset or maintenance.

Keywords: melatonin, circadian rhythm, sleep disorders, modified release, oral solid dosage forms, *per os* administration, experimental design

1. Introduction

Melatonin (MLT) is an indole amide hormone produced by the pineal gland, especially at night time and is mainly involved in the regulation of circadian and circannual rhythms. For clinical purposes (including Alzheimer's disease, insomnia, stroke, depression, Parkinson's disease, migraine, headache, etc.), exogenous MLT could be administered for alleviation of the symptoms. Irrespective of the pathological case, MLT's exogenous administration should mimic the typical nocturnal endogenous MLT levels. The release profile of these drug delivery systems should be in a controlled manner, due to MLT's short half-life of elimination and low bioavailability.

The development of novel pharmaceutical formulations with the optimal release profile is of great importance for cases like melatonin. To this purpose, a series of experiments should be performed, utilizing various excipients at different ratios. These trials could require plenty of working hours, with no guarantee that, indeed, the optimal formulation composition will be reached. Therefore, the employment of a statistical/chemometric approach, such as design of experiments (DoE), can be beneficial for complicated and demanding tasks like the development of modified-release formulations with a minimum number of experiments.

2. Advances on the melatonin *per os* administered modified-release solid formulations

Scientific research has been conducted for the purpose of MLT evaluation not only towards medicine and clinical evaluation but also towards pharmaceutics [1–3]. Many researchers have studied and managed to produce immediate-release formulations of MLT (tablets, creams, sublingual sprays, nasal preparations, injectables, etc.), in order to facilitate sleep-onset problems. MLT modified-release formulations are clinically more useful in initiating and maintaining sleep in elderly insomniacs than those designed for immediate release. More in particular, the sustained-release dosage form which delivers MLT in a time period over 8 h is of clinical value for those who have disordered circadian rhythms [4, 5]. Therefore, the modified release of MLT from oral solid dosage forms that alter the time and/or the rate of MLT release may provide an alternative for MLT delivery and be useful in the treatment of circadian rhythmic disorders, like insomnia, jet lag, seasonal affective disease, shift work syndrome, etc. [6]. The recent advantages of MLT modified-release *per oral* solid formulations are reviewed below and summarized in **Table 1**.

Aiming at this mode of release of MLT, a series of hydrophilic matrix tablets has been prepared and tested in vitro. The tablets comprised of combinations of excipients (hydroxypropyl methylcellulose K 15 M (HPMC), low-viscosity sodium alginate, Avicel PH 102, etc.) and a variety of cyclodextrins (MLT (guest)-cyclodextrin (host) complexes in 1:1 ratio). The release studies that were performed in two dissolution media (acidic pH 1.2 and basic pH 7.4) suggested that melatonin was released faster from the MLT-cyclodextrin complexes than from the matrix systems possibly due to their increased solubility [7]. In another investigation, a rather unexploited biomaterial for applications in the design of drug delivery systems, the algal sulphated polysaccharide ulvan was used as an excipient in MLT solid dosage forms. The dissolution tests showed that the MLT release from the ulvan-based tablets followed a sigmoidal pattern, which denoted that the drug release is controlled by polymer relaxation and/or erosion [8]. In a similar study, hydrophilic matrix tablets with various excipients (hydroxypropyl methylcellulose K15 M, low-viscosity sodium alginate, lactose monohydrate and polyvinylpyrrolidone M.W.: 10.000 and 55.000) were developed and tested in vitro at two dissolution media (pH 1.2 and 7.4) in order to examine the modified-release characteristics of MLT. The objective was to produce a formulation with a quick initial pace, aiming at a satisfactory sleep onset, followed by a prolonged release that could target poor sleep quality problems. The dissolution results indicated that the combination of the excipients with different physicochemical properties could alter the release of MLT from solid matrix systems [9].

Moreover, researchers have developed matrix tablets comprised of common hydrogels (hydroxypropyl methylcellulose and dextran) to study the influence on the release profile of MLT in vitro and liposomes (of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine and dipalmitoylphosphatidylglycerol) incorporating the hormone in order to compare

Drug release behaviour	Delivery system	Excipient (s)	Reference
Modified	Matrix tablets	HPMC K15 M, MCC, sodium alginate and various cyclodextrins	[7]
		Ulvan, HPMC K15 M, low-viscosity sodium alginate, LM, PVP	[8]
		PVP (M.W.: 10.000 and 55.000), sodium alginate (low viscosity 2%), HPMC K15 M, dextran, MCC, LM	[9]
		Dextran, MCC, HPMC K15 M, MCC, LM	[10],
	Liposomes	DPPC, DPPG	[10]
	Nanofibrous electrospun mats incorporated into monolayered and three-layered tablets	PVP (M.W.: 1.300.000), CA (M.W.: 50.000), HPMC K15 M, LM	[11]
	Electrospun nanofibres in capsules	PVP (M.W.: 1.300.000), CA (M.W.: 50.000), hypromellose 2910, PEO (M.W. 900.000 and 400.000)	[12]
	Calcium alginate beads in hard gelatin capsules		[13]
Slow	Matrix tablets	HPMC, Carbopol 971P, MCC, maize starch	[14]
Sustained	Matrix tablets	HPMC, Avicel, Primojel, Ac-Di-Sol, Polyplasdone, Mg stearate, Talc, Cab-O-Sil	[15]
	Solid lipid nanoparticles in hard gelatin capsules	Stearic acid, Epikuron 200, lactose	[16]
Controlled	Matrix tablets	HPMCK15 M, low-viscosity sodium alginate, LM, PVP (M.W.: 10.000 or 55.000)	[17]
	Beads	Sodium alginate, Eudragit® RS100, aluminum tristearate, polyethylene, glycol 400, liquid paraffin	[18]
Delayed	Compression-coated tablets	Dextran, PVP(M.W.: 10.000), ethyl cellulose (45cps), Avicel PH 102, LM, sodium alginate	[19]
Biphasic	Matrix tablets	Dextran, PVP (10.000), ethyl cellulose (45cps), MCC, LM, sodium alginate	[19]
Immediate and sustained	Bilayer tablets	Dextran, ethyl cellulose (45cps), MCC, LM, sodium alginate	[19]
		MT-b-CD, HPMC, Carbopol 971P, MCC, maize starch	[20]
Immediate and controlled	Coated beads in hard gelatin capsule	Core sugar spheres, Aquacoat®, dibutyl sebacate, triethyl citrate, PVP, HPMC	[20]

HPMC: Hydroxypropyl methylcellulose, MCC: Microcrystalline cellulose (avicel PH 102), PVP: Polyvinylpyrrolidone, PEO: Polyethylene oxide, CA: Cellulose acetate, LM: Lactose monohydrate, DPPC: 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, DPPG: Dipalmitoyl-phosphatidylglycerol.

Table 1.
An overview of the recent advantages of MLT modified-release per oral *solid formulations.*

their release profiles. The results indicated that both formulations (liposomal and solid matrix tablets) could be suitable alternatives for treating sleep-onset/maintenance problems [10].

Once more, aiming at the modified release of MLT, another group of researchers studied the MLT release from monolayered and three-layered tablets, incorporating nanofibrous mats composed of cellulose acetate and polyvinylpyrrolidone. The in vitro dissolution release studies of the MLT formulations in simulated gastrointestinal fluids revealed tableting pressure and pH dependence. Comparing the MLT release from the physical mixture tablets and from the nanofibre-based tablets, it was concluded that the release profile was generally slower than the latter, rendering the formulation suitable for both sleep-onset and maintenance dysfunctions [11]. The same group of researchers produced electrospun-MLT loaded nanofibres (with cellulose acetate, polyvinylpyrrolidone and hydroxypropyl methylcellulose, as excipients) and used them to fill hard gelatin and delayed-release (DRcaps™) capsules. The in vitro dissolution results revealed a modified-release profile of MLT from the fabricated matrices in gastrointestinal-like fluids and suggested that the MLT-loaded nanofibrous mats could exhibit a promising profile for treating sleep problems [12].

Calcium alginate beads were also prepared to investigate the MLT modified release. Excipients utilized in their preparation included calcium alginate, polyvinylpyrrolidone (M.W.: 10.000 and 55.000), hydroxypropyl methylcellulose (M.W.: 15.000 and 100.000), lactose monohydrate and, as a surfactant, sodium lauryl sulphate. The in vitro release of melatonin was investigated at two different pHs (acidic pH 1.2 and basic pH 6.8), and the results concluded that the hormone's release from the beads was reversibly proportional to the extent of their expansion, which depends on the molecular weight/viscosity of the biopolymers present in the beads; the higher the molecular weight/viscosity of the hydrogels, the greater the beads swelling and the less the MLT's release [13].

Another group of researchers prepared a slow-release tablet of MLT with varying quantities of hydroxypropyl methylcellulose K15 M and Carbopol 971P, as well as other excipients (microcrystalline cellulose, maize starch, magnesium stearate and purified talc). The formulations developed showed a slow release of MLT during an 8 h period [14].

To the same end, matrix tablets were formulated using hydroxypropyl methylcellulose and tested in vitro in relation to drug release, as a function of polymer viscosity, drug loading, type and amount of disintegrant, lubricant and glidant and aqueous polymeric coating level, and further compared with two commercial products. The release studies showed that as the polymer viscosity increased, the release decreased, and as the coating level increased, an increased lag time was observed [15]. Other researchers have examined the in vivo sustained release of MLT that was incorporated in solid lipid nanoparticles. The results indicated that solid lipid nanoparticles may act as a reservoir, permitting a constant and prolonged MLT release, after oral administration, which may indicate new possibilities for sustained delivery systems [16].

In another research project, controlled-release matrix tablets of MLT were developed by the use of a computer programme, D-optimal experimental design, aiming at affecting its modified release at simulated gastrointestinal media. The careful selection of the excipients (polyvinylpyrrolidone (M.W.: 10.000 and 55.000), hydroxypropyl methylcellulose K15 M and lactose monohydrate) at their appropriate quantity resulted to the optimal solution and the controlled release of melatonin with the minimal number of experiments [17]. Moreover, in another research, polymer-reinforced and polymer-coated alginate beads with various concentrations of polymer (Eudragit® RSI00) and plasticizer (aluminum tristearate)

were produced and evaluated in vitro in relation to their controlled-release characteristics as an alternative for oral delivery of MLT. The results indicated that the polymeric reinforcement offered an initial burst release in intestinal fluids, while the coating led to release retardation in both gastric and intestinal fluids. The results also showed that as the polymer concentration increased, the MLT release decreased in the intestinal fluids, due to coated alginate beads disintegration [18].

Researchers have also probed the MLT release from matrix and compression-coated tablets that were comprised of combinations of ethyl cellulose, polyvinylpyrrolidone, dextran, low-viscosity sodium alginate, Avicel PH 102 and lactose monohydrate. The results obtained revealed that the initial release of melatonin was more delayed from coated tablets than from the respective uncoated. The matrix tablets showed an initial fast release that followed a sustained mode, demonstrating that the use of various excipients results to different controlled-release behaviors [19].

Also, bilayered tablets incorporating an immediate-release layer and a sustained-release layer were developed by the same group of researchers using as excipients ethyl cellulose, dextran, low-viscosity sodium alginate, Avicel PH 102, lactose monohydrate, iron oxide pigment red 30 and magnesium stearate. The dissolution results revealed immediate and sustained drug release [19]. Another group of researchers also prepared bilayered tablets of MLT incorporating an immediate-release part consisting of MLT-*b*-cyclodextrin inclusion complex and a sustained-release part containing MLT in hydroxypropyl methylcellulose K15 M and Carbopol 971P. The results showed an initial burst followed by a near zero-order release pattern for a period of 8 h [20].

Furthermore, scientists have designed an MLT oral formulation to provide immediate and controlled release, which consisted of MLT-loaded sugar beads coated with 20% Aquacoat®. The in vivo results showed average peak plasma concentration at about 600 pg/ml that maintained at approximately 100 pg/ml over 8 h, indicating biphasic release [20].

Researchers have also utilized principles of nanotechnology to make micro–/nanoparticles containing MLT that could be further formulated to solid *per os* modified-release formulations. Thus, MLT was loaded in poly(*D*,*L*-lactide-co-glycolide) nanoparticles and microparticles (diameter of 200 nm and 3.5 mm, respectively). The cumulative release curves for nano- and microparticles revealed that for PLGA nano-10 and PLGA nano-20, approximately 30 and 20% of melatonin were released, respectively, within the first 24 h, as due to the diffusion of melatonin molecules located closer to the particle surface. At the end of 40 days, approximately 65% of the loaded melatonin was released from PLGA nanoparticles by diffusion mechanism [21]. Similarly, MLT was encapsulated into poly(lactic-co-glycolic acid) microspheres, and the release results indicated a dual pattern: a low initial burst release (around 40%) after the first 3 days and a relatively prolonged release over 25 days (around 85% of total MLT release) [22]. Furthermore, scientists have prepared MLT nanocapsules with Eudragit® S100. This formulation revealed a modified-release profile, which when fitted to a monoexponential model revealed that the MLT release mechanism was controlled by swelling and dissolution of the polymer [23].

3. Employment of DoE for the development of novel MLT modified-release formulations

In the vast majority of experimental procedures in all scientific fields, the optimal conditions are reached by modifying the levels of one factor at a time (OFAT) while keeping all the rest that seem to affect the response constant.

This classical strategy usually requires a large number of experimental runs and subsequent working hours. However, it ignores any potential interactions among the factors, and this is a major drawback, as it may result in an eventually ineffective procedure. On the contrary, a more organized way of conducting experiments, based on statistics, could be cost-effective and time-saving and also enable reaching the real optimal conditions. There are various chemometric approaches, but the most suitable for optimization of a procedure is the design of experiments.

DoE has been applied in many fields, including pharmaceutical product development [24–28]. It is gaining an increasing interest among pharmaceutical researchers, as more and more are becoming familiar with this approach, due to the relatively recent requirement for quality-by-design (QbD) principles. Furthermore, DoE has proven its usefulness in a variety of pharmaceutical applications in this field. Its major advantage has to do with obtaining the optimal conditions among factors for the desired values of responses by conducting a small number of experiments. That way DoE can resolve problems in complex systems, which cannot be easily managed by the trial-and-error approach.

Among the various types of designs like (fractional) factorial, Box–Behnken, central composite design (CCD), etc., the D-optimal design has been established as a robust design strategy. It enables the assessment of both numerical and categorical factors [29], and regarding numerical factors, the latter are examined at many different levels (design matrix), and not at 3–5, as the more classical designs. These levels are generated automatically by computer algorithms from relevant softwares, in order to satisfy the D-optimality criterion, aiming to minimize the generalized variance of the estimated regression coefficients without increasing the total number of experimental runs.

Such design was employed in the study presented by Vlachou et al. [17] regarding MLT controlled-release matrix formulations. One categorical factor, namely, the M.W. of polyvinylpyrrolidone (PVP), was chosen (M.W.: 10.000, low, and 55.000, high), and two numerical factors, namely, the mass (mg) of PVP and hydroxypropyl methylcellulose K15 M, were selected. When a modified release is the aim, as in the current study, setting the right responses is very critical. Herein, the need for a fully release melatonin in a controlled manner within 8 h was the reason for setting as responses the time for 50% drug dissolution at pH = 1.2 and the diffusional exponent (n) at pH values 1.2 and 7.4. Initially, a quick melatonin's release is needed for treating sleep-onset problems, while its subsequent slow release is needed to improve sleep quality and/or to assist maintain sleep. Therefore, T50% (pH: 1.2) should be ≤150 min, so that an initial dose will be released to aid the sleep onset of patients, and n (pH: 1.2) = 0.89, in order to achieve zero-order release kinetics and Case II diffusion, and n (pH: 7.4) = 0.80 for first-order release kinetics and anomalous diffusion.

The experiments were conducted as suggested by the experimental plan of Design-Expert software, and then suitable quadratic models were obtained for all (3) responses, satisfying all statistical criteria (ANOVA test, lack-of-fit test, R^2, *adj.* R^2 and *pred.* R^2 values). The next step was to estimate the overall optimal conditions, and thus Derringer's desirability function was employed [30], taking into account the necessity for simultaneous optimization of the aforementioned objectives/responses. Desirability function is a tool that is usually included in experimental design softwares and therefore very useful for projects in pharmaceutical development. Each predicted response $\hat{Y}i$ and experimentally obtained response Yi can be transformed to a desirability function di. The latter can have a value from 0 to 1, where $di = 0$ represents completely undesirable response and $di = 1$ represents completely desirable or ideal response. The individual desirability scores di can then

be combined on a single overall (global) desirability D, which is optimized to find the optimum set of input variables:

$$D = (d_1 \times d_2 \times \ldots \times d_n)^{\frac{1}{n}} \quad (1)$$

with n denoting the number of responses.

In order to reach to optimal solution, the importance of responses should be set by adjusting the importance coefficients. T50% (pH, 1.2) was set as the most important response for consideration, while the rest two were of equal importance. Furthermore, weights (which denote the desired trend of the response within itself) and the range of responses could be changed, according to defined objectives. The optimal solution was reached with a value of global desirability of 0.907, which can be considered as very satisfactory. The suggested solution was performed, and the obtained results were in agreement with the goals defined for the responses and the predictions of the software. Consequently, with just 17 experiments defined by Design-Expert software and few preliminary in order to set the limits of the factors for the experimental plan, a novel MLT modified-release oral solid dosage form was developed.

To the best of our knowledge, the previous study was the only attempt to develop novel and improved MLT formulations by utilizing DoE. There has been a previous study [31] in which a different chemometric tool, artificial neural networks (ANN), was utilized. In that study, researchers prepared 27 different tablet formulations with different amounts of hydroxypropyl methylcellulose, xanthan gum and Carbopol® 974P NF. These formulations were subjected to drug release studies, using dissolution test data as inputs for ANN. The authors suggest that ANN with nine neurons in the hidden layer had the best results, meaning that it could predict, after training, dissolution data. In other words, this was a completely different strategy, based on training of the network and prediction of response values for novel (but not very different from training data) excipient mixtures. The optimal solution may not be reached, as the new mixtures are suggested by the user (trial and error) and then tested by the software. On the contrary, DoE is a tool for optimization of a procedure and not prediction of response values, and therefore it is recommended for application in such projects.

4. Conclusions

This analysis aims at the review of the latest advances of MLT modified-release oral solid dosage forms including the design of experiments approach. Many scientists have focused on the different ways in manufacturing modified-release oral solid formulations by using various excipients, dosage forms (multilayer or bilayer, coated or uncoated tablets), liposomes, alginate beads, nanofibre mats and nano–/microparticles, or by employing a variety of techniques (i.e. dry coating, electrospinning, experimental design, etc.) in order to gain knowledge for the production of such dosage forms.

Conflict of interest

The authors declare no conflict of interest.

Author details

Angeliki Siamidi[1*] and Yannis Dotsikas[2]

1 School of Health Sciences, Department of Pharmacy, Division of Pharmaceutical Technology, National and Kapodistrian University of Athens, Athens, Greece

2 School of Health Sciences, Department of Pharmacy, Division of Pharmaceutical Chemistry, National and Kapodistrian University of Athens, Athens, Greece

*Address all correspondence to: asiamidi@pharm.uoa.gr

IntechOpen

References

[1] Vlachou M, Eikosipentaki A, Xenogiorgis V. Pineal hormone melatonin: Solubilization studies in model aqueous gastrointestinal environments. Current Drug Delivery. 2006;**3**:255-265. DOI: 10.2174/156720106777731073

[2] Vlachou M, Tsiakoulia A, Eikosipentaki A. Controlled release of the pineal hormone melatonin from hydroxypropylmethylcellulose/sodium alginate matrices in aqueous media containing dioctyl sulfosuccinate. Current Drug Discovery Technologies. 2007;**4**:31-38. DOI: 10.2174/157016307781115449

[3] Vlachou M, Ioannidou V, Vertzoni M, Tsotinis A, Afroudakis P, Sugden D. Controlled release from solid pharmaceutical formulations of two nalkanoyl-4-methoxybicyclo[4.2.0] octa-1,3,5-trien-7-ethanamines with melatoninergic activity. Letters in Drug Design & Discovery. 2015;**12**:259-262. DOI: 10.2174/15701808116661410240005226

[4] Zisapel N. Development of a melatonin-based formulation for the treatment of insomnia in the elderly. Drug Development Research. 2000;**50**(3-4):226-234. DOI: 10.1002/1098-2299(200007/08)50:3/4<226::aid-ddr6>3.0.co;2-s

[5] Lyseng-Williamson KA. Melatonin prolonged release: In the treatment of insomnia in patients aged ≥55 years. Drugs and Aging. 2012;**29**(11):911-923. DOI: 10.1007/s40266-012-0018-z

[6] Vlachou M, Siamidi A. Chapter 9: Melatonin modified release formulations designed for sleep disorders. In: Drăgoi MC, editor. Melatonin - Molecular Biology, Clinical and Pharmaceutical Approaches. Rijeka. ISBN 978-1-78984-505-1: InTechOpen; 2018. DOI: 10.5772/intechopen.78337

[7] Vlachou M, Papamichael M, Siamidi A, Fragouli I, Afroudakis PA, Kompogennitaki R, et al. Comparative in vitro controlled release studies on the chronobiotic hormone melatonin from cyclodextrins-containing matrices and cyclodextrin: Melatonin complexes. International Journal of Molecular Sciences. 2017;**18**:1641. DOI: 10.3390/ijms18081641

[8] Vlachou M, Tragou K, Siamidi A, Kikionis S, Chatzianagnostou AL, Mitsopoulos A, et al. Modified in vitro release of the chronobiotic hormone melatonin from matrix tablets based on the marine sulfated polysaccharide ulvan. Journal of Drug Delivery Science and Technology. 2018;**44**:41-48. DOI: 10.1016/j.jddst.2017.11.019

[9] Vlachou M, Stavrou G, Siamidi A, Flitouri S, Ioannidou V, Mavrokordopoulos S. N-acetylserotonin vs melatonin. In vitro controlled release from hydrophilic matrix tablets. Letters in Drug Design & Discovery. 2018;**15**:347-352. DOI: 10.2174/1570180815666180404125519

[10] Zampakola A, Siamidi A, Pippa N, Demetzos C, Vlachou M. Chronobiotic hormone melatonin: Comparative in vitro release studies from matrix tablets and liposomal formulations. Letters in Drug Design & Discovery. 2017;**14**(4):476-480. DOI: 10.2174/1570180813666161006162246

[11] Vlachou M, Kikionis S, Siamidi A, Tragou K, Ioannou E, Roussis V, et al. Modified in vitro release of melatonin loaded in nanofibrous electrospun mats incorporated into mono-layered and three-layered tablets. Journal of Pharmaceutical Sciences. 2019;**108**(2):970-976. DOI: 10.1016/j.xphs.2018.09.035

[12] Vlachou M, Kikionis S, Siamidi A, Tragou K, Kapoti S, Ioannou E, et al.

Fabrication and characterization of electrospun nanofibers for the modified release of the chronobiotic hormone melatonin. Current Drug Delivery. 2019;**16**(1):79-85. DOI: 10.2174/1567201815666180914095701

[13] Vlachou M, Siamidi A, Goula E, Georgas P, Pippa N, Sentoukas T, et al. Probing the release of the chronobiotic hormone melatonin from hybrid calcium alginate hydrogel beads. Acta Pharmaceutica. 2020:**70**. DOI: 10.2478/acph-2020-0037

[14] Kumar A, Agarwal SP, Khanna R. Modified release bi-layered tablet of melatonin using b-cyclodextrin. Die Pharmazie. 2003;**58**(9):642-644

[15] Lee BJ, Ryu SG, Cui JH. Formulation and release characteristics of hydroxypropyl methylcellulose matrix tablet containing melatonin. Drug Development and Industrial Pharmacy. 1999;**25**(4):493-501. DOI: 10.1081/DDC-100102199

[16] Priano L, Esposti D, Esposti R, Castagna G, De Medici C, Fraschini F, et al. Solid lipid nanoparticles incorporating melatonin as new model for sustained oral and transdermal delivery systems. Journal of Nanoscience and Nanotechnology. 2007;**7**:3596-3601. DOI: 10.1166/jnn.2007.809

[17] Vlachou M, Siamidi A, Konstantinidou S, Dotsikas Y. Optimization of controlled release matrix formulation of melatonin via experimental design. The Journal of Pharmaceutics and Drug Delivery Research. 2016;**5**(6):1-5. DOI: 10.4172/2325-9604.1000159

[18] Lee BJ, Min GH. Oral controlled release of melatonin using polymer-reinforced and coated alginate beads. International Journal of Pharmaceutics. 1996;**144**(1):37-46. DOI: 10.1016/S0378-5173(96)04723-0

[19] Vlachou M, Siamidi A, Pareli I, Zampakola A, Konstantinidou S. An account of modified release of melatonin from mono- and bi-layer solid pharmaceutical formulations. Journal of Pharmaceutical Sciences. 2016;**1**(4):10-14. DOI: 10.24218/vjpps.2016.19

[20] Lee BJ, Parrott KA, Ayres JW, Sack RL. Design and evaluation of an oral controlled release delivery system for melatonin in human subjects. The International Journal of Pharmaceutics. 1995;**124**(1):119-127. DOI: 10.1016/0378-5173(95)00088-Z

[21] Altındal DÇ, Gümüşderelioğlu M. Melatonin releasing PLGA micro/nanoparticles and their effect on osteosarcoma cells. Journal of Microencapsulation. 2015;**33**(1):53-63. DOI: 10.3109/02652048.2015.1115901

[22] Zhang L, Zhang J, Ling Y, Chen C, Liang A, Peng Y, et al. Sustained release of melatonin from poly (lactic-co-glycolic acid) (PLGA) microspheres to induce osteogenesis of human mesenchymal stem cells in vitro. Journal of Pineal Research. 2012;**54**(1):24-32. DOI: 10.1111/j.1600-079x.2012.01016.x

[23] Schaffazick SR, Pohlmann AR, Mezzalira G, Guterres SS. Development of nanocapsule suspensions and nanocapsule spray-dried powders containing melatonin. Journal of the Brazilian Chemical Society. 2006;**17**(3):562-569. DOI: 10.1590/s0103-50532006000300020

[24] Vatsaraj N, Zia H, Needham T. Formulation optimization of a sustained-release tablet of ketorolac tromethamine. Drug Delivery. 2002;**9**(3):153-159. DOI: 10.1080/10426500290095656

[25] Choi du H, Shin S, Khoa Viet Truong N, Jeong SH. A new experimental design method to optimize formulations focusing on a

lubricant for hydrophilic matrix tablets. Drug Development and Industrial Pharmacy. 2012;**38**(9):1117-1127. DOI: 10.3109/03639045.2011.641563

[26] Furlanetto S, Cirri M, Maestrelli F, Corti G, Mura P. Study of formulation variables influencing the drug release rate from matrix tablets by experimental design. European Journal of Pharmaceutics and Biopharmaceutics. 2006;**62**(1):77-84. DOI: 10.1016/j.ejpb.2005.07.001

[27] Martinello T, Kaneko TM, Velasco MV, Taqueda ME, Consiglieri VO. Optimization of poorly compactable drug tablets manufactured by direct compression using the mixture experimental design. International Journal of Pharmaceutics. 2006;**322**(1-2):87-95. DOI: 10.1016/j.ijpharm.2006.05.034

[28] Dey S, Mahanti B, Khila S, Mazumder B, Gupta SD. Formulation development and optimization of bilayer tablets of aceclofenac. Expert Opinion on Drug Delivery. 2012;**9**(9):1041-1050. DOI: 10.1517/17425247.2012.707187

[29] de Aguiar PF, Bourguignon B, Khots MS, Massart DL, Phan-Than-Luu R. D-optimal designs. Chemometrics and Intelligent Laboratory Systems. 1995;**30**(2):199-210. DOI: 10.1016/0169-7439(94)00076-X

[30] Malenović A, Dotsikas Y, Mašković M, Jančić-Stojanović B, Ivanović D, Medenica M. Desirability-based optimization and its sensitivity analysis for the perindopril and its impurities analysis in a microemulsion LC system. Microchemical Journal. 2011;**99**(2):454-460. DOI: 10.1016/j.microc.2011.06.022

[31] Martarelli D, Casettari L, Shalaby KS, Soliman ME, Cespi M, Bonacucina G, et al. Optimization of melatonin dissolution from extended release matrices using artificial neural networking. Current Drug Delivery. 2016;**13**(4):565-573. DOI: 10.2174/1567201812666150608101528