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The Release Kinetics of Melatonin from Innovative Dosage Forms: The Role of the Fractal Geometry of the “Vehicle”

Natassa Pippa and Costas Demetzos

Abstract

Melatonin (N-acetyl-5-methoxytryptamine) is an antioxidant active pharmaceutical ingredient with numerous applications as medicine and nutraceutical. Melatonin, a hormone synthesized by the pineal gland, has a significant role in the regulation of the circadian biological clock. The aim of this chapter is to present the conventional solid and liquid forms (i.e., tablets, capsules, suspensions, etc.) and the nanoformulations (i.e., liposomes, niosomes, polymeric nanoparticles, chitosomes, calcium alginate beads, etc.) of melatonin and to give special attention to its release kinetics from the pharmaceutical vehicle. These systems have been designed and developed as platforms for the delivery and release of melatonin. In all cases, the controlled release of melatonin is the main goal of its loading into drug delivery platforms. Fractal analysis is a mathematical tool to quantify nature and physical systems' complexity. These systems have been characterized as fractal objects, due to their fractional dimensions. In this chapter, we are probing the interrelationship between the fractal dimension of pharmaceutical vehicle and the release profile of melatonin. Several examples will be given in order to understand in depth the reason of controlled-release profile of melatonin and its added value for the development of a new medicine and/or nutraceutical.

Keywords: nanosystems, drug delivery, kinetics, release, fractals

1. Introduction

According to the Drug and Lactation Database, “Melatonin is the hormone produced by the pineal gland that plays a role in regulating sleep and circadian rhythm as well as a possible role in gut-brain signaling.” As it is stated in the Drug and Lactation Database, melatonin (methoxyindole) is used for the organization of the circadian rhythms, especially core temperature and sleep–wake rhythms [1]. Melatonin is also characterized as a full-service anticancer agent due to its functions: inhibition of initiation, progression, and metastasis phases of tumors [2]. Aside from its antioxidant, anticancer, antitumor, anti-inflammatory, antiaging, antidiabetic, antiviral, and neuroprotective activities, melatonin exhibits a therapeutic potential in the treatment of asthma, respiratory diseases or infections, chronic obstructive pulmonary disease, lung cancer, pleural cavity diseases, as well

as vascular pulmonary diseases [3]. Melatonin is also used as a food supplement and nutraceutical. The dosage and release profile of melatonin are very crucial factors that affect the effectiveness of treatment, especially in older adults [4]. According to a recent critical analysis, in older adults, the use of the lowest possible dose of immediate-release formulation of melatonin is appropriate to best mimic the normal physiological circadian rhythm of melatonin and to avoid prolonged, supra-physiological blood levels [4].

Melatonin has been encapsulated in different conventional and nanotechnological systems [5]. In the majority of the cases, the aim of the incorporation of melatonin into formulations is to achieve controlled or sustained release. The aim of this chapter is to present the conventional solid and liquid forms (i.e., tables, emulsions, suspensions, etc.) and the nanoformulations of melatonin and to give special attention to release kinetics from the pharmaceutical vehicle.

Furthermore, fractal analysis is a mathematical tool to quantify nature and physical systems' complexity [6]. Fractals have been observed in powdered drug substances, in excipients, and in their mixtures, as well as in semifluid dosage forms like gels and emulsions [6]. Fractals have been used to describe the dimensions of dosage forms, such as tablets, matrix tablets, and spheres [6]. The application of fractal geometry for the quantification of the dimensionality of advanced drug delivery nanosystems (aDDnS) recently appeared in the literature [5, 6]. For example, liposomes, micelles, polymersomes, and other nanosystems are fractal objects [6]. Additionally, the fractal and fractional kinetics can model very close to the reality the release of drugs from polymeric matrices and other dosage forms, both solid and liquid [7–9].

In this chapter, we are going to find the interrelationship between the fractal dimension of pharmaceutical vehicle and the release profile of melatonin. Several examples will be given in order to understand in depth the reason of controlled-release profile of melatonin and its added value for the development of a new medicine and/or nutraceutical.

2. Dosage forms of melatonin

2.1 Conventional dosage forms of melatonin

Reiter et al. summarized what is known about the function of melatonin in the oral cavity [10]. Melatonin is released into the saliva by the acinar cells of the major salivary glands and via the gingival fluid [10]. Functions of melatonin in the oral cavity are likely to relate primarily to antioxidant activities [10]. A case series study revealed that the light level and duration of exposure determine the impact of self-luminous tablet on melatonin suppression [11]. Hydrophilic polymer matrices composed of hydroxypropyl methylcellulose, xanthan gum, and Carbopol® 974P NF in different amounts were formulated in tablet forms [12]. These tablets exhibited a prolonged-release profile of melatonin [12]. Monolayered and three-layered tablets, incorporating nanofibrous mats composed of cellulose acetate and polyvinylpyrrolidone loaded with MLT, were prepared and exhibited a prolonged-release profile of melatonin, too [13]. The release profile of Circadin® tablets is presented in the recent literature. Circadin® is a prolonged-release tablet, the only licensed melatonin formulation available in the UK [14]. According to Chua et al., the division of tablet into two or four halves did not affect the prolonged-release characteristics [14]. We can observe this kinetics in **Figure 1**. Furthermore, immediate-release tablets are available in Greek markets as food supplements. This formulation investigation is a composition of natural ingredients, which have a relaxant,

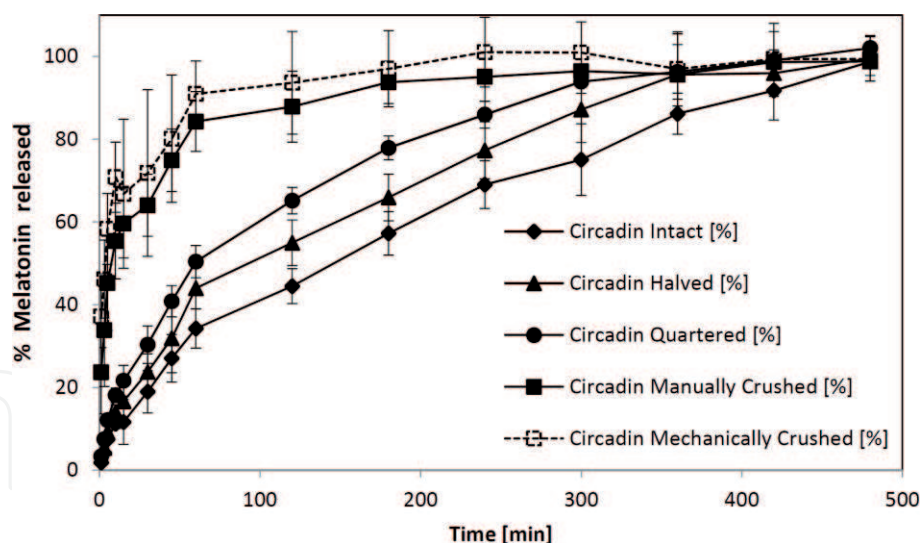


Figure 1.
Comparison of dissolution profiles for the release of melatonin from Circadin tablets in an intact, halved, quartered, and crushed form (data represented mean \pm SD, $n = 6$) (adapted from [14]). The division of tablet into two or four halves did not affect the prolonged-release characteristics.

anxiolytic, and sleep-inducing action. The active ingredients of the formulations are melatonin, L-tryptophan (an important amino acid, which is converted in the brain to serotonin (neurotransmitter that contributes to a normal sleep cycle; serotonin is sequentially converted to melatonin)), passiflora, valeriana, hawthorn, and eschscholtzia that increase GABA levels in the brain. For an immediate-release formulation, the amount of drug released should not be less than 80% of the labeled amount at 30 minutes [15]. This release profile of melatonin is suitable for alleviating certain insomnia-related problems and particularly those arising from sleep-onset difficulties [15].

Other conventional dosage forms that are used for the melatonin delivery are soft capsule gels [16, 17]. Soft gel capsules improved the bioavailability of melatonin in humans even when the administered dose was reduced [16]. Considering the number of conditions in which melatonin supplementation is recommended, this evidence could support a broader use of melatonin in clinical practice, especially in the field of nutraceuticals [16]. Sublingual solution and hard capsules of melatonin have been also appeared as dosage forms in the literature [17]. The sublingual solution was prepared with glycerin, ethyl alcohol, stevia powder extract, and tutti-frutti flavor. The concentration of melatonin was equal to 10 mg/ml [17]. The hard capsules are composed of Methocel E4M and lactose anhydrous, and the amount of melatonin was 3 mg per capsule. Both of the aforementioned formulations were found to be stable in accelerated conditions [17].

2.2 Advanced drug delivery nanosystems of melatonin

2.2.1 Liposomes and lipid drug delivery nanosystems

Liposomes are bilayers composed of phospholipids [6]. They are used as drug and vaccine delivery systems and as cellular membrane models [6]. They are biocompatible and biodegradable [6]. The location of melatonin in 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) model membranes was investigated by several techniques such as small-angle neutron scattering (SANS) and molecular dynamics (MD) simulations [18]. The location of melatonin in those lipid membranes is illustrated in **Figure 2** [18]. The interactions of melatonin with cellular membrane models can be a road map to

elucidate the protective effect of melatonin against the formation of amyloid-beta ($A\beta$) proteins of Alzheimer's disease [19]. In **Figure 2**, we can also observe that melatonin is located in the interface between head groups and lipid tails, while cholesterol is located parallel to lipid chains [18]. The “stabilizing” effect of melatonin, a naturally occurring hormone produced by the brain's pineal gland, on phase-separated model membranes mimicking the outer leaflet of plasma membranes was also investigated [20]. For example, melatonin stabilizes the liquid-ordered/liquid-disordered phase coexistence over an extended range of temperatures. Melatonin appeared to induce re-ordering effects in liposome and Langmuir monolayers [20].

Melatonin-loaded liposomes (MLL) were successfully prepared using rapid expansion of supercritical solution technology [21]. The system is composed of phosphatidylcholine-cholesterol-melatonin at 20:2:1 molar ratio, and the size of the liposomes was found to be around 100 nm [21]. The release kinetics of melatonin shows slow-release features in early digestive stages and more through characteristics in later stages of simulated gastric fluids [21]. Furthermore, vesicular (liposomal and nanoencapsulated) forms of melatonin efficiently downregulate sodium fluoride-induced rat hepato- and broncho-TNF- α , TGF- β expressions, and associated oxidative injury, as well as oxidative damage in sodium fluoride (NaF)-treated lungs and liver [22]. The nanoencapsulated melatonin was evaluated as a more powerful remedial therapy in comparison with liposomes, in terms of its efficacy in regulating NaF-intoxicated oxidative injury [22]. Melatonin also encapsulated into liposomes produced using supercritical carbon dioxide (an easier technique compared to thin-film hydration method) [23]. The release profile of melatonin is more or less the same for the liposomes prepared by the two techniques (i.e., supercritical carbon dioxide and thin-film hydration method), but exhibits differences compared to tablets [23].

The hypotensive melatonin analogue 5-methoxycarbonylamino-N-acetyltryptamine (5-MCA-NAT) was loaded into hybrid liposomes (i.e., polymer-grafted liposomes). These hybrid formulations were combined with mucoadhesive (sodium hyaluronate or carboxymethylcellulose) or amphiphilic block thermosensitive (poloxamer) polymers to prolong the release profile of the active ingredient. The prepared hybrid liposomes' size was found to be between 150 to 200 nm with low polydispersity and zeta potential near zero. The release profile of the 5-MCA-NAT melatonin was found to be dependent on the nature of the polymeric guest. In the presence of the polymer, the released ratio of melatonin was also decreased

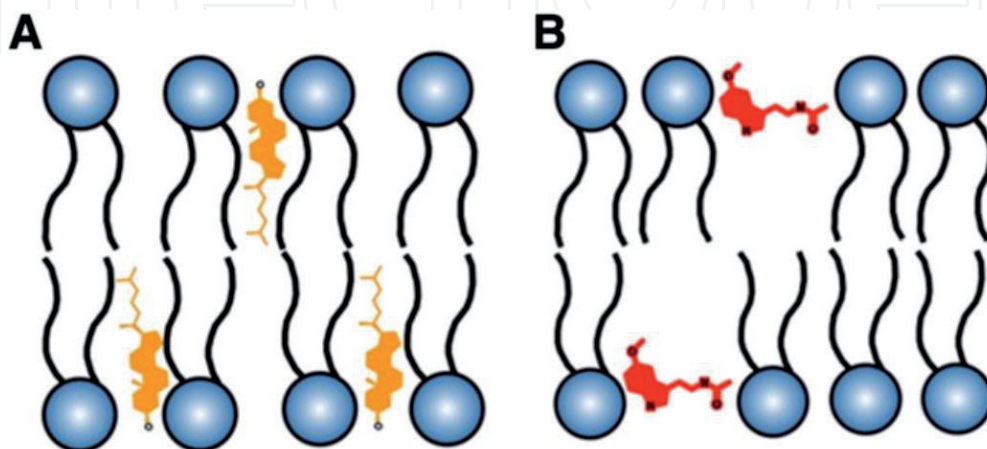


Figure 2. Schematics illustrating the proposed locations of cholesterol and melatonin in the lipid membrane: (A) cholesterol, (B) melatonin. (adapted from [18]).

in comparison with the pure liposomes. The hypotensive effect of the prepared systems was further investigated in rabbit eyes.

Melatonin was also incorporated into chitosomes [24]. Chitosomes are chitosan-coated liposomes that represent an alternative to conventional liposomes since they present better stability and bioadhesivity [24]. Chitosomes are prepared by using a different molar ratio of the active ingredient and the polymer. In all cases, the amount of the phosphatidylcholine was constant [24]. These formulations exhibit size between 200 and 250 nm, with negative zeta potential and encapsulation efficiency between 30 and 60% [24]. The amount of chitosan exhibited the key role for the stability of the polymer-coated liposomes and their loading properties [24].

Last but not least, melatonin and its structural analogues do not possess antioxidant properties on Fe(2+)-initiated peroxidation of sonicated liposomes made of retinal lipids [25]. The in vitro protective effects of melatonin against oxidation of 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine (PLPC) liposomes and low-density lipoproteins (LDL; 3 g/L total concentration) by hydroxyl radicals produced by water gamma radiolysis were also investigated [26].

Solid lipid microparticles were designed as an oral pulsatile system for the delivery of melatonin to pediatric patients [27].

2.2.2 Niosomes

Niosomes are composed of surfactants (Tweens and Spans) and are vesicular drug delivery systems. The unique structure of niosome presents an effective novel drug delivery system with the ability of loading both hydrophilic and lipophilic drugs [28, 29]. A transmucosal niosome gel was developed to improve the pharmacokinetics of exogenous melatonin [30]. The melatonin niosome gel was characterized by several physicochemical techniques, and melatonin levels were determined in healthy volunteers [30]. Oral transmucosal melatonin niosome gels, at different molar ratio of the active ingredient, topically applied in 14 healthy volunteers in a randomized double-blinded crossover design with a 7-day washout, gave dose-proportional pharmacokinetics, with improved absorption and prolonged systemic circulation [30]. Additionally, melatonin-loaded elastic niosomes were prepared and lyophilized [31]. The lyophilized niosomal system was incorporated into the Pickering emulsion. Ex vivo permeation studies revealed 58% of melatonin were permeated through the rat skin while 37% of melatonin accumulated in the skin after 24 hours. This formulation is effective for UV-induced skin damage [31].

2.2.3 Polymeric nanoparticles and polymer-based drug delivery nanosystems

Polymeric nanoparticles include a variety of systems composed of polymers. The polymers should be biocompatible for drug delivery applications. Generally, polymers exhibit nice drug loading and release properties. Some polymers like Pluronic[®] are FDA approved. Pohlmann et al. (2010) have shown that the in vitro antioxidant effect of melatonin against lipid peroxidation in microsomes and liposomes can be improved by encapsulation of the antioxidant drug in polymeric nanoparticles. Polymeric nanoparticles (nanocapsules or nanospheres) have been used to improve the melatonin efficacy and release, too [32]. For example, incorporation in polymeric nanocapsules improves the antioxidant effect of melatonin against lipid peroxidation in mice brain and liver. It should be pointed out that the melatonin-loaded polysorbate 80-coated nanocapsules caused a marked reduction on lipid peroxidation levels in all studied tissues and increased the total antioxidant reactivity in the hippocampus [32].

Two types of polymeric nanoparticles have been also investigated for ocular administration of melatonin [33]. For example, lecithin/chitosan nanoparticles with size around 250 nm and Pluronic® F127/chitosan micelles with size around 20 nm have been designed and developed in order to improve the bioavailability of melatonin in the eyes [34]. According to the authors, “the permeability study results confirmed the permeation enhancing effect of F127, which was hindered in the presence of chitosan. Lecithin/chitosan nanoparticles were characterized by prominent mucoadhesive properties and prolonged melatonin release, which was shown to control melatonin permeation across an in vitro corneal epithelial model. Such properties demonstrate the potential for nanoparticles to provide an extended pre-corneal residence time of melatonin, ensuring higher eye-related bioavailability and extended intraocular pressure reduction compared to melatonin in both aqueous and micelle solutions” [34].

Poly(D,L-lactide-co-glycolide) (PLGA) polymers have been used for the preparation of nanoparticles and microparticles loaded with melatonin [35, 36]. Both of them are prepared by the emulsion-diffusion-evaporation method and the addition of 0.2% (w/v) melatonin in the aqueous phase. The size of nanoparticles was about 200 nm and the entrapment efficiency around 14%, while the size of microparticles was about 3.5 micrometers and the encapsulation efficiency 27% [35, 36]. The toxicity and the effectiveness of the prepared systems are also evaluated using cell lines. According to the results, melatonin could be an adjunct to the routine chemotherapy of osteosarcoma by encapsulating it into PLGA polymeric delivery platform [36]. Additionally, PLGA nanoparticles and polysorbate 80-coated PLGA nanoparticles (PLGA-PS80) increase the in vitro antioxidant activity of melatonin [36]. The sizes of the PLGA-PS80 and PLGA nanoparticles were 212 and 187 nm, and the encapsulation entrapment of melatonin was 26 and 41%, respectively [37]. The release kinetics of melatonin followed the second-order model during studies from nanoparticles, while PLGA-PS80 presented more prolonged melatonin release [37]. The spherical shape of nanoparticles and the strong interactions due to negative zeta potential are the possible explanation of the release kinetics of melatonin from nanoparticles [37].

Furthermore, melatonin was loaded into chitosan-tripolyphosphate nanoparticles [38]. Melatonin nanoparticles protect against etoposide-induced genotoxicity in the HepG2 cell line (etoposide is one of the most effective chemotherapeutic agents used in the treatment of various types of tumors) [38]. An increased nose-to-brain delivery of melatonin mediated by polycaprolactone nanoparticles for the treatment of glioblastoma has been also designed and developed [39]. No cytotoxic effect was observed against non-tumor cells [39]. Another interesting formulation of melatonin is hybrid hydrogels composed of calcium alginate beads and combinations of polymers such as polyvinylpyrrolidone (PVP₁₀₀₀₀ and PVP₅₅₀₀₀), hydroxypropyl methylcellulose (HPMC₁₅₀₀₀ and HPMC₁₀₀₀₀₀) at different molar weights, lactose monohydrate, and as a surfactant sodium laureth sulfate (SLS) [40]. In all cases, the encapsulation efficiency of melatonin was very high, around 80% [40]. The swelling studies and the release profile were found to be dependent on the presence of the polymer [40]. Fickian diffusion mechanism and burst release were also observed [40]. The nature (architecture and molecular weight) of the polymeric guest altered the physicochemical behavior of the calcium alginate beads and the release of melatonin, too [40].

2.3 Release kinetics of melatonin from delivery platforms

Melatonin has been encapsulated in different delivery carriers, as mentioned above. The majority of these carriers are summarized in **Table 1**. We should highlight

Conventional forms	Advanced drug delivery systems
Tablets	Liposomes/lipid carriers
• Polymeric matrices	• Chitosan-coated liposomes (chitosomes)
• Three-layered tablets	• Polymer-grafted liposomes
Sublingual suspensions	• Solid lipid microparticles as pulsatile system
Capsules	Niosomes
• Soft gels	Polymeric nanoparticles
• Hard gels	• PLGA nanoparticles and microparticles
	• Polysorbate 80-coated PLGA nanoparticles

Table 1.
The delivery systems of melatonin.

that the reason of the design and the development of different formulations of melatonin is the achievement of its controlled/programmed release. There are systems of immediate release of melatonin, i.e., tablets and some others where the release of melatonin is prolonged, i.e., polymeric nanoparticles. The treatments of sleep-onset problems and/or sleep maintenance discomforts are the goals of preparing a delivery platform of melatonin as medicine or as food supplement/nutraceutical. Recently, a comparative study of the in vitro release of melatonin from matrix tablets and liposomal formulation appeared in the literature [41]. The matrix tablets used were comprised of HPMC and dextran. Moreover, melatonin was encapsulated into conventional liposomes composed of DPPC and dipalmitoyl-phosphatidyl glycerol (DPPG), in an attempt to compare the hormone's release profile from liposomal formulations with its respective release from matrix tablets [41]. Some of the formulations of the matrix tablets and the liposomes exhibit the same release behavior ideal for the maintenance of sleep [41]. On the other hand, the burst release of melatonin from some other matrix tablet formulations is ideal for the fast sleep onset [41].

In order to design and develop an ideal drug delivery platform combining a burst release of melatonin accompanied by a prolonged release, mathematical modeling and simulations are needed. In that case, the fractal nature of the formulation should be taken into consideration [6–8]. The last one is very important because the fractal dimensionality of these systems is closer to their real dimensions [6–8]. Both conventional systems like tablets and capsules and nanosystems like liposomes and polymeric nanoparticles are fractal objects. In other words, the mechanistic explanation of the release profile of melatonin should be based on the fractal dimensions of the drug delivery systems.

3. Conclusions

Melatonin is a chronobiotic hormone used for the treatment of sleep problems and disorders. In this chapter, we presented the formulations of melatonin that appeared in the literature. Tablets, capsules (hard and soft gels), and the advanced drug delivery systems of pharmaceutical nanotechnology offer the possibility of encapsulation efficiency of melatonin and ideal release properties. Several routes of administration have been proposed, but in the majority of the cases, the per os administration is the most popular route of administration, especially of the nutraceuticals. The interrelationship between the fractal dimension of pharmaceutical vehicle and the release profile of melatonin is the key point for the development of delivery platforms of melatonin as medicines and food supplements/nutraceuticals.

Conflict of interest

The authors confirm that this chapter content has no conflict of interest.

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Author details

Natassa Pippa* and Costas Demetzos
Division of Pharmaceutical Technology, Department of Pharmacy, School of Health
Sciences, National and Kapodistrian University of Athens, Athens, Greece

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

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