MELATONIN DOSAGE FORMS WITH AN EMPHASIS ON MODIFIED RELEASE DOSAGE FORMS

Dissertation to obtain the academic degree of Master in Pharmaceutical Research

Submitted to the Department of Biology, Chemistry, and Pharmacy of the Free University of Berlin submitted by

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List of abbreviations

BCS – Biopharmaceutics classification system

DCP – Dibasic calcium phosphate

DSC – Differential scanning calorimetry

EC – Ethylcellulose

GI – Gastrointestinal

HPMC – Hydroxypropylmethylcellulose

ICH – International Conference on Harmonization

IPA – Isopropanol

MC – Moisture content

MCC – Microcrystallinecellulose

PVA – Polyvinyl acetate

PVP – Polyvinylpyrrolidone

SLS – Sodium lauryl sulphate

S-TM – Sulphatoxymelatonin

1. INTRODUCTION

1.1. Melatonin

Melatonin (N-Acetyl-5-methoxytryptamine) (Figure 1) is a hormone produced principally by the pineal gland in the brain. Secretion of this hormone depends on exposure to darkness and light, being the highest during the night. Therefore, melatonin plays an important role in regulation of the circadian rhythm and the sleep-wake cycle (Filali et al. 2017; Martins et al. 2017; Chua et al. 2016; Albertini et al. 2014; Hoffmann et al. 1999; Daescu et al. 2020; Girgin et al. 2016).

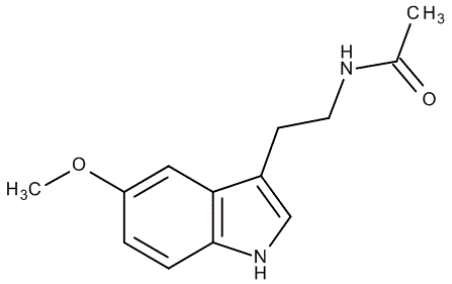

Figure 1. Chemical structure of melatonin.

Melatonin has important anti-inflammatory and antioxidative properties (Daescu et al. 2020; Albertini et al. 2014; Mahal et al. 1999), improves immune function, and scavenges free radicals (Mahal et al. 1999; Lin et al. 2012; Daescu et al. 2020). It has been suggested that Melatonin has a neuroprotective role against diseases such as Alzheimer and Parkinson (Martins et al. 2017).

Exogenous melatonin has therapeutical application in treatment of circadian rhythm disorders, such as jet-lag syndrome, seasonal depression and insomnia in shift worker, elderly, blind people and patients with Alzheimer (Albertini et al. 2014; Hoffmann et al. 1999; Lee et al. 1995). Melatonin has been studied to treat children with sleep and neurodevelopmental disorders (Albertini et al. 2014).

Furthermore, melatonin has been studied to treat COVID-19, due to its significant antioxidant, anti-inflammatory and immunoregulatory properties, which may be used against the generation of free radicals, inflammation, and neuroinvasion caused by COVID-19 (Romero et al. 2020; Acuña-Castroviejo et al. 2020; Tan and Hardeland 2020; Zhang et al. 2020).

Melatonin is considered a dietary supplement by the FDA in the United States, while it is categorized as a medicine in the European Union. In Germany the product Circadin 2 mg prolonged release tablets is marketed, which is indicated as "monotherapy for the short-term treatment of primary insommia characterized by poor quality of sleep in patients who are aged 55 or over" (European Medicines Agency).

1.1.1. Melatonin pharmacokinetics and mechanism of action

Melatonin reaches the maximum plasma level between 2:00 and 4:00 with concentrations between 0.2 to 0.3 pmol/mL and then it decreases towards the morning with low plasma concentrations below 0.04 pmol/mL (<10 pg/mL) during the day (Filali et al. 2017; Albertini et al. 2014; Hoffmann et al. 1999; Lee et al. 1995; Tordjman et al. 2017).

Oral absorption of melatonin shows peak plasma levels rapidly after 20 to 60 minutes of ingestion (Chua et al. 2016; Albertini et al. 2014; Tordjman et al. 2017). Melatonin has a high first-pass effect, resulting in a bioavailability lower than 20%. It is metabolized mainly by the liver with 80 to 90% to the inactive metabolite, 6-sulphatoxymelatonin (6-S-TM), which is excreted in the urine (Albertini et al. 2014; Touitou 2001; Martins et al. 2017; Girgin et al. 2016; European Medicines Agency). Melatonin has an in-vitro plasma protein binding of approximately 60% (European Medicines Agency) and an elimination half-life of approximately 47 minutes (Tordjman et al. 2017). A small percentage (2-5%) of melatonin is excreted in the urine as unchanged active substance (Tordjman et al. 2017; European Medicines Agency).

The activity of melatonin is mediated through receptors MT1, MT2 and MT3, mainly with MT1 and MT2 which are involved in the regulation of circadian rhythms and sleep regulation and contribute to melatonin sleep-promoting properties (European Medicines Agency; Tordjman et al. 2017).

1.1.2. Melatonin stability

It has been found that pH is not an important variable in melatonin stability (Moussaoui and Bendriss 2014; Cavallo and Hassan 1995; Daya et al. 2001). Melatonin solutions are stable at 4°C under vacuum for at least 6 months (Cavallo and Hassan 1995) and at 20°C and 37°C

for 2 days, showing less than 30% of decrease after 21 days (Daya et al. 2001), suggesting that melatonin is relatively stable at body temperature after oral ingestion.

1.2. Tableting manufacturing

Tableting is the densification process, in which loose powder becomes a consistent mass by compaction or molding (Bellini 2018). During compaction low forces produce rearrangement of the particles, while higher forces lead to plastic or elastic deformation of the particles or combination of both. Inter-particular bonds are created, producing cohesion of the particles and tablet strength (Grund 2013).

Tablet manufacturing is widely used in the pharmaceutical industry, with almost 70% of oral dosage forms manufactured for human use (Bellini 2018). Tablet manufacturing is a simple and low-cost process compared to other dosage forms.

Selection of the manufacturing process depends on, among others, factors such as compression properties of the ingredients, physicochemical stability of the drug, particle size of the ingredients, equipment availability and cost of manufacturing.

Manufacturing of tablets is performed by direct compression or after wet or dry granulation.

1.2.1. Direct compression

Direct compression is the preferred method of tablet manufacture for controlled release and immediate release tablets, because it has a low cost, higher time effectiveness and requires less equipment and operational space than other techniques (Timmins et al. 2014; Katikaneni et al. 1995a; Gohel and Jogani 2005). Direct compression is more appropriate for heat and moisture sensitive drugs, due to it avoid wetting and drying steps, thus, the negative effects over the drug stability are reduced (Gohel and Jogani 2005; Jivraj et al. 2000).

Ingredients used in direct compression should have good flowability and compactibility properties (Bolhuis et al. 2003). For that reason, excipients with these characteristics have been developed to compensate the poor compression properties of active ingredients. Examples of these excipients are Flowlac (lactose), Emcompress (calcium phosphate), Ludipress (lactose, PVP, crospovidone), etc. In addition, there are carrier materials used in direct compression for

prolonged release matrices, such as HPMC, ethylcellulose, Eudragit RS, Kollidon SR, glycerol behenate, carnauba wax and others.

1.2.2. Granulation

Granulation is the process in which powder particles become merged into large agglomerates called granules. During this process it is necessary to employ binders to get the desired adhesivity between the powders. Granulation prior to the compaction has advantages such as reduction of particles segregation, decrease of dust during tableting, enhance flow properties of the powder blend and improve compression characteristics of the drug. The disadvantages of granulation processes are the higher costs and longer duration in comparison with direct compression. The granulation technique can be classified in two types, dry and wet granulation, based on the method employed to facilitate the agglomeration.

Dry granulation forms the bridge between the powder particles by the deformation of a plastic material under high pressure (Grund 2013). The most common process used in dry granulation is roller compaction.

Wet granulation is more used than dry granulation, despite involving more processes and being more time consuming (Shanmugam 2015). Wet granulation is a method that includes a binder solution which forms the wet mass when it is added to other ingredients. The solid bridge is created for example due to recrystallization of the dissolved ingredients during drying (Grund 2013). Thus, this technique is not suitable for humidity-sensitive drugs or drugs with polymorphs.

1.2.3. Compaction properties

Tablet compaction is the most widespread manufacturing process which uses tablet presses to apply a force or pressure on to the loose powder in an enclosed space. This technique has four steps: rearrangement, deformation, compaction, and relaxation.

During the rearrangement step, the powder particles move inside the die cavity to fill voids spaces between particles when the pressure is rising, giving as a result a decrease in volume due to the close packing structure generated. Once all the spaces are filled and more

particle rearrangement is not possible, a reversible elastic deformation is experienced (Jivraj et al. 2000; Bellini 2018).

After the elastic limit of the material is exceeded, particles undergo a permanent deformation. However, depending on the nature of the material or blend, a plastic deformation or particle fragmentation may occur. Plastic deformation produces bonds due to the increased contact area between particles and new surfaces and strong bonds are created due to the fragmentation (Jivraj et al. 2000). Finally, the pressure is removed and the tablet experiences an elastic relaxation (Bellini 2018).

Tablet manufacturing success depends on the right balance of plastic and brittle behaviour within the blend, which depends on compressional characteristics of the drug and excipients. In theory, plastic deformation occurs by compression of materials such as MCC and brittle fracture on materials such as dicalcium phosphate dihydrate, however, in practice, the majority of the drugs and excipients experiment a combination of both mechanism during compaction (Jivraj et al. 2000; Wang et al. 2010).

The behaviour of the materials has an important effect in tableting processes. Plastic behaviour is observed in amorphous materials, which keep an elastic and linear behaviour while the stress does not surpass the elastic limit or yield point. After the yield point, particles suffer a permanent deformation.

Brittle behaviour is observed in crystalline materials, which undergo fragmentation after the compression stress, without any considerable plastic deformation. Fractures and cracks produce new bonding surfaces, improving the tablet strength (Bellini 2018).

During compaction brittle materials frequently break, while ductile materials present a plastic deformation. Most of the ductile pharmaceutical materials have a higher compaction force than the yield strength and lower compaction force than the fragmentation strength, therefore, plastic materials deform without fracture (Bolhuis et al. 2003). Blending plastic and brittle materials increased surface area, due to the fragmentation of the brittle materials which produces a larger surface area for inter-particulate bonding with the plastic materials. The compressibility is higher for plastic/brittle blends excipients compared to plastic/ elastic and much higher than elastic/brittle mix (Mousa Al-Ibraheemi et al. 2013).

1.3. Oral controlled release dosage form

The oral route is subjected to more variety than other routes of administration. However, solid oral dosage forms (i.e., tablets and capsules) are the most widespread and the preferred route of delivery to patients, due to the minimal invasion of this route. Tablets are the most common dosage form, and there are several types of them, including, compressed, coated and controlled release (prolonged or delayed) (Mastropietro et al. 2017; Wen and Park 2010; Riis et al. 2007; Jivraj et al. 2000).

Oral controlled release delivery system is the most widely used in pharmaceutical industry. The most used controlled release formulations are delayed release, prolonged release and repeated action release (Wen and Park 2010). A delayed release formulation, commonly used, is enteric coated tablets, in which the drug releases after reaching the intestine (Wen and Park 2010). Prolonged release formulations release the drug loading gradually over a period of time (Wen and Park 2010). Prolonged release formulations have many considerable advantages, such as improved therapeutic effect, reduction of dosing frequency or less number or intensity of adverse effect (Riis et al. 2007). Oral controlled release dosage form can be designed among others in a matrix system or membrane-controlled release system and both of them often use polymers to achieve the desired release rate (Wen and Park 2010).

Excipients are used in oral dosage forms due to the benefits obtained during the manufacturing process and for the final product. Several of these excipients are polymeric materials which help to overcome oral delivery barriers and to obtain a reproducible delivery (Mastropietro et al. 2017). One of the main benefits of using polymeric materials for drug delivery is that they have a wide range of different physical and chemical properties, which can be chosen in accordance with the purpose of a formulation, such as enhancement of administration, bioavailability improvements or manufacturing of dosage forms (Mastropietro et al. 2017). During manufacturing processes, polymers can be used to enhance compaction and powder flow. Polymers have different roles, such as, drug protection (i.e. environment), delayed release (i.e. specific region in the GI tract) or drug release (i.e. modified release as a membrane, matrix or barrier) (Mastropietro et al. 2017).

Other excipients are added to the formulation to improve the process by enhancing the powder flow, compressibility, and lubricity. Excipients commonly used in matrices are filler, binders, lubricants and glidants. If necessary, other excipients may be added, such as buffering

agents, stabilisers, surfactants to enhance and optimise the drug release and solubility, and stabilize the formulation (Timmins et al. 2014).

Fillers behave as bulking agents and enhance flow, compressibility, and manufacturing features. Fillers may have a considerable effect on drug release, the extent of which depends on the type, content, and other factors. Common fillers used in matrix tablets are lactose, MCC, dicalcium phosphate, mannitol and pregelatinized starch. Water soluble fillers, such as lactose or mannitol lead mainly to a faster release rate, whereas insoluble fillers like MCC and dicalcium phosphate tend to produce a slower release profile (Timmins et al. 2014). Lactose is the most used filler in tablets, due to its cost-effectiveness, easy availability, physical and chemical stability, water solubility and low hygroscopicity. Spray-dried lactose is widely used for direct compression, because of its good flow and binding properties (Gohel and Jogani 2005).

Lubricants are chemically inert, odor- and tasteless. They help the compaction process by reduction of the friction between the tablet and the die wall and aiding the tablet ejection. Lubricants prevent the adhesion of the tablet material to the tooling (Wang et al. 2010; Grund 2013). Some widely used lubricants are magnesium stearate, aluminum stearate, calcium stearate, sodium stearate and zinc stearate. A range between 0.25 to 1.0% of lubricant is commonly employed and they are usually added to the formulation as the last step prior to the tablet compression (Wang et al. 2010).

Additionally, the properties of the active ingredient affect the mechanism, speed, etc. of release, and should be considered during the development and manufacturing process of oral dosage forms. Some of these properties are solubility, particle size, and the presence of polymorphs.

According to the solubility and permeability of the drug, the biopharmaceutics classification system (BCS) classifies drug substances in four categories. A drug substance is classified as highly soluble “if the highest single therapeutic dose is completely soluble in 250 ml or less of aqueous media over the pH range of 1.2–6.8 at 37±1°C” (European Medicines Agency 2020). Drug solubility is an important factor in the release mechanism and rate. The absorption of a poor water-soluble drug is limited by dissolution rate. On the other hand, drugs with a very high solubility and loading higher than 80% could be a challenge, because the small amount of polymer cannot control the diffusion in the matrix system (Wen and Park 2010). Drug solubility can affect the release profile in HPMC matrices. Some studies show that low

soluble drugs present slower release rates than soluble drugs with the same formulation (Timmins et al. 2014).

Particle size is also an important factor which affects the release of the drug, including the polymer and drug particle size. HPMC particle size has shown an effect on drug release. Timmins et al., (2014) have shown how the release rate of propranolol hydrochloride is slower if the polymer particle size is decreased from 355 to 150-210 µm. Coarse particles size of HPMC hydrate very slowly, resulting in a burst release which is not suitable for prolonged release. Also, large particles allow water penetration which produces disintegration before the gel layer formation. Coarse particles may create bigger pores sizes, decreasing the gel structure stability, while smaller particles permit fast hydration and steady gel formation (Timmins et al. 2014). Drug release from water-insoluble matrices is mainly by diffusion through the matrix. The polymer particle size affects the drug-release rate. Crowley et al., (2004) found that guaifenesin release rate was vastly dependent on EC particle size, showing slower release rate when smaller particle sizes were employed.

Also, the presence of polymorphs is an important factor to be considered. According to the International Conference on Harmonization (ICH) Guideline Q6A polymorphism is defined as "the occurrence of different crystalline forms of the same drug substance. This may include solvation or hydration products (also known as pseudopolymorphs) and amorphous forms". These crystalline forms differ in their physical properties, which may affect the quality or performance of the drug product (European Medicines Agency - CHMP/ICH 2000). An understanding of the polymorph forms of the drug is relevant due to the variation in physical properties, such as compaction, flow properties, aqueous solubility, and therefore bioavailability (Potter et al. 2017). Moreover, polymorphs have differences in melting point or heat capacity.

1.4. Matrix tablets

Matrix tablets are composed of the drug homogeneously embedded in a carrier (inert material) and are frequently used in controlled release dosage forms due to their ease of manufacture. Matrix formers are frequently non-swellable hydrophobic or swellable hydrophilic polymers. The rate of drug release is governed by permeation, diffusion and/or dissolution according with the polymer properties (Mastropietro et al. 2017; Wen and Park 2010; Reza S. et al. 2003).

Due to the drug must be dissolved for its release, the rate of water penetration into the tablet matrix is related to the capacity of the drug to dissolves and diffuse out of the matrix and into the medium. The rate of release and absorption of the drug is affected by diffusional distance of the matrix. The drug moves from a region with high concentration to one with low concentration in the medium (solution) around (Mastropietro et al. 2017; Wen and Park 2010).

1.4.1. Water-soluble matrices (erodible)

Hydrophilic matrices hydrate after getting in contact with an aqueous medium and form a gel layer on the tablet surface. The gel layer behaves as a barrier for the water penetration and the drug diffusion. Over time, the gel layer will become thicker, due to the permeation of the water into the tablet and dissolve or erode after being completely hydrated (Mastropietro et al. 2017; Wen and Park 2010; Timmins et al. 2014; Dabbagh M.A. et al. 1996; Reza S. et al. 2003).

Depending on the solubility of the drug the release is different, thus, for soluble drugs the release is predominantly by diffusion of the dissolved drug through the gel layer, while for low soluble drugs the release occurs mainly by erosion of the matrix. Therefore, an important characteristic of the hydrophilic polymer matrix is the gel forming ability. The drug release is not only affected by drug solubility, but it is also influenced by polymer physical and mechanical properties (Mastropietro et al. 2017; Wen and Park 2010; Ali et al. 2017).

One of the hydrophilic polymers more widely used in modified release is hydroxypropyl-methylcellulose (Figure 2). HPMC is suitable for direct compression, due to its good compressibility properties. The hydration and gel formation depend on the molecular weight and substitution of the polymer (Mastropietro et al. 2017; Wen and Park 2010; Timmins et al. 2014).

Ratios of methoxyl and hydroxypropoyl substitution and molecular weight affects properties such as powder flow, compactability and compressibility, solubility, swelling, diffusion, and drug release (Wen and Park 2010; Timmins et al. 2014). HPMC has 3 subdivisions used in controlled release, marketed as Methocel® (Table 1), classified according to the methoxy or hydroxypropyl substitution. However, Methocel K and Methocel E are more often used in prolonged release formulation than Methocel F, due to the more rapid hydration and gel formation (Wen and Park 2010; Timmins et al. 2014).

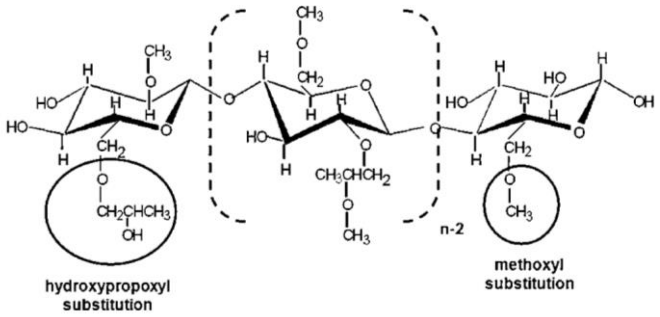

Figure 2. Chemical structure of HPMC with examples of methoxyl and hydroxypropoxyl groups. (Timmins et al. 2014)

Increasing the number of substituents on the cellulose chain will decrease the polymer hydrophilicity, due to the replacement of hydroxyl groups. Methoxyl groups are more hydrophobic and decrease polymer swelling more than hydroxypropyl groups. HPMC matrix formulation with low viscosity grades present greater erosion rate with thinner gel layer, resulting in faster drug release (Timmins et al. 2014).

Table 1. Methoxy and hydroxypropyl substitution of Methocel.

Methocel	Methoxy Content (%)	Hydroxypropyl Content (%)
K	22	8
F	29	6
E	29	10

It has been reported that more robust matrices with less susceptibility to erosion during the gastrointestinal transit are obtained with higher viscosity grades of HPMC. Also, more viscous, and entangled gel layers are obtained with high viscosity grade of HPMC which are less affected under agitation during the in vitro testing (Timmins et al. 2014).

The drug release rate in HMPC matrices is controlled mainly by the content of HMPC. It has been demonstrated that drug release is slower as the polymer content increases in the formulation. A more porous and weaker gel layer may be obtained from a relatively small content of polymer (less than 20 – 30%), resulting in a fast drug diffusion and matrix erosion.

For that reason, HPMC content of around 30% w/w has been recommended to enhances the robustness of these matrices (Timmins et al. 2014).

1.4.2. Water-insoluble matrices (non-erodible)

The drug release from water-insoluble matrix tablets starts after contact with the dissolution medium as drug particles in the surface of the matrix are first released into the medium creating pores and cracks. Afterwards, the medium diffuses through the porous matrix and reaches the drug, the drug dissolves and diffuses through these medium-filled pores (Mastropietro et al. 2017; Reza S. et al. 2003).

Modified release dosage forms have been prepared using several water-insoluble polymers as matrix materials, some examples of these polymers are EC, Eudragit RS and Kollidon SR.

1.4.2.1. Ethylcellulose (Ethocel®)

Ethylcellulose (EC) is a hydrophobic cellulose ether used widely as a coating material, binder, and matrix former in controlled release dosage forms. EC (Figure 3) is an non-swellable polymer, insoluble in water and soluble in organic solvents such as isopropanol (IPA), ethanol, acetone, dichloromethane, methanol and chloroform (Wen and Park 2010; Rekhi and Jambhekar 1995; Katikaneni et al. 1995a; Upadrashta et al. 1994; Dabbagh M.A. et al. 1996).

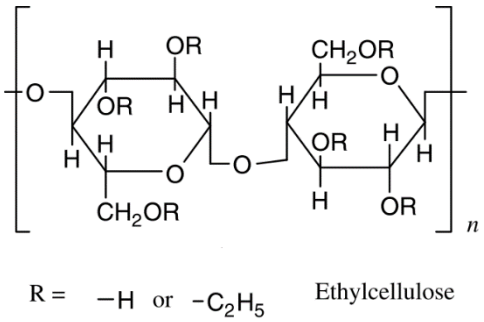

Figure 3. Chemical structure EC (Wen and Park 2010).

The release of the drug from an insoluble EC matrix is mainly controlled by diffusion. Once the matrix gets in contact with the dissolution medium, it penetrates into the pores and the drug dissolves in the filled pores, which afterwards diffuses out of the matrix, through these pores (Dabbagh M.A. et al. 1996; Streubel A. et al. 2000). The insoluble EC matrices remain intact, producing a decline in drug concentration inside the medium-filled pores of the matrix, causing a drug concentration gradient and as a result the driving force for diffusion dwindles (Streubel A. et al. 2000).

High compaction forces and small particles sizes reduce the drug release rate in EC matrix tablets due to the decrease in the tablet porosity (Mastropietro et al. 2017; Katikaneni et al. 1995a; Dabbagh M.A. et al. 1996). Due to the fact that a drug mainly diffuses through the medium-filled channels, a decrease of the pore size will result in a reduced release rate (Streubel A. et al. 2000). Pruthvipathy et al. (1995) reported a decrease in tablet hardness and a rise in release rate when the particle size was increased, using a constant compression force.

1.4.2.2. Polymethacrylates (Eudragit®)

Eudragit polymers are copolymers derived from esters of acrylic and methacrylic acids (Figure 4). Eudragit grades have different physicochemical properties according to the proportion of alkaline, acid, and neutral groups (Wen and Park 2010).

Eudragit L/S are enteric-type methacrylate polymers which act as a barrier against drug release in the stomach and allow the release in the intestine. Another enteric-type polymer is Eudragit FS which is used for colon targeting (Wen and Park 2010).

Eudragit RS and RL (ammonio methacrylate copolymer type A and B) are methacrylate copolymers with cationic quaternary trimethyl-ammonio groups which determine their hydrophilicity (Steiner 2011; Korbely et al. 2012). Both types are water insoluble, pH independent and capable of swelling and hydration, due to the presence of the ionized quaternary ammonium groups (Bodmeier et al. 1996; Wen and Park 2010; Knop 1996; AlKhatib and Sakr 2003). However, the percentage of quaternary ammonium groups is different between them, being between 4.5% and 6.8% for Eudragit RS and between 8.8% and 12% for Eudragit RL. Thus, Eudragit RL is more hydrophilic and takes up more water (more permeable) than Eudragit RS (Wen and Park 2010; Korbely et al. 2012; Siepmann et al. 2008; Boza et al. 1999).

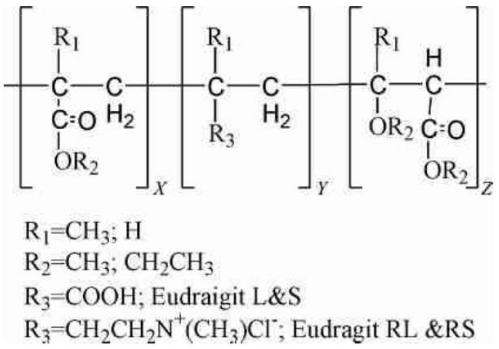

Figure 4. Chemical structure of Eudragit (Wen and Park 2010)

The release of the drug occurs by diffusion and erosion of the matrix tablet and the release rate is directly related to the liquid penetration into the matrix and subsequent drug dissolution (Korbely et al. 2012).

1.4.2.3. Kollidon® SR

Kollidon® SR is a polymeric blend of 80% polyvinyl acetate (PVA), 19% polyvinylpyrrolidone (PVP), 0.8% sodium lauryl sulphate (SLS) and 0.2% silica, commonly used in direct compression matrix tablets (Figure 5) (Bühler 2008; Sakr et al. 2011). PVP is water soluble and dissolves producing pores in the PVA insoluble matrix (Mastropietro et al. 2017; Strübing et al. 2008).

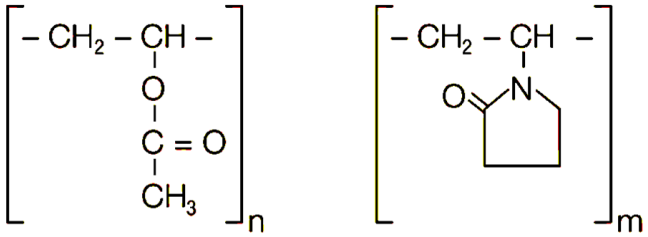

Figure 5. Chemical structure of the main components of Kollidon® SR (PVA, PVP) (BASF 2015)

Kollidon SR has high compressibility, due to its great dry binding properties. Tablets containing Kollidon SR show very high hardness levels because of the combination of PVA with plastic characteristics and PVP with greatly binding properties (Bühler 2008; Hauschild and Picker-Freyer 2006; Riis et al. 2007; Strübing et al. 2008). Prolonged release matrix tablets based on Kollidon SR have shown release-independency from pH, ion strength, compression

force and tablet hardness (Bühler 2008; Reza S. et al. 2003). It has been found that particle size of the active ingredient has an important effect on the release rate (Bühler 2008).

After matrix tablets based on Kollidon SR contact the medium, the liquid penetrates into the matrix and the water soluble PVP leaches out forming channels through which the drug diffuses out. (Engineer et al. 2004; Draganoiu 2003; Kranz et al. 2005; Sahoo et al. 2008; Sakr et al. 2011; Shao et al. 2001).

1.5. Pulsatile release system

Pulsatile drug-release system is characterised by a complete drug release after a specified lag time. A lag time is considered as a time period without drug release or with less than 10% release of the total amount of drug. This kind of delivery system is beneficial for drugs with a high first-pass effect or those which present biological tolerance, drugs with specific chrono-pharmacological requirements or targeting of a specific site of absorption in the intestinal tract (Bussemer et al. 2001; Maroni et al. 2005; Maroni et al. 2013; Bussemer et al. 2003; Jain et al. 2011; Mohamad and Dashevsky 2006; Sungthongjeen et al. 2004).

In a pulsatile drug delivery system, the drug should be released rapidly and completely after the lag time, as shown in Figure 6 A. However, drugs frequently show a prolonged release over time after the initial lag time (Figure 6 B&C) (Bussemer et al. 2001).

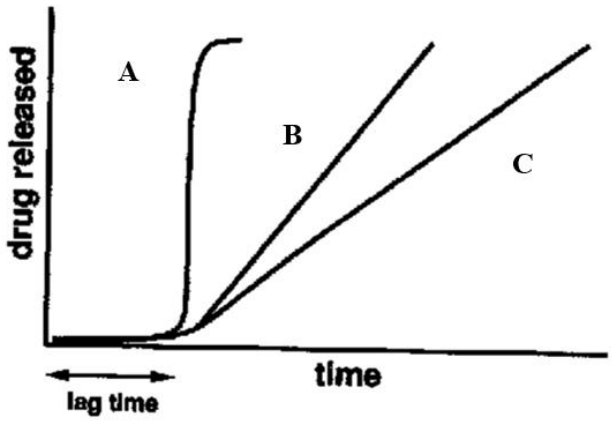

Figure 6. Pulsatile release profiles. A: Sigmoidal release; B & C: Delayed release after initial lag time.

Pulsatile drug delivery systems can be divided into site-specific systems and time-controlled systems. Site-specific systems release the drug in a specific desired location in the intestinal tract and it is regulated by factors, such as enzymes or pH in the intestinal region. Conversely, time-controlled systems, should not be affected by environment factors, such as pH, enzymes, ion strength, gastrointestinal transit time or food intake (Bussemer et al. 2001; Maroni et al. 2005; Sungthongjeen et al. 2004).

Time-controlled systems are classified as multiparticle systems (i.e., pellets) or single unit systems (i.e., tablets, capsules) (Bussemer et al. 2001). Coating with different features can be found in pulsatile drug delivery system, such as erodible, rupturable or permeable (Maroni et al. 2005). The majority of the systems are composed by a drug reservoir and a coating barrier which dissolves, erodes or ruptures (Bussemer et al. 2003; Sungthongjeen et al. 2004).

1.5.1. Erodible coating

Erodible coatings are barriers that swell, erode and/or dissolve after a programmed period (lag time). Afterwards, the drug is released fast from the core. The lag time can be regulated by the thickness of the coating layer (Bussemer et al. 2001; Maroni et al. 2005; Maroni et al. 2013). However, a possible problem with this system is the delay and non-immediate drug release after the barrier function is lost or the possibility of early drug release, especially with high soluble drugs (Bussemer et al. 2003; Mohamad and Dashevsky 2006).

1.5.2. Rupturable coating

Rupturable coating systems are composed of a drug-containing core, an optional swelling layer and the external layer-coating consisting of an insoluble, but permeable polymer (Dashevsky et al. 2004). The release of the drug in this kind of coating occurs after the coating layer breaks up, allowing the release of the drug from the core. The coating rupture is generated by hydrostatic pressure produced inside the system. This pressure can be reached using effervescent excipients or swelling agents, such as cellulose ethers or superdisintegrants (Bussemer et al. 2001; Maroni et al. 2013; Jain et al. 2011; Sungthongjeen et al. 2004).

Therefore, the lag time is increased by raising the coating level and increasing the core hardness (Bussemer et al. 2001; Maroni et al. 2013). The drug release from the majority of

rupturable systems depends on the core and also on the drug employed (i.e., solubility and dose) (Dashevsky et al. 2004). The lag time prior to the breakup of the system is defined mainly by the water (medium) permeability and mechanical properties of the outer layer (coating) and the swelling properties of the core or swelling layer (Bussemer et al. 2003).

One of the excipients use in the external layer-coating is Compritol 888 ATO (glyceryl behenate), a hydrophobic mixture of mono-(12 – 18% w/w), di-(45-54% w/w) and tri-(28-32% w/w) behenate of glycerol (Figure 7) (Aburahma and Badr-Eldin 2014). Compritol 888 ATO is a water insoluble excipient which does not swell or erode after contact with aqueous medium. It is not susceptible to physiological changes such as pH, digestion or alcohol (Gattefosse 2016). Drug release from matrix based on Compritol 888 ATO is produced by slow diffusion out of the inert matrix. The matrix tablet does not change the shape during the dissolution test (Gattefosse).

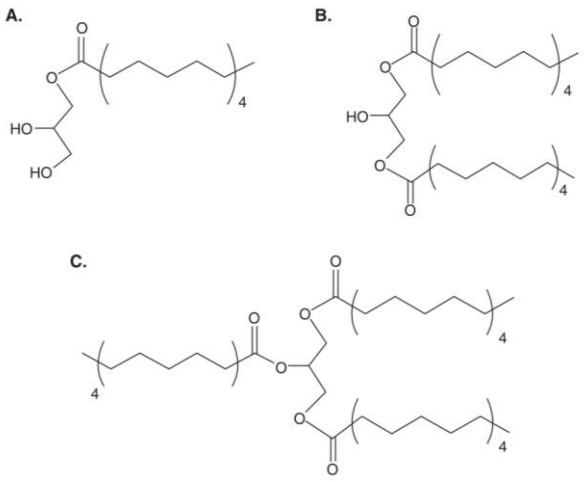

Figure 7. Chemical structure of Compritol ATO 888; A. Glyceryl monobehenate; B. Glyceryl dibehenate and C. Glyceryl tribehenate (Aburahma and Badr-Eldin 2014).

Other excipient commonly used in the core to generated the hydrostatic pressure inside the system and break the coating is Ac-Di-Sol (sodium croscaramellose), a superdisintegrant (substance with a high swelling potential), which has shown to be better than other swellable materials and could breakup completely the coating layer, due to its notable swelling energy, leading to a fast drug release after the programmed lag time (Maroni et al. 2005; Mohamad and Dashevsky 2006).

1.6. Research objectives

a. Evaluate physicochemical properties of melatonin, such as solubility, particle size and possible polymorphism.

b. Investigate the effect of:

- Polymer viscosity, loading, permeability, and particle size,
- preparation method and variation of agitation rate during dissolution test,

on melatonin release from matrix tablets based on different polymers.

c. Assess the effect of polymer loading on the lag time and melatonin release from pulsatile release tablets based on a rupturable-lipid coating.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Drug

Melatonin (Flamma S.p.A., Italy)

2.1.2. Matrix formers

Hydroxypropylmethylcellulose: Methocel® K15M Premium CR Grade, Methocel® K4M Premium CR, Methocel® K100 Premium LV, Methocel® E50 Premium LV and Methocel® E15 Premium LV (Colorcon Ltd, Dartford, Kent, UK); ethylcellulose: Ethocel® Standard 10 FP Premium and Ethocel® Standard 10 Premium (Colorcon Ltd, Dartford, Kent, UK); Kollidon® SR (BASF AG, Ludwigshafen, Germany); Polymethacrylates: Eudragit® RL PO and Eudragit® RS PO (Evonik Röhm GmbH, Darmstadt, Germany); glyceryl behenate: Compritol® 888 ATO(Gattefosse, France).

2.1.3. Other excipients

Lactose monohydrate, (FlowLac® 100, Meggle Wasserburg GmbH & Co. KG, Wasserburg, Germany); magnesium stearate (Baerlocher GmbH, Unterschleißheim, Germany); dicalcium phosphate dihydrate (Emcompress® Premium, JRS Pharma GmbH & Co. KG, Rosenberg, Germany); colloidal silicon dioxide, (Aerosil® 200, Evonik Industries AG, Essen, Germany); polyvinyl pyrrolidone, PVP (Kollidon® 30, BASF SE, Ludwigshafen, Germany); croscarmellose sodium (Ac-Di-Sol®, FMC, Philadelphia, USA); lake pigment HT® (5285 FD&C Yellow N 6 Aluminium lake, Colorcon Ltd, Dartford, Kent, UK).

2.2. Methods

2.2.1. Drug solubility

Excess of melatonin was added to glass containers with lid, containing either 10 mL of hydrochloric acid (HCl) 0.1 N or buffer phosphate pH 6.8. Afterwards, the samples were vortexed and agitated at 37°C for 48 h, taking a sample at 24 h. Following by centrifugation for 10 min at 3200 rpm to separate the undissolved drug. The supernatant solutions were diluted

(1:100) to a suitable concentration, and the dissolved drug was analysed at wavelengths of 220 nm and 273 nm in a UV-Vis spectrophotometer (2101 PC, Shimadzu Scientific Instruments, USA). The test was performed in triplicate.

2.2.2. Particle size

Particle size of melatonin was evaluated in a Zeiss polarized light microscope (Axioskop), measuring one by one the particle size for a total of 164 particles (Figure 8). The data obtained was used to obtain the particle size distribution.

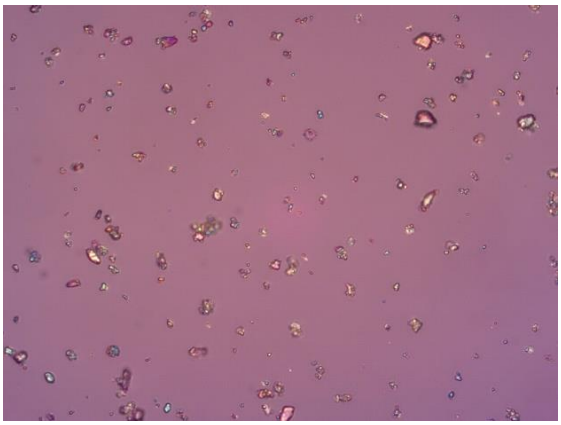

Figure 8. Melatonin particles observed in a polarized light microscope.

Polymers size distribution was evaluated by laser diffraction (Sympatec particle size analyzer HELOS BF). The material was sieved prior to the measurement to avoid agglomeration. The sample was initially analysed using the lens with a range of 875 µm and according to the results, the sample was evaluated afterwards, with the lens of 175 µm or 35 µm.

2.2.3. DSC analysis

Differential scanning calorimetry (DSC) was performed on the powder of melatonin and the crystals (recrystallization) obtained after oversaturation of a solution of IPA:water (80:20) with melatonin, then drying the crystals in an oven at 50°C for 10 hours. The analysis was done in a DSC 6000 (Perkin Elmer) in a temperature range of 29 °C to 195 °C, at a rate of 10°C/min. The samples weighed 5.569 mg and 7.456 mg for the powder and crystals, respectively.

2.2.4. Preparation of tablets

2.2.4.1. Direct compression

The ingredients were weighed and sieved through a 250 μm mesh. The drug, matrix former and filler in different ratios (Table 2) were mixed manually, using a bowl and a spatula. Afterwards, magnesium stearate 1%w/w was added and further blended. A single punch tableting machine (Korsch EK0, Korsch Pressen GmbH, Berlin, Germany) was equipped with a 12 mm flat faced punch. 500 mg of the blend was weighed and compressed manually into a tablet, keeping the compression force and hardness (hardness tester Erweka GmbH, Heusenstamm, Germany) constant in each formulation (Table 3).

Table 2. Formulations of prolonged release matrix tablets containing 20 mg melatonin.

Component	**%**	**%**	**%**
Melatonin	4	4	4
Lactose	75	60	45
Magnesium stearate	1	1	1
Matrix former	20	35	50

Table 3. Compression force and hardness of prolonged release matrix tablets.

Tablets based on	**Compression force ± 1 kN**	**Hardness**
Eudragit	15	78 ± 5 N
Methocel Ethocel 10	11	87 ± 5 N
Kollidon SR (35%) Ethocel 10 FP (20%)	9	104 ± 1 N
Kollidon SR (50%) Ethocel 10 FP (35%)	9	126 ± 2 N

2.2.4.2. Wet granulation

The ingredients were weighed and sieved through a 250 μm mesh. Melatonin (4% w/w; 20 mg per tablet), matrix former (Eudragit RS or Ethocel 10) (35% w/w), and lactose (60% w/w), were mixed manually, obtaining 30 g of blend. The powder blend was transferred into a granulator (Diosna P1/6 Mixer/Granulator) and a mix of IPA: water (80:20) was added steadily and slowly while employing a rotation speed of 50 rpm, until a mass with suitable consistency

was obtained. Blends containing Eudragit RS and Ethocel 10, required 2.00 g and 3.76 g of liquid, respectively, to obtain an appropriate mass. The wet mass was sieved through a 900 µm sieve and the resulting granules were dried for 18 h in an oven at 50°C. The granules were weighed before and after drying.

Humidity of the granules was measured with a moisture tester (Mettler Toledo). Subsequently, the granules were mixed with magnesium stearate 1%w/w and compressed manually in a single punch tableting machine (Korsch EK0, Korsch Pressen GmbH, Berlin, Germany) equipped with 12 mm flat faced punch, taking 500 mg of the blend per each tablet. The compression force and hardness (hardness tester, Erweka GmbH, Heusenstamm, Germany) were kept constant with 20 ± 1 kN; 67 ± 2 N for Ethocel 10, and 18 ± 1 kN; 82 ± 2 N for Eudragit RS.

2.2.4.3. Press coated tablets

The ingredients (Table 4) of the cores were weighed, sieved (250 µm) and blended manually using a bowl and spatula. Afterwards, Magnesium stearate and Aerosil® were added and mixed. A single punch tableting machine (Korsch EK0, Korsch AG, Berlin, Germany) was equipped with a 5 mm flat faced punch (cores 1) and with 8 mm flat faced punch (cores 2). Each core was weighed manually, containing 60 mg each core 1, and 150 mg each core 2. Cores were compressed one by one setting the compression force at 9 ± 1 kN (core 1) and 15 ± 1 kN (core 2). The hardness was measured employing a hardness tester (Erweka GmbH, Heusenstamm, Germany), obtaining 60 ± 2 N for cores 1 and 84 ± 1 N for cores 2.

Table 4. Core formulations containing 20 mg melatonin.

Component	Core 1 (%)	Core 2 (%)
Melatonin	33.33	13.33
Lactose	45.17	64.00
Polyvinylpyrrollidone	6.00	7.00
Sodium croscamellose	14.00	14.00
Magnesium stearate	0.80	1.00
Silicon dioxide	0.50	0.50
Pigment	0.20	0.20

The coating blends were prepared by manual mixing of the sieved (250 µm mesh) materials (Table 5 and 6). Afterwards, magnesium stearate was added and mixed.

Table 5. Coating formulation and hardness of pulsatile release tablets containing Dibasic calcium phosphate.

Component	**%**
Glyceryl behenate	40.00
Dibasic calcium phosphate	50.00
Polyvinylpyrrollidone	8.40
Magnesium stearate	1.00
Silicon dioxide	0.60
Hardness of the press-coated tablets	78 ± 2 N

Table 6. Coating formulations and hardness of pulsatile release tablets containing lactose.

Component	**%**	**%**	**%**	**%**
Glyceryl behenate	50.00	40.00	30.00	20.00
Lactose	40.00	50.00	60.00	70.00
Polyvinylpyrrollidone	9.50	9.50	9.50	9.50
Magnesium stearate	0.50	0.50	0.50	0.50
Hardness of the press-coated tablets	82 ± 2 N	96 ± 2 N	101 ± 2 N	117 ± 2 N

The coating of the formulation containing Dibasic calcium phosphate (Table 5), was approximately 6 times the weight of the core, weighing of 340 mg (coating). The proportion of the coating per tablet in respect to the core weight was evaluated (Figure 9), resulting in the best option 3 times the weight of the core, with a weight of 450 mg, for the coating of the formulations containing lactose (Table 6).

A single punch tableting machine (Korsch EK0, Korsch Pressen GmbH, Berlin, Germany) was equipped with a 9 mm flat faced punch for the formulation containing Dibasic calcium phosphate (containing core 1) and with a 12 mm flat faced punch for the formulation

containing lactose (containing core 2) resulting in 4 mm of difference between the core and the final tablet for all the formulations.

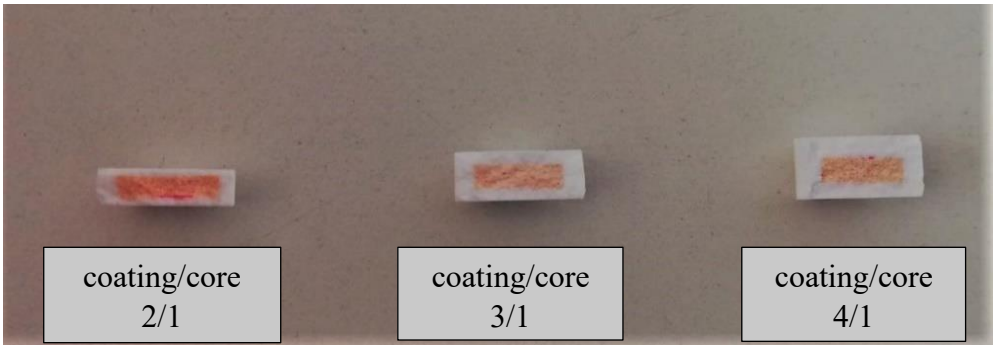

Figure 9. Cross section of tablets with different coating/core ratio.

The coating was compressed setting 40% of the blend per tablet in the die, and setting a low compression force, with the aim of reducing the volume of the blend. The tablet core was centrally placed, and the die filled with the rest of the blend per tablet, equivalent to 60%. Finally, the tablet was compressed with a compression force of 11 ± 2 kN for a final weight of 400 mg per tablet for the formulation containing Dibasic calcium phosphate and 20 ± 2 kN for a final weight of 600 mg per tablet the formulations containing lactose. The hardness of the tablets was measured in a hardness tester (Erweka GmbH, Heusenstamm, Germany) (Tables 5 and 6).

2.2.5. Characterization

2.2.5.1. Release studies

Release studies were performed using a USP paddle apparatus (VK 7000, Agilent Technologies Deutschland GmbH, Böblingen, Germany) 50 rpm, 37°C, 500 ml, phosphate buffer pH 6.8. Press coated tablets were also analyzed in HCl 0.1N pH 1.2. Samples were taken at predetermined times and drug release was evaluated by UV spectrophotometer (2101 PC, Shimadzu Scientific Instruments, USA) at a wavelength of 273 nm.

The agitation rate evaluation on matrix tablets was performed at 10, 25, 50 and 100 rpm.

2.2.5.2. Disintegration test

Disintegration was evaluated using a disintegration tester (PharmaTest PTZ-Auto), at 37°C, n=3. During the first hour the tablets were immersed in HCl 0.1 N, and afterwards the intact tablets were transferred to a phosphate buffer pH 6.8 medium, until the tablets disintegrated. The time for each tablet to get disintegrated was registered automatically by the machine.

3. RESULTS AND DISCUSSION

3.1. Drug solubility

Drug solubility plays an important role in the release mechanism and rate from matrix tablets. Therefore, melatonin solubility was analysed in HCl 0.1 N and buffer pH 6.8 at 37°C. The results show a marginal difference between melatonin solubility in HCl 0.1N and buffer pH 6.8, getting a slightly better solubility in HCl 0.1N than in buffer pH 6.8 (Table 7).

Table 7. Melatonin solubility in phosphate buffer pH 6.8 and HCl 0.1 N, pH 1.2.

Solubility	**24h (mg/mL)**	**48h (mg/mL)**
HCl 0.1 N	2.61	3.15
Buffer pH 6.8	2.42	2.90

The most common doses of marketed melatonin are 2 mg and 3 mg, but there are some clinical trials with higher doses such as 10 or 20 mg (clinicaltrials.gov). Therefore, the highest single therapeutic dose of melatonin is soluble in 250 mL or less of an aqueous medium at pH of 1.2 and 6.8 at 37±1°C (European Medicines Agency 2020). According to the BCS and the results found, melatonin is considered a highly soluble substance with a solubility around 3 mg/mL in both mediums, which means approximately 750 mg/250 mL.

3.2. Particle size

Particle size is also an important factor in solid dosage forms which affects the drug release rate. The particle size of melatonin and polymers, posteriorly used in this study, were analyzed. Melatonin particle size was analyzed by microscopy (Figure 10), showing a D50 of 4.1 µm and D90 of 8.1 µm (Table 8), which indicates that it is a micronized powder.

The particle size of 10 polymers were analysed by laser diffraction, obtaining similar results for all of them with a range of D50 between 60 and 96 µm, except for Ethocel which has 2 completely different particle size. Ethocel 10 FP has fine particles (micronized) with a D50 of 5.2 µm and Ethocel 10 has coarse particles having a D50 of 164.3 µm.

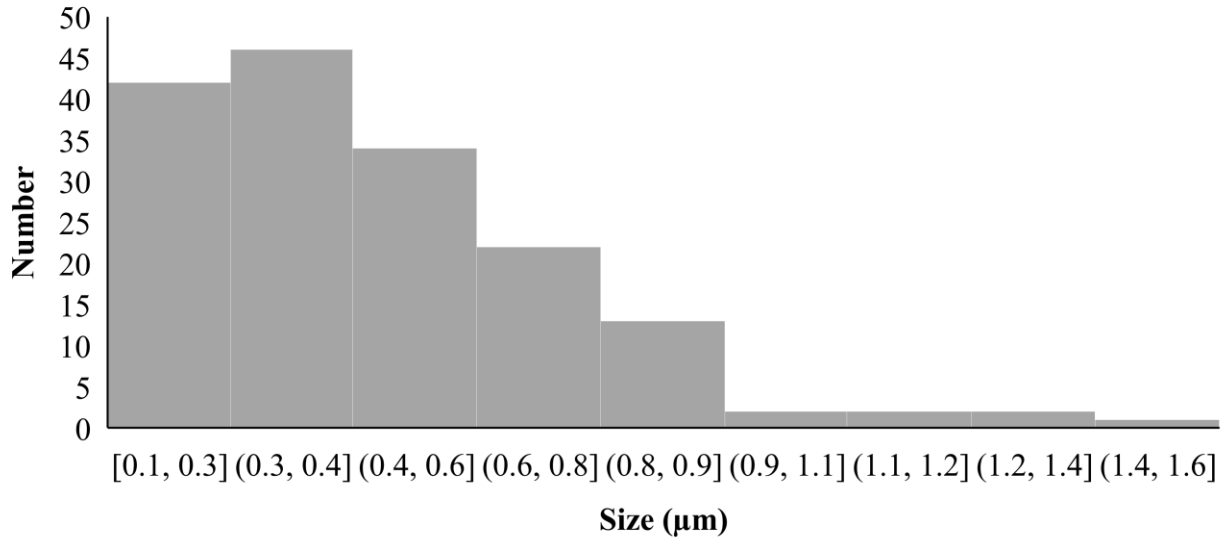

Figure 10. Melatonin particle size distribution

Table 8. D50 and D90 of the materials

Materials	D50 (µm)	D90 (µm)
Melatonin	4.1	8.1
Ethocel 10 FP	5.2	9.7
Ethocel 10	164.3	290.0
Kollidon SR	74.0	149.2
Eudragit RS	60.7	197.0
Eudragit RL	86.2	231.2
Methocel E15LV	67.3	174.5
Methocel E50LV	68.9	178.0
Methocel K15MCR	67.8	168.1
Methocel® K100LV	70.4	171.0
Methocel K4MCR	95.6	227.8

3.3. DSC analysis

DSC is a useful tool for detecting and quantifying different crystalline forms. DSC measures temperatures and enthalpy associated with transitions in materials (TA Instruments). DSC analysis was performed to evaluate the presence of polymorphism in melatonin, before preparing the tablets by wet granulation.

The samples present the same endothermic reaction, the melting that occurs at 118.5°C (Figure 11), which confirms the recrystallization in the same polymorphic form. This result is consistent with the information shown in the assessment report of Slenyto, EMEA/H/C/004425/0000 (European Medicines Agency- CHMP 2018).

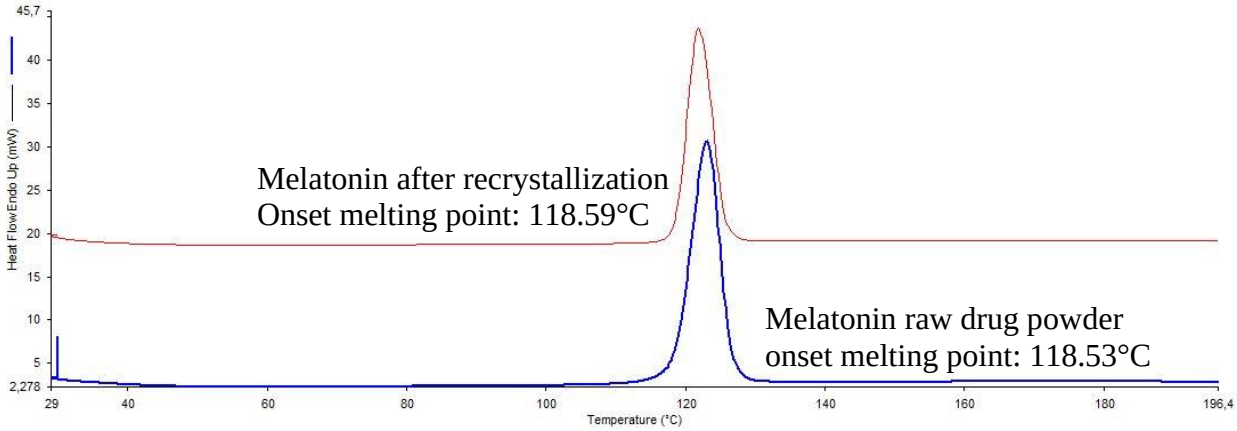

Figure 11. DSC Thermogram of Melatonin.

Therefore, wet granulation method could be used to prepare tablets containing melatonin, due to the absence of melatonin polymorphs forming. After dissolution and recrystallization of the drug by this method other crystalline forms will not be observed and therefore, no changes in physical properties, quality or performance of the drug are expected.

3.4. Prolonged release tablets

Prolonged release matrix tablets prepared and analysed in this study are based on polymers with different characteristics such as solubility, permeability, swellability and so on. Melatonin release was evaluated when changing polymer types and loading in the formulation. The filler used in these formulations was lactose (Flowlac® 100), a water-soluble ingredient and as a lubricant, magnesium stearate was employed.

A blank with the excipients was analysed in both wavelengths, 220 nm and 273 nm. Kollidon® SR showed an absorption at 220 nm, due to the presence of PVP in its composition, which has the main absorption peak at 213.5 nm (Tavlarakis et al. 2011). Therefore, the wavelength 273 nm was chosen to analysis all the formulations and avoid interferences.

3.4.1. Erodible matrix tablets

Erodible matrices are based on hydrophilic/soluble polymers, such as hydroxypropyl-methylcellulose, which is chemically stable over the pH range of 3 to 11 and widely used in modified release. These matrices hydrate when contact with aqueous medium, forming the gel layer, which acts as a barrier for water penetration and drug diffusion. Over the time, the gel layer dissolve or erode after being completely hydrated (Mastropietro et al. 2017; Wen and Park 2010; Timmins et al. 2014; Dabbagh M.A. et al. 1996; Reza S. et al. 2003).

HPMC (Methocel®) has different viscosity grades which can modify the drug release rate. Thus, higher viscosity grade produces greater gel viscosity, and the drug release diffusion will be slower, as well as the gel layer will be more resistant to erosion (Timmins et al. 2014). In addition, it has been demonstrated that greater content of HPMC in a matrix tablet, produce slower drug release rate (Timmins et al. 2014).

Methocel® was selected to prepare the erodible matrix tablets and evaluate the effect of polymer viscosity on melatonin release. Five types of Methocel® with different viscosity grades were chosen. These polymers belong to two categories, K and E, with different degrees of methoxyl and hydroxypropoxyl substitution (Table 1). The preparation of these tablets was performed by direct compression, due to the ease and simplicity in manufacturing. The formulations chosen for this evaluation contain 35% of polymer.

During the test, the tablets containing Methocel E15LV and Methocel E50LV increased slightly in size, and eroded rapidly, with the tablets breaking in 2 parts, around 30 min and 1 hour, respectively after staring the test. The results obtained by UV analysis showed an 80 % release within the first 2 and 4 hours, respectively. After 30 min and 1 hour, the release is abrupt for Methocel E15LV and E50LV respectively (Figure 12), which is explained by the erosion of the matrix at this point, leading to an increase in the surface area and a faster release of melatonin.

The rapid release in these two kinds of matrix tablets can be explained by two reasons. On one hand, Methocel E is less hydrophilic than Methocel K, due to the grades of substitution, and produces less swelling in these tablets. Thus, the gel layer formation is slower, and the drug started release before complete gel formation. On the other hand, the grade of viscosity of these two tablets is low in comparison with the others producing a less viscous and a thinner gel layer which erodes fast.

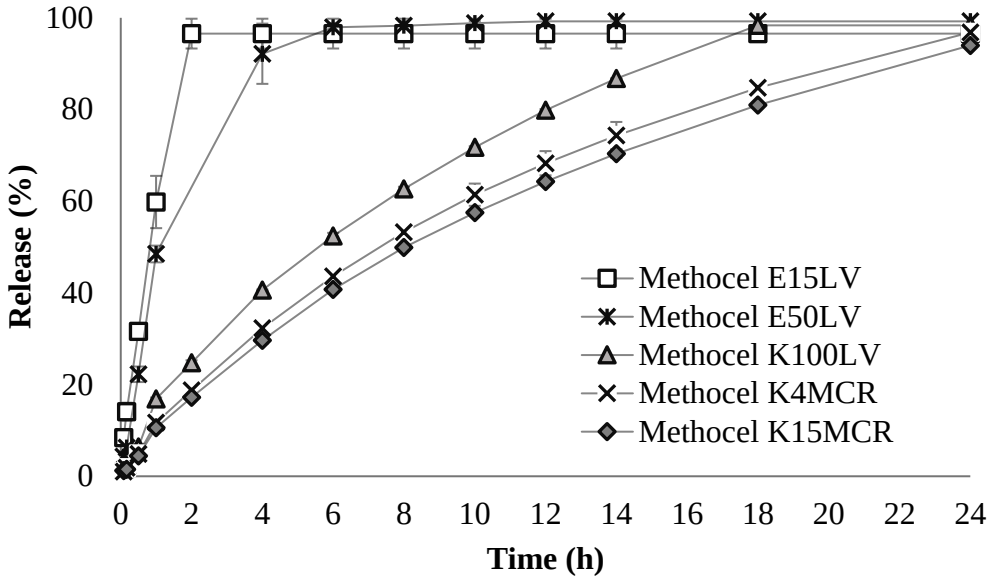

Figure 12. Effect of Methocel viscosity on melatonin release from matrix tablets.

In the tablets containing Methocel K100LV, Methocel K4MCR and Methocel K15MCR, the release was slower than tablets with Methocel E. During the dissolution test the fast formation of a thick gel layer for all of them was observed. The tablets swell and increase in size, without complete erosion or dissolution until the end of the test for Methocel K4MCR and K15MCR (Figure 13), but in the case of Methocel K100LV, it disintegrates 18 hours after starting the dissolution test.

The 80% of melatonin release from the matrix tablets containing Methocel K100LV, K4MCR and K15MCR was obtained at 12, 16 and 18 hours, respectively (Figure 12). As expected, the release is slower when the polymer viscosity increases.

Figure 13. Matrix tablets containing Methocel K15MCR before (left) and after (right) dissolution test at 50 rpm.

Melatonin is a soluble drug, for that reason, the release from HPMC matrix tablets is via diffusion through the gel layer, instead of by erosion of the matrix, which is the case for insoluble drugs. After the medium penetrates the matrix through pores, melatonin dissolves and diffuses through these channels. Thus, the release rate is controlled by the speed of gel formation and the thickness and viscosity of it.

Methocel with higher viscosity rapidly forms a thicker layer producing a slower melatonin release, while in the case of Methocel E15 and E50, the viscosity is low and the gel layer is not formed fast enough, thus, the drug release faster due to the incomplete and weaker gel formation.

3.4.2. Non-erodible matrix tablets

The release of the drug from non-erodible matrices is primarily controlled by diffusion through the pores. Common examples of these matrix formers are Kollidon SR, Eudragit and Ethylcellulose. All of them are water-insoluble and pH independent polymers.

EC is a non-swellable polymer, while Eudragit, due to the presence of quaternary ammonium groups is able to swell and hydrate. Kollidon SR is composed of two main ingredients, the insoluble PVA with plastic characteristics and the soluble PVP with significant binding properties. Grund et al. (2014) found that the compactibility of these polymers follow the order: Kollidon SR>ethylcellulose>Eudragit RS. The main consolidation mechanism of these polymers is plastic deformation.

Non-erodible matrix tablets were prepared based on Ethocel 10, Ethocel 10 FP, Eudragit RS, Eudragit RL and Kollidon SR, to evaluate the effect of the polymer loading, particle size, permeability, and method of preparation on the release of melatonin.

The hardness of the tablets was measured (Table 3), getting the highest values with Kollidon SR and Ethocel 10 FP, increasing the tablet strength when polymer content was risen. Kollidon SR is known for its high compactibility properties, and in the case of Ethocel FP, due to its small particle size it has a better packing density and increased number of contact points in the powder, giving more particle-particle interaction and bonding surface area, which increase the tablet hardness. Therefore, strength and porosity of tablets based on EC are dependent on the initial particle size, due to EC consolidating through plastic deformation (Katikaneni et al. 1995b).

The evaluation of the polymer loading on melatonin release from matrix tablets was performed with Eudragit RS, Kollidon SR and Ethocel 10, with 2 different loadings in the formulation, 35% and 50%.

Ethocel 10 (D50 164.3 µm) and Ethocel 10 FP (D50 5.2µm) we chosen to evaluate the polymer particle size (Table 8) on melatonin release from the matrix tablets. The loading selected for this evaluation was set at 35% and 50% for Ethocel 10, and 20% and 35% for Ethocel 10 FP.

Permeability was evaluated with matrix tablets based on Eudragit RS and Eudragit RL with 35% and 50% loadings.

The method of preparation of the matrix tablets was evaluated using Eudragit RS and Ethocel 10 with 35% loading in the formulation. Matrix tablets were prepared by direct compression and wet granulation.

3.4.2.1. Effect of polymer loading on melatonin release from non-erodible matrix tablets

During the dissolution test the tablets containing Kollidon SR swelled and grew for both amounts 35% and 50% and remained this size until the end of the test, forming a rubbery tablet, difficult to break (Figure 14).

Tablets containing Eudragit RS 35% disintegrated partially, while the matrix tablets containing 50% of this polymer grew slightly in size (swelling) and remained intact until the end of the test (Figure 14). However, the matrix tablets based on Kollidon SR grew more in size than tablets containing Eudragit RS, 50% (Figure 14).

Matrix tablets based on Ethocel 10 containing 35% of polymer showed a partial disintegration during the test, whereas matrix tablets containing 50% of this polymer, remained intact, without increasing the size of the tablet, since EC is a non-swellable polymer (Figure 14).

Results show that the release rate of melatonin is faster when less polymer is employed. Employing 35% of polymer, the release of 80 % melatonin was within the 3, 4 and 7 hours for Kollidon SR, Eudragit RS and Ethocel 10, respectively (Figure 15). Employing 50% of

polymer decreases the release rate, giving as a result an 80% melatonin release within 7, 11, and 19 hours for Kollidon SR, Eudragit RS and Ethocel 10, respectively (Figure 16).

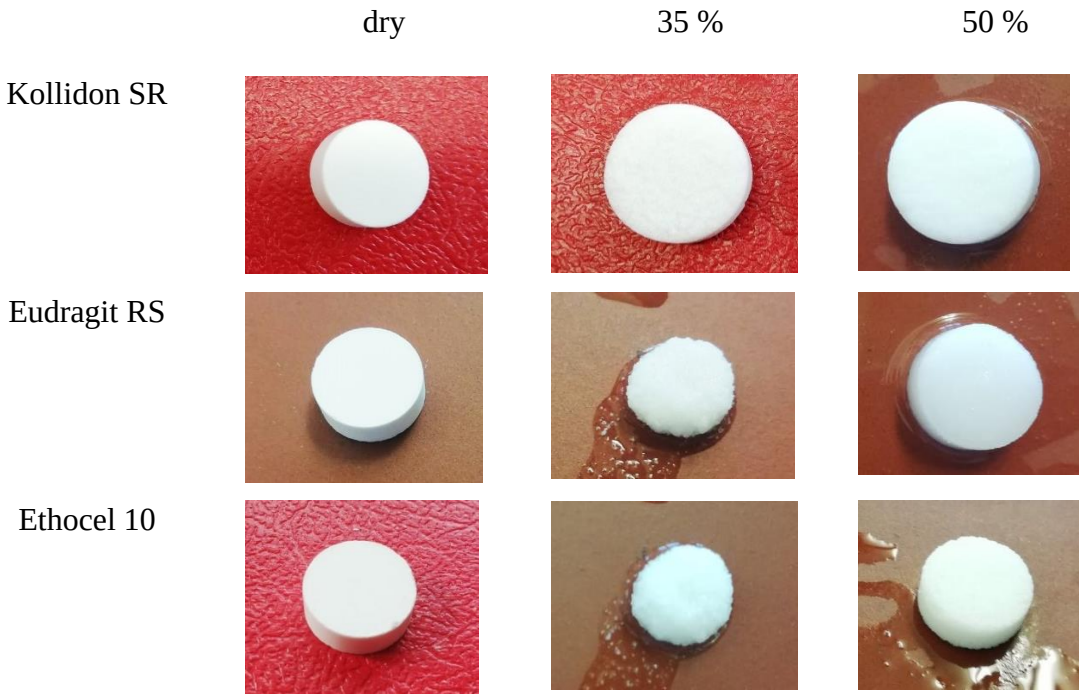

Figure 14. Pictures of matrix tablets containing Kollidon SR, Eudragit RS and Ethocel 10, before (dry) and after dissolution test, with 35% and 50% loading.

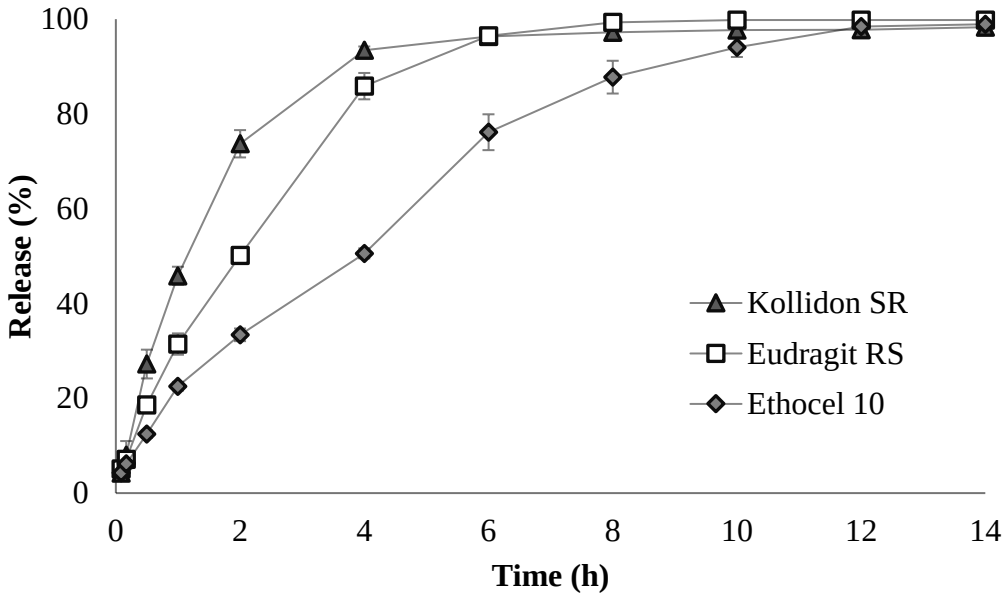

Figure 15. Melatonin release behaviour from matrix tablets containing 35 % of different insoluble polymers.

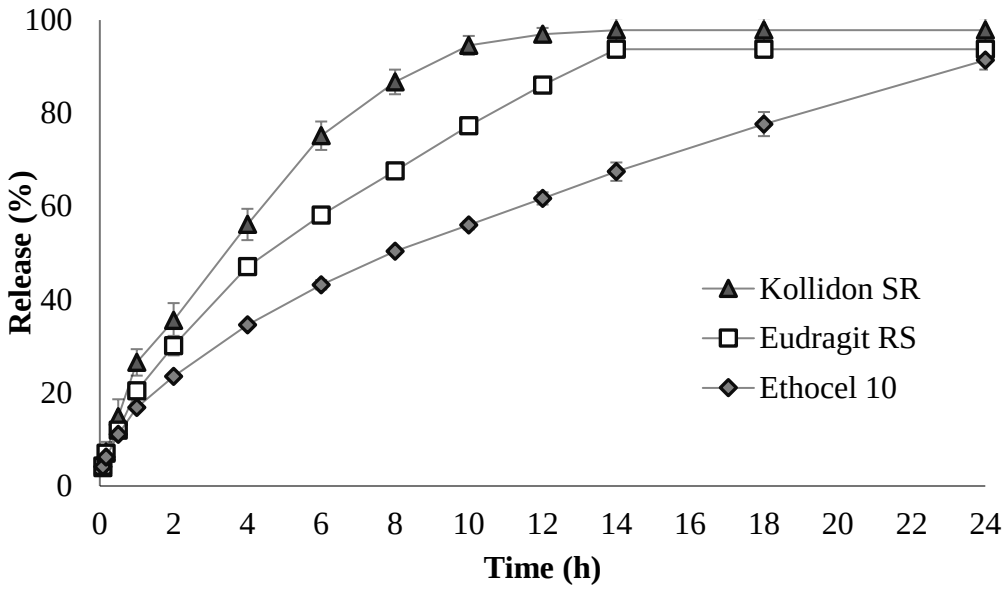

Figure 16. Melatonin release behaviour from matrix tablets containing 50 % of different insoluble polymers.

The fastest release of melatonin was observed with matrix tablets containing Kollidon SR, the most permeable polymer due to the content of PVP (19%), a soluble ingredient. Followed by Eudragit RS, the second most permeable polymer which allows the medium diffusion through the hydrated quaternary ammonium groups. The slowest release of melatonin was observed from matrix tablets based on Ethocel 10, because it is an impermeable polymer.

As mentioned above, the faster release of the matrix tablets based on Kollidon SR can be explained due to the presence of PVP, a soluble component of Kollidon SR, which leaches out of the matrix forming pores through which the drug diffuses, and which decreases the tortuosity of the matrix and increases the porosity of it. Additionally, the formulation of these tablets contains lactose (FlowLac® 100), a water-soluble pore-forming excipient which increases the porosity of the matrix tablet, raising the release rate of melatonin. Agnese et al. (2010), Kranz et al. (2005) and Shao et al. (2001), have shown that adding lactose to a formulation based on Kollidon SR, increase the release rate of the drug in comparison with matrix tablets containing only Kollidon SR, without a pore former. (Agnese et al. 2010; Kranz et al. 2005; Shao et al. 2001).

Kollidon SR has low hydration and water uptake properties (Reza S. et al. 2003), which keep the matrix shape due to the main component of Kollidon which is the insoluble PVA. However, a slow hydration of the polymer, could produce a significant percentage of the drug

to be release during the beginning of the release study (Reza S. et al. 2003), which may explain the faster initial release from Kollidon SR matrix tablets.

Eudragit RS swells and hydrates, due to its composition containing quaternary ammonio groups which makes Eudragit RS more hydrophilic (permeable) and allows water penetration into the matrix tablet and faster release of the drug by diffusion through these channels.

Ethocel 10 is a non-swellable and impermeable polymer, which remains intact during the dissolution test, producing a reduction in release rate since the release occurs by diffusion, and produces a drug concentration gradient inside the medium-filled pores of the matrix, giving as a result a decrease on diffusion driving force (Streubel A. et al. 2000).

Regarding the percentage of polymer in the formulation, the release rate of melatonin decreases when the polymer amount was increased, due to the decline in matrix porosity. These results are consistent with those presented by Reza et al. (2003), who showed a decrease in the drug release rate after increasing the polymer content in the formulation, having three different polymers.

In addition, the release rate of melatonin was faster from matrix tablets containing 35% of Ethocel 10 and Eudragit RS, due to the partial disintegration of the matrix during the dissolution test, which lead to an increase in the surface area and a faster release of melatonin.

3.4.2.2. Effect of polymer particle size on melatonin release from matrix tablets based on Ethocel

During the 24 hours of the dissolution test the matrix tablets containing 35% Ethocel 10FP and 50% Ethocel 10 remained intact (Figures 14 and 17), while reducing the amount of both polymers produces a partial disintegration of the tablets.

Tablets based on Ethocel 10FP 20% showed a partial disintegration at the end of the dissolution test (Figure 17). In the case of matrix tablets containing 35% of Ethocel 10, a partial disintegration of the tablet began after 4 hour and carried on until the end of the dissolution test of 24 hours (Figure 14). Consequently, these tablets show an abrupt release between 4 and 6 hours, due to the partial disintegration of the matrix tablets, which led an increase in surface area and rather uncontrolled release in this point of the dissolution test (Figure 18).

Figure 17. Matrix tablets containing Ethocel 10FP, 20% (left) and 35% (right), respectively, after 24 hours of dissolution test, at 50 rpm.

The slowest release was observed from the matrix tablets containing 35% of Ethocel 10FP, which achieved 79% in 24 hours. In contrast, tablets containing the same amount of Ethocel 10 in the formulation, showed the fastest release, with 80% release within 7 hours of dissolution test. Matrix tablets containing Ethocel 10 FP 20% and Ethocel 10 50% show 80% melatonin release at 12 and 19 hours, respectively (Figure 18).

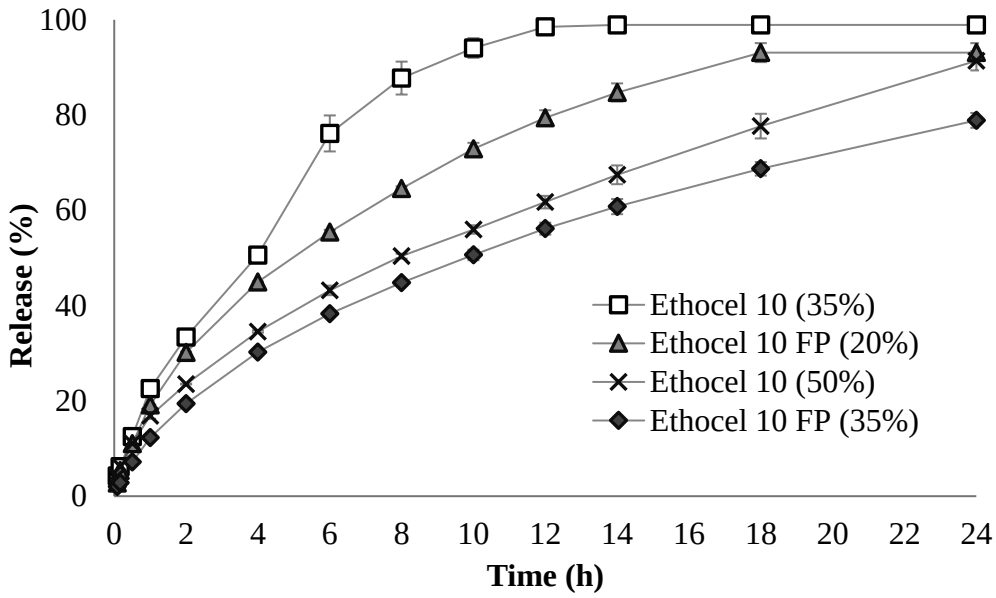

Figure 18. Effect of Ethocel particle size and loading on melatonin release from matrix tablets.

Increasing the amount of polymer by 15% within this formulation produces a slower melatonin release, requiring approximately 12 hours more to get the 80% release with both polymers (Figure 18). Changing the particle size of the polymer from Ethocel 10 with D50 of 164.3 µm to Ethocel 10FP with D50 of 5.2µm, having the same amount in the formulation,

give as a result a much slower melatonin release, taking 17 hours more to get the 80% release, with Ethocel 10FP in comparison with Ethocel 10.

Slower melatonin release from matrix tablets based on Ethocel with a smaller particle size is explained due to the higher amount of EC particles which forms fewer cluster of a soluble drug (in this case, melatonin), resulting in a less large and a more tortuous pore network (Crowley et al. 2004) and a decrease in the matrix porosity, producing a slower melatonin release. Therefore, when a larger particle size of Ethocel is employed, water can easier penetrate the matrix, because the mechanism of drug release from matrix based on EC is mainly by diffusion through the medium-filled channels.

The results obtained are consistent with the ones reported by Mohammad A. et al., (1996) who found that matrix tablets containing EC with less than 125 μm particle size show slower release than matrices with coarser particles. Therefore, the release rate declines when particle size decreases. Likewise, Katikaneni et al (1995), found that the release of pseudoephedrine hydrochloride from matrix tablets prepared by direct compression and based on EC was controlled by EC particle size and compression force.

3.4.2.3. Effect of the preparation method on melatonin prolonged release non-erodible matrix tablets

The granules obtained by wet granulation showed a weight loss of 0.78 g and 2.42 g for Eudragit RS and Ethocel 10, respectively after drying. The moisture content (MC) obtained was 1,49% MC and 1,10% MC for the granules containing Eudragit RS and Ethocel 10, respectively.

Matrix tablets prepared by wet granulation for both polymers disintegrated after 5 hours of dissolution testing, leaving the granules loose. In the case of matrix tablets prepared by direct compression, as was mentioned previously in section 3.4.2.1, disintegrated partially after around 4 hours of dissolution test.

The release rate of melatonin is faster when tablets were prepared by direct compression than by wet granulation in both cases (Ethocel 10 and Eudragit RS). Matrix tablets based on Ethocel 10 showed an 80% release within 7 and 10 hours for direct compression and wet granulation method of preparation, respectively (Figure 19).

A constant and steady release from tablets prepared by wet granulation can be seen (Figure 19), while after 4 hours tablets prepared by direct compression show an abrupt release, which is induced by the partial disintegration observed at this point of time during the dissolution test.

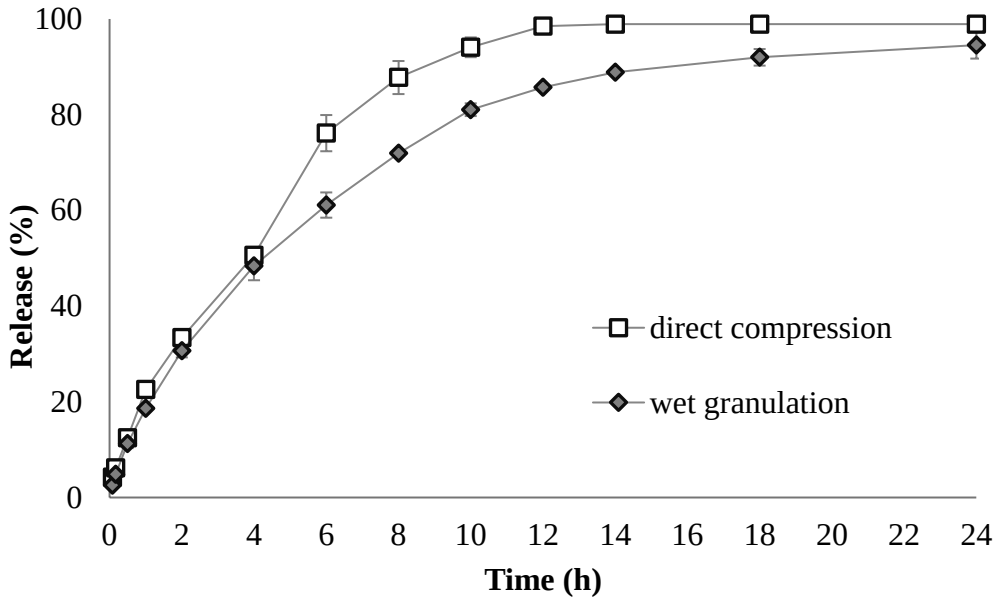

Figure 19. Effect of the preparation method on melatonin release from matrix tablets based on Ethocel 10, 35%.

Matrix tablets based on Eudragit RS presented an 80% release within 4 and 5 hours for direct compression and wet granulation method of preparation, respectively. The tablets obtained by the two methods get complete release at the same time in 6 hours (Figure 20). However, the release before this time is slower in tablets prepared by wet granulation.

After partial disintegration of the tablets obtained by direct compression, the particles get more area of contact, which allows a faster and abrupt release at this time, while the tablets prepared by wet granulation disintegrate into the granules containing the blend of ingredients, thus, the release is governed by the shape and size of the granules, which is larger compared to the direct compressed particles.

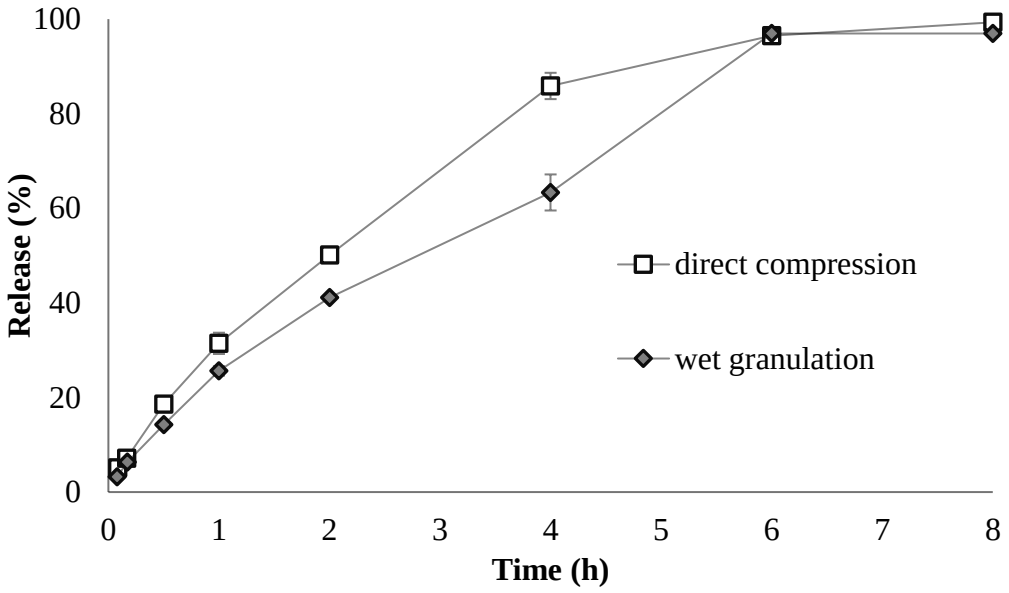

Figure 20. Effect of the preparation method on melatonin release from matrix tablets based on Eudragit RS, 35%.

Additionally, the decrease in the release rate when wet granulation was employed instead of direct compression could be explained, due to the improvement in granule compressibility, which results in lower porosity. It may produce a better interparticle cohesion, giving as a result a slower drug release rate, due to the decline in the water penetration rate through the matrix pores. These results differ from the ones found by Grund et al. (2014), who reported no effect in Eudragit RS and ethylcellulose matrices when prepared by wet granulation and direct compression, but they are consistent with the results obtained from Khan and Meidan (2007), who reported much slower release from EC matrix tablets prepared by wet granulation, in comparison with direct compression (Khan and Meidan 2007).

3.4.2.4. Effect of polymer permeability on melatonin release from matrix tablets based on Eudragit.

The matrix tablets based on Eudragit RL disintegrated rapidly during the dissolution test, in the case of the tablets containing 35% of polymer, the disintegration started immediately after getting in contact with the medium but reaching complete disintegration after around 1 hour. Tablets containing 50% of this polymer began after 1 hour a slow disintegration and getting it almost complete after 2 hours.

The release rate of matrix tablets based on Eudragit RL was extremely fast, achieving 80% melatonin release within 1 and 1.5 hours, for the tablets containing 35% and 50% respectively (Figure 21).

Matrix tablets based on Eudragit RS, showed a slower release than tablets based on Eudragit RL, reaching 80% melatonin release within 4 and 11 hours for 35% and 50% content, respectively (Figure 21). These tablets do not disintegrate as fast as the ones containing Eudragit RL. As explained above, tablets based on 35% Eudragit RS showed partial disintegration after the dissolution tests and tablets containing 50% of this polymer swelled and kept the shape after finishing the test (Figure 14).

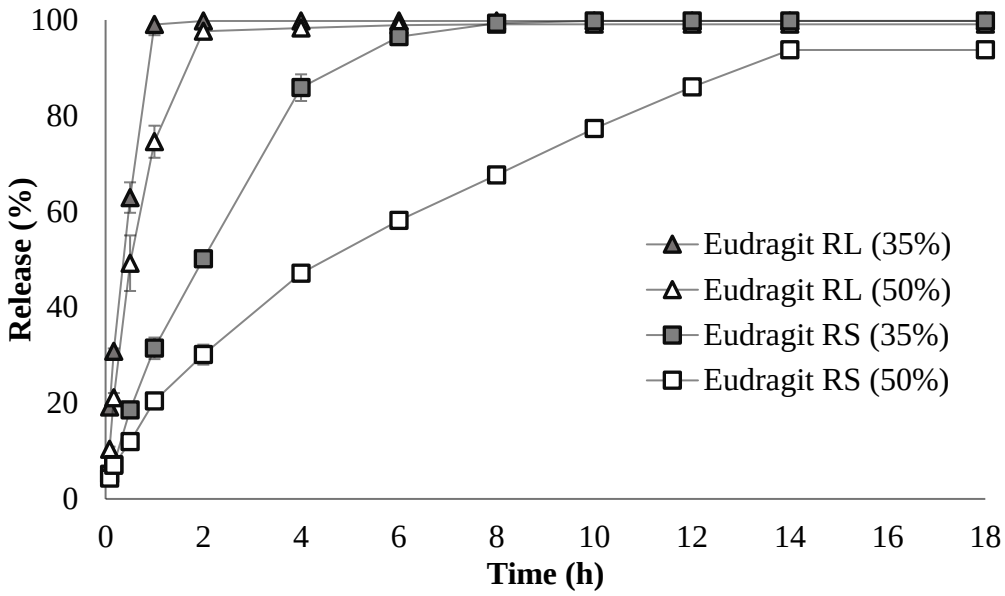

Figure 21. Effect of Eudragit permeability and loading on melatonin release from matrix tablets.

A disintegration test was performed, showing an almost immediate disintegration of tablets based on Eudragit RL containing 35% polymer, requiring only 1.4 (± 0.1) minutes for complete disintegration. The matrix tablets with 50% of this polymer, presented a complete disintegration in 7 (±0.5) minutes.

Disintegration took longer time for matrix tablets containing Eudragit RS than for matrix tablets based on Eudragit RL, showing complete disintegration at 14 (±0.5) minutes and 85 (± 5) minutes, for 35 and 50% polymer content, respectively. Tablets based on Eudragit RS disintegrated completely during the disintegration test, while they only partially or not

disintegrated during the dissolution test, which could be explained due to the higher mechanical forces applied during the disintegration test.

Eudragit RS showed slower release rate and disintegration time than Eudragit RL for both percentages of polymer, which could be explained by the fact that the amount of quaternary ammonium groups in Eudragit RS is less than in Eudragit RL. For that reason, Eudragit RS is less hydrophilic and permeable than Eudragit RL. Eudragit RL takes up more water and the release by diffusion of the drug through the medium-filled pores is faster. Less water uptake by Eudragit RS could be the reason that the tablet presented higher strength during the disintegration test that the matrix tablets containing Eudragit RL.

Considering the results obtained, a combination of both Eudragit is suggested, as employing only Eudragit RL did not show an appropriate behaviour for prolonged release tablets.

3.4.3. Effect of agitation rate during dissolution test on melatonin release from erodible and non-erodible matrix tablets.

Important factors within dissolution test conditions are hydrodynamic conditions and mechanical destructive forces, which can change the behaviour of the dosage form during the dissolution test (Ali et al. 2017). The effect of variation on the agitation rate during the dissolution test was evaluated with five polymers, Methocel K15 MCR, Methocel K100LV, Ethocel 10FP, Kollidon SR and Eudragit RS, containing 35% loading each formulation. The agitation rate was set at 10, 25, 50 and 100 rpm.

Matrix tablets containing Methocel K15MCR formed a gel, and do not completely erode until the end of the test with the four different agitation speeds (Figure 22). Increasing the agitation rate showed a slight rise in the melatonin release rate from Methocel K15MCR at the four different speeds (Figure 23). The matrix tablets show an 80% melatonin release at 18 hours for agitation rate of 25, 50 and 100 rpm, but the most significant change was observed for 10 rpm, with 80% melatonin release at 21 hours, which is probably due to a slower erosion of the outer layer.

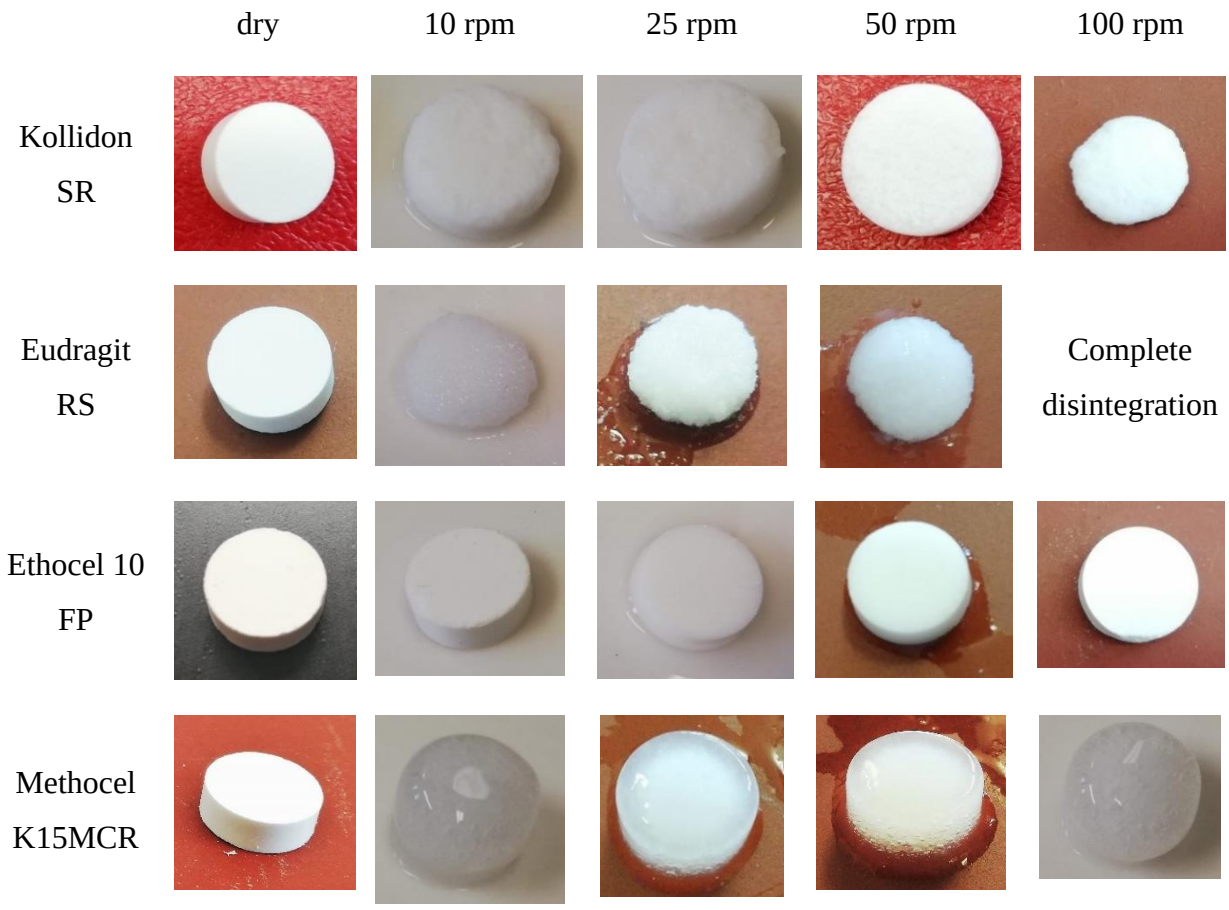

Figure 22. Pictures of matrix tablets containing Kollidon SR, Eudragit RS, Ethocel 10 and Methocel K15MCR, before (dry) and after dissolution test with 10, 25, 50 and 100 rpm.

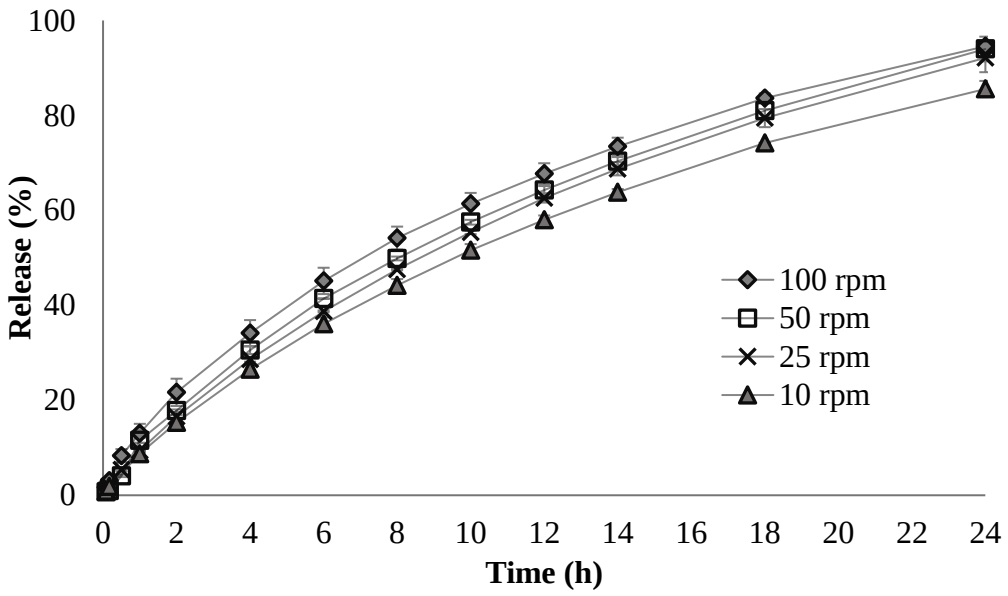

Figure 23. Effect of agitation rate variation on melatonin release from matrix tablets containing Methocel K15MCR.

Matrix tablets based on Methocel K100LV, the tablets formed the gel, but it erodes and dissolves around 18 hours with 10, 25 and 50 rpm and after only 6.5 hours with 100 rpm. A slight change in the release rate obtained with 10 rpm is observed, in comparison with the release obtained with 25 and 50 rpm, getting 80 % melatonin release at 12 hours with 25 and 50 rpm and at 14 hours with 10 rpm. There is a significant increase in melatonin release rate at 100 rpm, showing 80% release at 4.5 hours (Figure 24), which could be explained for a rise in erosion rate, shortens the diffusion pathway and given a faster dissolution from Methocel K100LV matrix tablets with this variation in agitation rate.

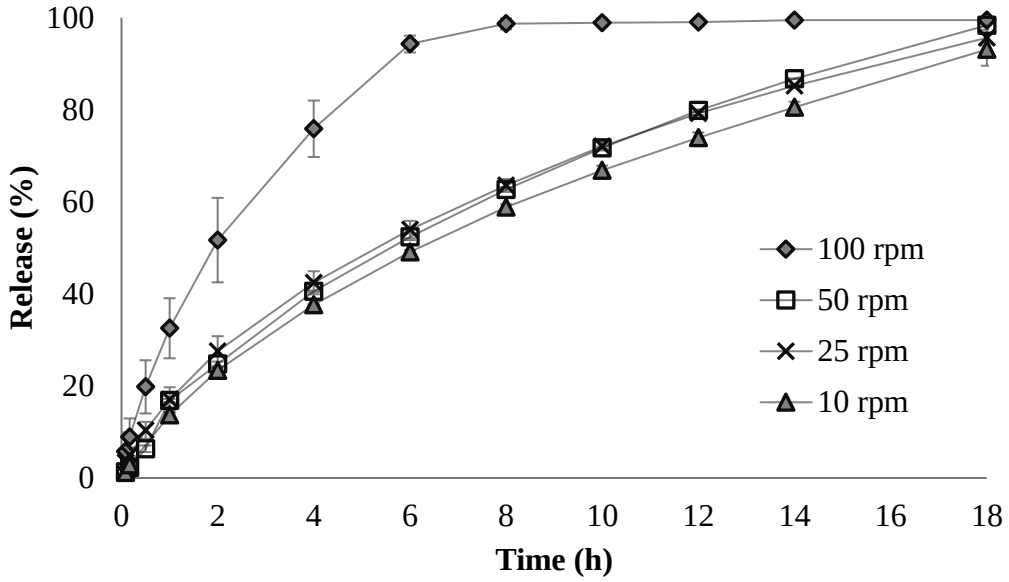

Figure 24. Effect of agitation rate variation on melatonin release from matrix tablets containing Methocel K100LV.

The different results between these two Methocel types can be explained with the change of agitation rate, because the higher viscosity Methocel K15MCR, which forms a thicker and viscous gel layer, being more robust to changes in hydrodynamic conditions.

The third polymer evaluated was Ethocel 10 FP, the matrix tablets containing this polymer remained intact until the end of the test with all the agitation speeds (Figure 22). The matrix tablets presented insignificant changes in the release rate with almost 80% release after 24 hours of dissolution test with 10, 25, 50 and 100 rpm (Figure 25).

Matrix tablets based on Ethocel 10FP are not significantly affected by hydrodynamic conditions, due to EC is an insoluble and non-swellable polymer, which keep the original shape and size of the matrix tablet.

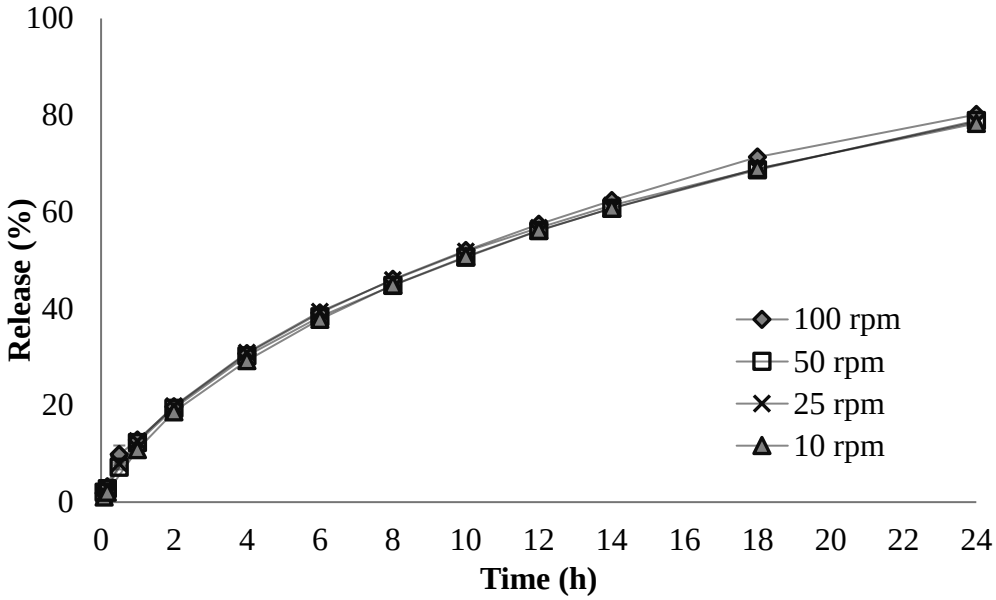

Figure 25. Effect of agitation rate variation on melatonin release from matrix tablets containing Ethocel 10FP.

The fourth polymer assessed was Eudragit RS. The matrix tablets based on this polymer disintegrated partially during the complete dissolution test at 10, 25 and 50 rpm (Figure 22). However, using 100 rpm the matrix tablets disintegrated completely after 6.5 hours. Variation on melatonin release rate in tablets based on Eudragit RS was observed between the different agitation rates. The 80 % melatonin release was achieved after 3.2, 3.7, 6.6 and 8.8 hours for 100, 50, 25 and 10 rpm, respectively (Figure 26).

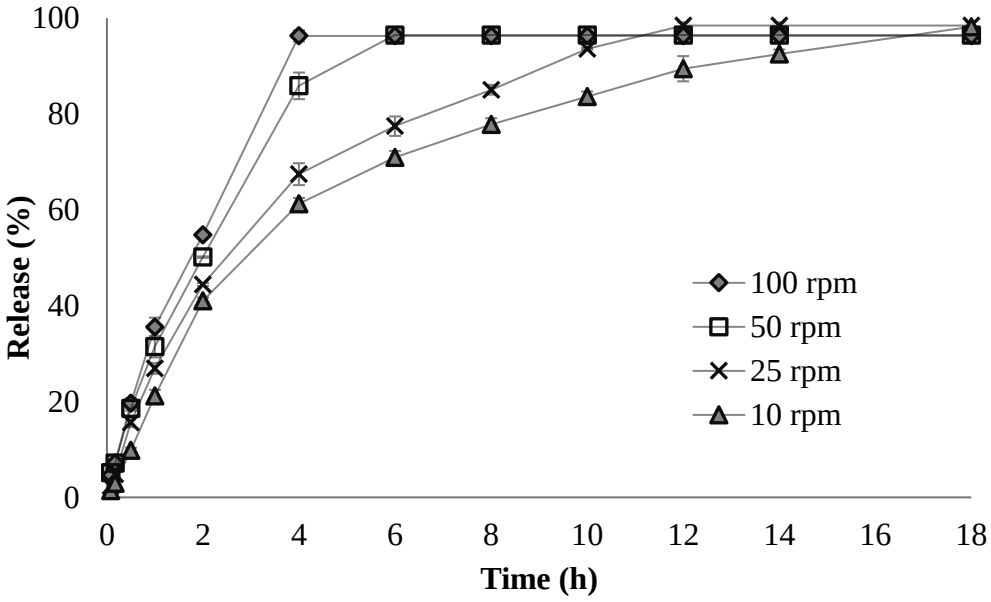

Figure 26. Effect of agitation rate on melatonin release from tablets containing Eudragit RS.

Matrix tablets based on Eudragit RS are significantly affected by hydrodynamic conditions, which can be explained due to its composition, which gives to Eudragit a capacity of swelling and hydration. Increasing the agitation rate may produce a faster hydration of the matrix tablet and subsequent disintegration, giving as a result a faster release, due to the continued circulation of the medium through the pores and the higher surface area after partial disintegration.

The last polymer tested was Kollidon SR, the matrix tablets containing this polymer swelled and grew, keeping the shape, using the agitation speed of 10, 25 and 50 rpm, whereas using 100 rpm the matrix tablets disintegrated partially (Figure 22). An insignificant change on the release rate between agitation rate of 100 rpm and 50 rpm is observed, with 80 % melatonin release at 2.6 hours. A slightly change with 25 rpm is observed, presenting 80% melatonin release at 3.5 hours, while the major change is observed with 10 rpm, getting the same percentage of release at 12 hours (Figure 27).

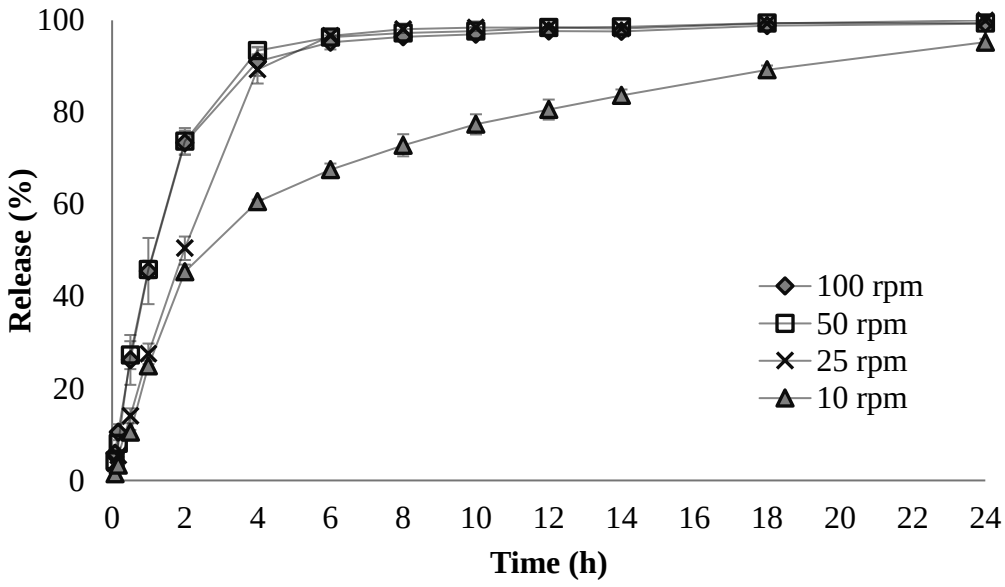

Figure 27. Effect of agitation rate variation on melatonin release from matrix tablets containing Kollidon SR.

A significant effect is observed with reduction of the agitation speed to 10 rpm, probably, due to a slower water-penetration rate into the matrix, producing a slower dissolution and diffusion of the soluble components.

3.5. Pulsatile release tablets

Pulsatile release tablets prepared and analysed in this study are based on a rupturable lipid coating employing Compritol 888 ATO, a water-insoluble, pH-independent excipient. The core contains melatonin, PVP as a binder and Ac-Di-Sol, a superdisintegrant chosen to produce the hydrostatic pressure inside the system, due to its known high swelling behavior.

A blank with the excipients was analysed in both wavelengths, 220 nm and 273 nm. A significant absorption was observed at 220 nm, due to the presence of PVP in the formulation, which has the main absorption peak at 213.5 nm (Tavlarakis et al. 2011). Additionally, melatonin presents higher absorption at 220 nm, thus, with 50% release during the dissolution test, the absorption observed in the UV-visible spectrophotometer analysis, was higher than 2 AU, showing noise in the spectrum. Therefore, the wavelength 273 nm was chosen to analysis the formulations and avoid interferences.

3.5.1. Formulation containing dibasic calcium phosphate

The first formulation prepared and evaluated has a small size with 5 mm core and 9 mm total tablet. The core contains a dye to facilitate visualization after breaking the coat. The coating contains 50% of dibasic calcium phosphate (DCP) as a filler and 40% Compritol 888 ATO, and the coating weight is approximately 6 times the core weight.

During the dissolution test the tablets broke up in HCl 0.1 N after a long lag time of more than 12 hours (Figure 28 and 29). The tablets tested in buffer pH 6.8 did not rupture after 18 hours of testing. One of the tablets, tested in HCl 0.1 N, presented a prolonged release after the lag time, while the other tablet showed a complete and fast release after the lag time.

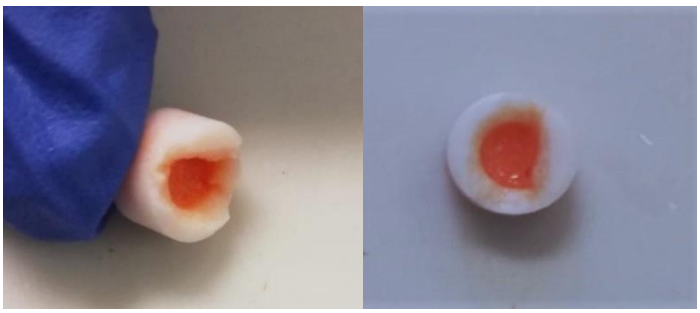

Figure 28. Pulsatile release tablets first formulation after 18 hours of dissolution test in HCl 0.1 N, pH 1.2.

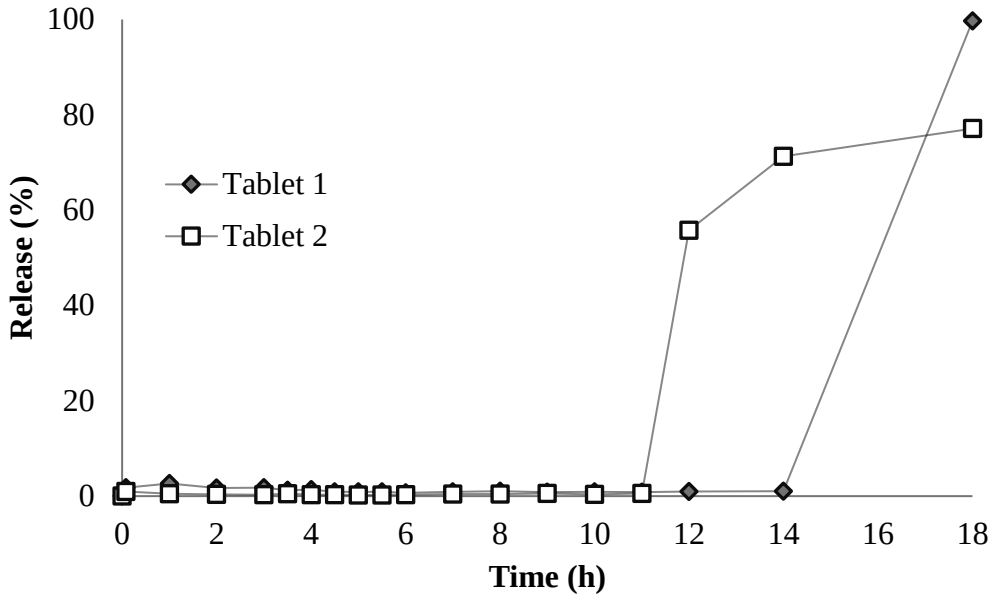

Figure 29. Release of melatonin pulsatile release tablets first formulation in HCl 0.1 N, pH 1.2.

The lack of release of this formulation in buffer pH 6.8 is explained by the presence of DCP, since this excipient is insoluble in water, but dissolves in HCl 0.1 N. DCP is dissolved in HCl 0.1N medium and produces a faster swelling of the core and a weaker coating layer, creating enough pressure inside the core to rupture of the outer layer easier. The swelling forces developed in the core during the test in buffer phosphate pH 6.8 were not strong enough to break the coating layer, possibly due to the slower water uptake from the core compared to the acidic medium.

Although the presence of DCP helped to a faster release from tablets tested in HCl 0.1N, the water uptake and the pressure generated inside the core was not enough to rupture the thick coating layer in a short time, producing an extensive lag time. Due to the results obtained, a reformulation of the pulsatile drug delivery system was necessary.

3.5.2. Effect of polymer loading on the lag time and melatonin release from tablets based on a rupturable coating containing lactose.

The first point to change in this formulation was the presence of DCP for a water-soluble filler, in this case, lactose (FlowLac® 100). The second point was the amount of coating layer in relation to the core weight, due to the extended lag time of more than 12 hours with the first formulation.

The relation between coating weight and core weight was changed from 6 times to 3 times, by preliminary testing. The tablet size was increased to 12 mm keeping the same relation of 4 mm between core and final tablet.

Pulsatile release tablets were evaluated modifying the percentage of Compritol 888 ATO and lactose (FlowLac® 100) in the formulation. The hardness of the tablets with the same compression force increased with decreasing the percentage of Compritol, due to the rise in lactose amount which results in a better compressibility characteristic of the finished tablet.

During the dissolution test the tablets broke up partially after the lag time (Figure 30) followed by a complete rupture and division of the tablet into two parts (Figure 31). The core dissolves completely at the end of the dissolution test.

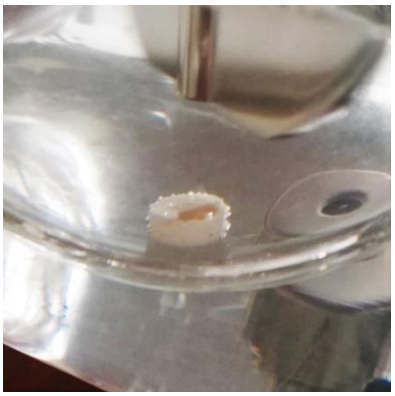

Figure 30. Pulsatile release tablets with coating layer of Compritol ATO 888 after rupture, during dissolution test.

Figure 31. Pulsatile release tablets with coating layer of Compritol ATO 888 after dissolution test.

After contact with both release mediums, the water penetrates through the outer layer, reaching the core which hydrates and swells, due to the presence of Ac-Di-Sol, producing enough pressure inside the tablet to finally rupture the coating and release the drug. Showing

that the thickness of the coat layer was sufficient to break up after an appropriate lag time in both mediums.

The results show that the formulations presented a sigmoidal release, without drug release during the lag time and followed by a complete and rapid release (Figures 32, 32, 34 and 35) in both mediums, HCl 0.1 N and buffer pH 6.8.

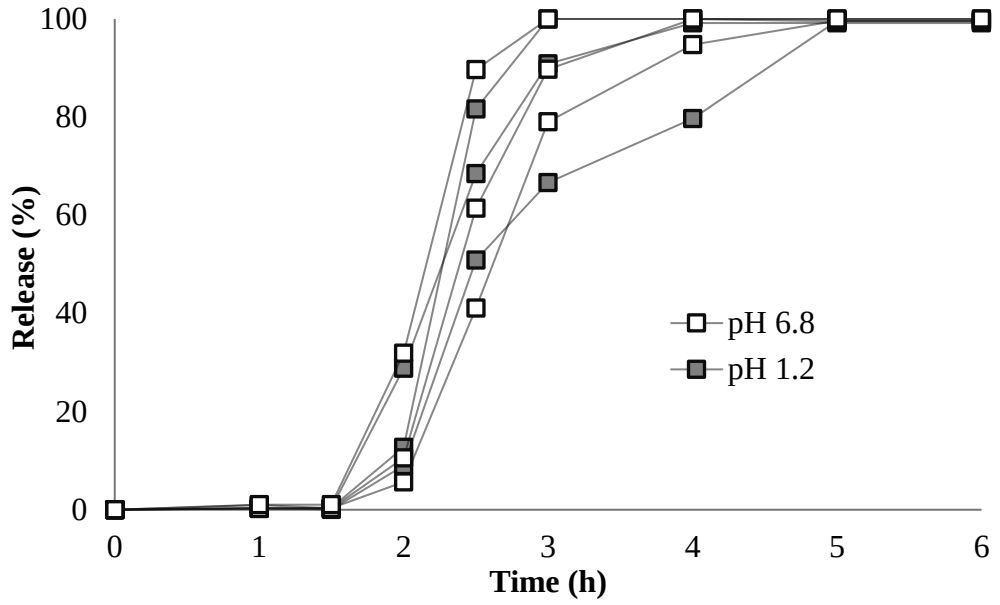

Figure 32. Melatonin release from pulsatile release tablets containing 20% Compritol 888 ATO.

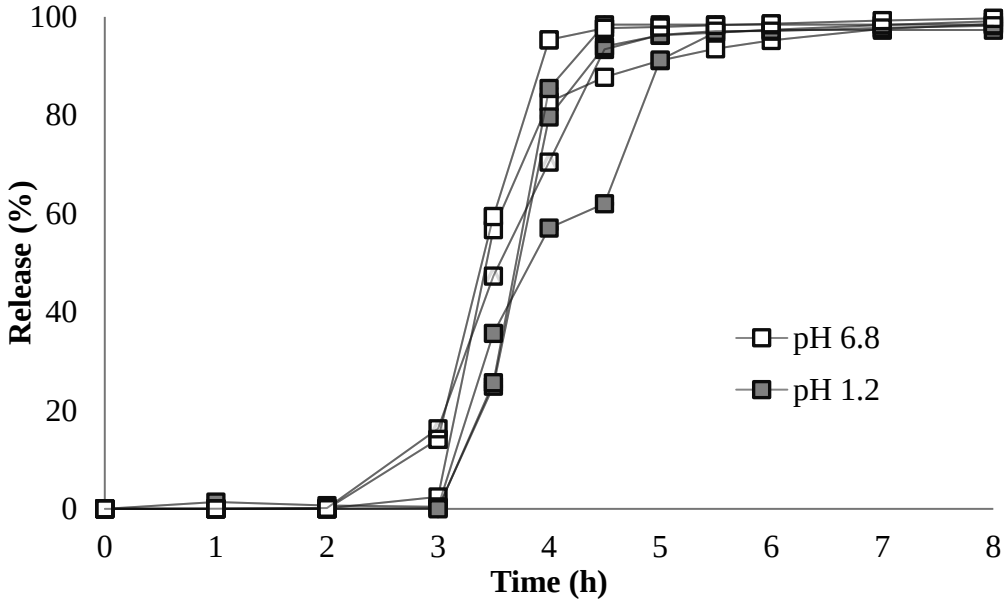

Figure 33. Melatonin release from pulsatile release tablets containing 30% Compritol 888 ATO.

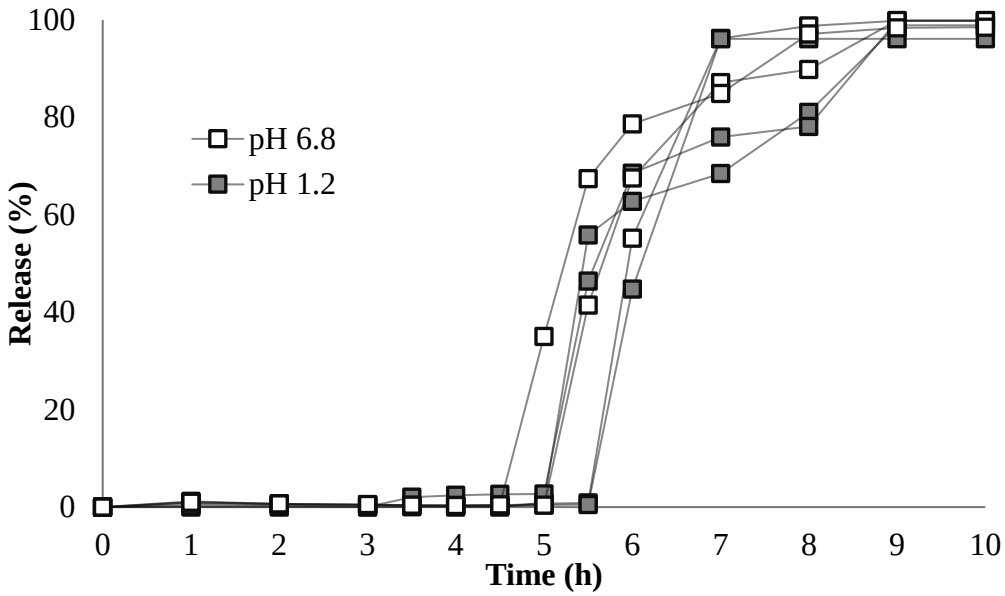

Figure 34. Melatonin release from pulsatile release tablets containing 40% Compritol 888 ATO.

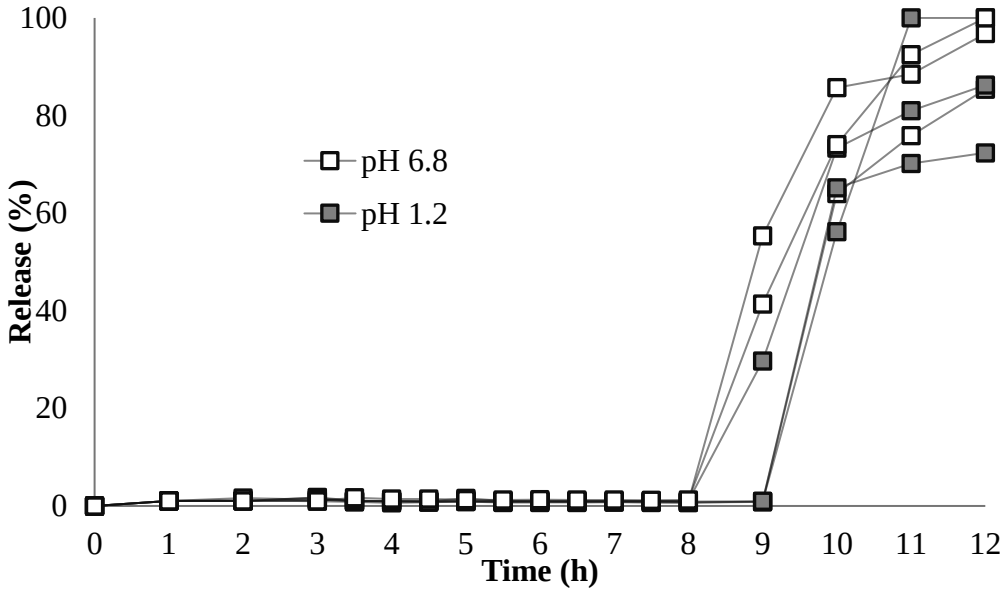

Figure 35. Melatonin release from pulsatile release tablets containing 50% Compritol 888 ATO.

The lag time increased with increasing the percentage of Compritol 888 ATO, due to a reduction in permeability of the tablet coating. A lag time around 2.2, 3.3, 5.5 and 9.5 hours for tablets containing of 20, 30, 40, and 50 % Compritol 888 ATO, respectively, was obtained (Figure 36).

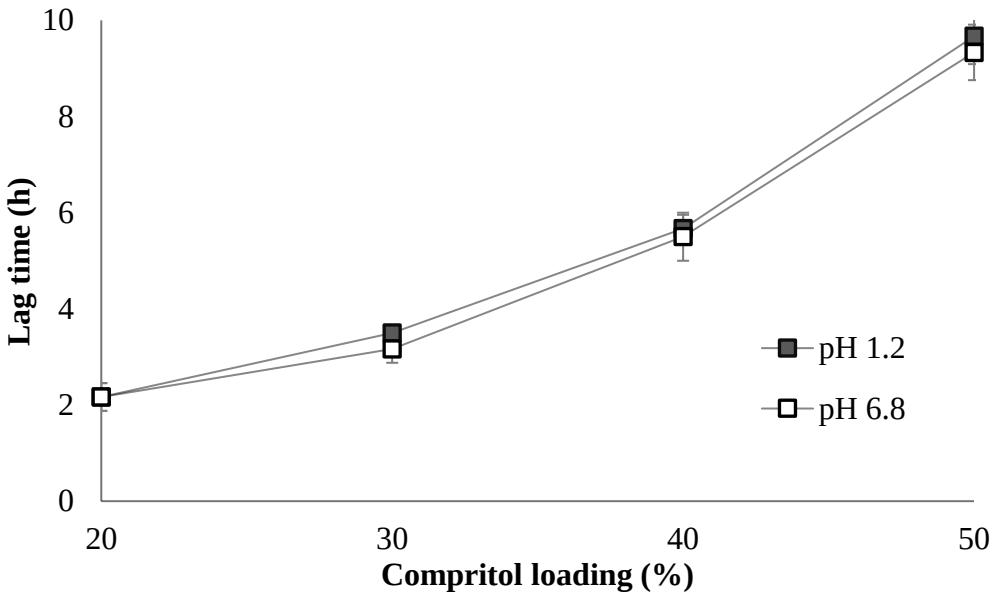

Figure 36. Effect of Compritol 888 ATO amount on the lag time of pulsatile release tablets in pH 1.2 and 6.8.

The lag time was slightly affected by the pH of the medium, which can be explained because Ac-Di-sol is dependent on the pH of the release medium. Therefore, the lag times were slightly longer in HCl 0.1N (pH 1.2) than in buffer phosphate pH 6.8. Mohamad and Dashevsky (2006) found that the swelling volumen of Ac-Di-Sol was approximately 750% in pH between 1.2 and 5.4, while it rose up to 1000% in pH between 5.4 and 7.4, showing a higer swelling energy of Ac-Di-Sol in buffer than in HCl 0.1N.

Compritol loading versus the lag time presents an exponential curve (Figure 36). Increasing the amount of Compritol in the formulation by 10% results in approximately the double of the lag time. Changing the percentage of Compritol from 20 to 30% presents an increase in lag time around 1 hour, from 30 to 40% the rise is approximately 2 hours and from 40 to 50% the lag time grows 4 hours. Therefore, higher loading of Compritol in the formulation show major sensitivity, giving much higher lag times.

After the lag time the release behaviour was different in both mediums with the changes in the amount of Compritol 888 ATO in the formulation (Figure 37). In the case of tablets containing 20 and 30% Compritol 888 ATO, the release was rapid, getting 80% around 1 hour after the lag time in both mediums.

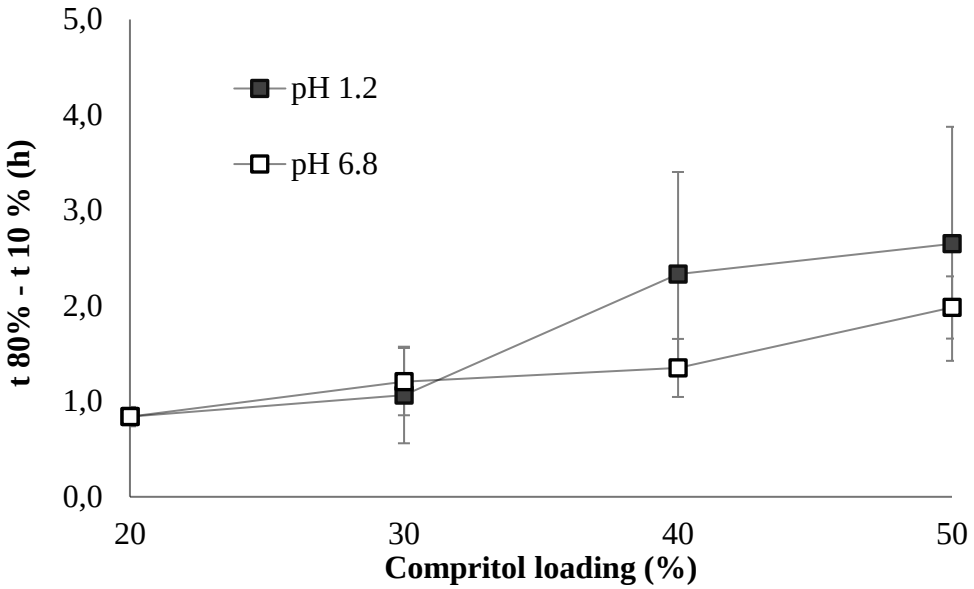

Figure 37. Effect of Compritol 888 ATO amount on the melatonin release after the lag time in pulsatile release tablets with pH 1.2 and 6.8.

Increasing the amount of Compritol to more than 40% produces changes in the release time according to the medium. The tablets tested in buffer phosphate pH 6.8 present an 80% release around 1.5 and 2 hours after the lag time for 40 and 50% Compritol content, respectively. Therefore, it can be concluded that the release rate after the lag time is rapid for all formulations in buffer pH 6.8 but decreases slightly for higher Compritol loadings, due to a decreased coating porosity and permeability.

The tablets tested in HCl 0.1 N, pH 1.2, show an increase in the release time, getting 80% release around 2.5 hours after the lag time for tablets with 40 and 50% Compritol 888 ATO. This behaviour could be explained by the fact that Ac-Di-Sol swells less in HCl 0.1 N than in buffer pH 6.8 and due to the reduction in permeability after increasing the percentage of Compritol in the tablets, this produces a lower degree of rupture of the coating layer, giving a slower release in HCl 0.1 N than in buffer.

4. CONCLUSIONS

a. Melatonin is a soluble drug, which has a micronized particle size and recrystallization in the same polymorphic form.

b. Type, viscosity, loading, particle size and permeability of the polymer, method of preparation and hydrodynamic conditions were identified as formulation parameters for controlled release matrix tablets. Thus,
   - A decrease on melatonin release rate is obtained by:
     - Increasing polymer viscosity and polymer loading in the formulation,
     - Decreasing particle size and polymer permeability,
   - Tablets prepared by wet granulation produce a decrease on melatonin release rate in comparison with tablets prepared by direct compression,
   - Variation in agitation rate during dissolution test can change the behaviour of the dosage form, depending on the polymer employed.

c. Pulsatile release tablets were obtained with different lag times by varying the amount of Compritol 888 ATO in the formulation. A longer lag time was obtained with higher amount of Compritol 888 ATO. The release after the lag time was slightly different in the two mediums, explained by the swelling behaviour of Ac-Di-Sol in the two pH.

5. SUMMARY

The first aim of this work was to evaluate important melatonin properties for the manufacture of oral controlled release tablets. Firstly, solubility was evaluated on HCL pH 1.2 and buffer pH 6.8, at 37°C. According to the results and the definition of the BCS, melatonin is considered a highly soluble substance. Secondly, particle size was evaluated by microscopy, showing that melatonin is a micronized powder with a D50 of 4.1 µm. Lastly, a DSC analysis was performed, which showed the same endothermic reaction for melatonin powder and recrystallized, indicating the recrystallization in the same polymorphic form.

The second objective was to evaluate the effect of polymer properties, agitation rate and manufacturing method on melatonin release from prolonged release erodible and non-erodible matrix tablets. Firstly, erodible matrix tablets were prepared containing 35% Methocel with 5 grades of viscosity and 2 different ratios of methoxyl and hydroxypropoyl substitution. As a result, matrix tablets based on Methocel E15LV and E50LV, erode completely after short time and release rapidly. Tablets containing Methocel K15MCR, K4MCR and K100LV formed a thicker gel and presented a slower melatonin release when a higher viscosity was employed. The release rate of melatonin is controlled by the speed of gel formation and the thickness and viscosity of it, thus, increasing the polymer viscosity, decrease the melatonin release rate.

Secondly, non-erodible matrix tablets based on Eudradit, Kollidon and Ethocel were prepared with 35 % and 50% loading. Tablets based on Kollidon SR showed the fastest release, due to its soluble component PVP, which leaches out forming pores through which the drug diffuses. Followed by Eudragit RS, explained by its composition containing quaternary ammonio groups, which make it more hydrophilic, allowing water penetration and faster drug release by diffusion. Matrix tablets containing Ethocel 10, presented the slowest release, due to its non-swellable and impermeable behavior. The order of release was the same with both loadings in the formulation. Increasing the polymer loading showed a decrease on melatonin release rate, explained for the decline in matrix porosity.

The evaluation of the polymer particle size on melatonin release from matrix tablets was performed with Ethocel 10 (D50 164.3 µm; 35% and 50% loading) and Ethocel 10 FP (D50 5.2µm; 20% and 35% loading). Slower release was obtained from tablets based on Ethocel 10FP, due to its smaller particle size, the matrix porosity is decreased, and the water penetration and drug diffusion are slower. Thus, decreasing the polymer particle size decrease the melatonin release rate.

The effect of preparation method was evaluated with matrix tablets based on Eudragit RS and Ethocel 10, with 35% loading, prepared by direct compression and wet granulation. A steady melatonin release is observed with tablets based on Ethocel 10, prepared by wet granulation, while tablets prepared by direct compression showed an abrupt release, which is induced by the partial disintegration during the dissolution test. Tablets based on Eudragit RS obtained by the two methods get complete release at the same time, with slower release behavior on tablets prepared by wet granulation. After partial disintegration of the tablets obtained by direct compression, the particles get more area of contact, which allows a faster and abrupt release at this time. Also, wet granulation improves granules compressibility, which results in lower porosity and better interparticle cohesion, giving as a result a slower drug release. Thus, tablets prepared by wet granulation showed a decrease on melatonin release rate compared with direct compression.

The effect of polymer permeability on melatonin release was evaluated with matrix tablets based on Eudragit RS and RL with 35% and 50% loadings. A considerable difference on melatonin release rate from the matrix tablets based on these two polymers is observed, showing an extremely fast release from matrix containing Eudragit RL. Eudragit RL is more hydrophilic and permeable than Eudragit RS, due to its amount of quaternary ammonium groups. Therefore, matrix tablets based on Eudragit RL take up more water and produced a faster release by diffusion of melatonin. Therefore, increasing the polymer permeability increasing considerably the melatonin release rate.

The evaluation of the agitation rate on melatonin release was performed with five polymers, Methocel K15 MCR and K100LV, Ethocel 10FP, Kollidon SR and Eudragit RS, containing 35% loading each formulation. The agitation rate was set at 10, 25, 50 and 100 rpm.

The melatonin release rate from matrix tablets based on Methocel K15MCR are slightly affected by the agitation rate; the release rate is similar with all the agitation rates. In the case of tablets based on Methocel K100LV slight differences between 10 rpm, 25 rpm and 50 rpm are observed, but showing a much faster melatonin release with 100 rpm, explained by a rise in erosion rate, which shortens the diffusion pathway. The higher viscosity Methocel K15MCR, produces a thicker and viscous gel layer, being more robust to changes in hydrodynamic conditions than Methocel K100LV.

Ethocel 10 FP matrix tablets presented insignificant changes on the melatonin release rate with all the agitation rates, explained by the fact that EC is an insoluble and non-swellable

polymer, which keep the original shape and size of the matrix tablet. Eudragit RS matrix tablets showed increase on melatonin release rate when increasing the agitation rate. Faster agitation produces a faster hydration of Eudragit matrix tablet, due to its capacity of swelling and hydration, giving as a result a faster release due to the higher surface area after partial disintegration. Kollidon SR matrix tablets showed a slight change on the melatonin release rate between agitation rate of 100 rpm, 50 rpm and 25 rpm. However, a much lower melatonin release is observed with 10 rpm, due to the slower water-penetration rate, which produces a slower dissolution and diffusion of the soluble components. In conclusion, variation in agitation rate during dissolution test can change the behavior of the dosage form, depending on the polymer employed.

The third aim was to evaluate the effect of polymer loading on the lag time and melatonin release from pulsatile release tablets based on a rupturable coating containing Compritol 888 ATO (glyceryl behenate), a water insoluble excipient. Pulsatile drug-release system is beneficial for drugs with a high first-pass effect, which is the case of melatonin.

A formulation containing 50% DCP as a filler and 40% compritol 888 ATO on the coating, with a coating/core ratio of 6/1, was developed. A lag time of 12 and 18 hours on HCl pH 1.2 was obtained. Tablets tested on buffer pH 6.8 did not break nor release during the test, explained by the presence of DCP, a water-insoluble excipient, but soluble in HCl pH1.2. In addition, the thick coating layer of the tablets produces extensive lag times from the tablets tested in HCl pH1.2.

A reformulation of the coating was necessary, replacing the DCP for lactose a water-soluble component and decreasing the coating/core ratio from 6/1 to 3/1. Compritol 888 ATO loading was evaluated with 20%, 30%, 40% and 50% content in the formulation. The formulations presented a sigmoidal release, without drug release during the lag time and followed by a complete and rapid release in both mediums, HCl pH1.2 and buffer pH 6.8.

The lag time increased with increasing the percentage of Compritol 888 ATO, due to a reduction in permeability of the coating. The lag time was slightly longer in HCl pH 1.2 than in buffer pH 6.8, due to the swelling behavior of Ac-Di-sol, which is dependent on the pH of the medium. Compritol loading versus the lag time presents an exponential curve, thus, increasing the amount of Compritol by 10% results in approximately the double of the lag time. Therefore, higher loading of Compritol showed major sensitivity, giving much higher lag times.

After the lag time the release behaviour was similar in both mediums with 20 and 30 % Compritol, but it showed differences with 40% and 50% Compritol ATO, presenting a decrease on release rate in tablets tested on HCl pH1.2, which is explained by the different swelling behavior of Ac-Di-Sol in both mediums.

In conclusion, increased lag time was obtained when loading of Compritol 888 ATO was risen in the formulation, and the release rate after the lag time was slightly different in the two mediums, explained by the swelling behaviour of Ac-Di-Sol.

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