J. Kor. Pharm. Sci., Vol. 23, No. 3, S9-S18 (1993)

Development and Evaluation of an Oral Controlled Release Delivery System for Melatonin

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(Received April 10, 1993)

ABSTRACT

Sugar spheres loaded with melatonin (MT) were coated with Aquacoat® to control the release rate of MT over 8 hours. A zero-order release pattern over 8 hours was obtained with 20% coating on 8-10 mesh beads in USP basket dissolution studies. MT in 20% coated beads was quite stable at room temperature with less than 5% MT degraded during 6 months' storage. Dissolution profiles were also unchanged after 6 months. An oral preparation containing MT-loaded uncoated beads for immediate release and 20% coated beads with Aquacoat® for controlled release over 8 hours was evaluated in six human subjects. When total 0.5 mg MT as low dose (immediate release portion of MT, 0.1 mg) was administered to four subjects, average peak plasma MT concentration was reached at about 600 pg/m*l* and maintained at about 10 pg/m*l* over 8 hours. Plasma MT concentration-time profiles were similar in shape to computer-simulated profiles. However, maximal plasma MT concentrations were three times greater compared to computer simulated curve. These results suggest that MT dose, ratio of immediate and controlled release MT, and pharmacokinetic parameters selected are adjusted to mimic endogenous MT concentration-time curve. In another study, 0.2 mg MT having 10% of immediate release portion and 80% controlled release portion produced plasma MT concentration-time curve which is more similar to endogenous profiles. A low bioavailability (<20%) may result from extensive first pass metabolism and remaining amounts of MT from controlled beads. A good correlation between plasma MT concentration and urinary excretion rate of 6-sulphatoxymelatonin (6-STMT), a major metabolite of MT was observed. As plasma MT concentration increased, urinary excretion rate of 6-STMT increased concomitantly. The linear relation between plasma MT and urinary excretion rate of 6-STMT was statistically significant. This result suggests that urinary 6-STMT may be used as an index of circadian rhythms of MT in humans.

1. INTRODUCTION

Melatonin (MT) is an indole amide neurohormone (Lerner and Case, 1959). It is primarily secreted by the pineal gland in a circadian rhythm (Waldhauser and Dietzel, 19 85; Lewy and Newsome, 1983). MT concentration is very low during the daytime (<10 pg/m*l*). MT concentrations start to rise in the late evening and are maintained at 25-120 pg/m*l* during the night (over 8 hours) until MT levels return to the daytime baseline. Although the full potential significance of introducing exogenous MT at physiological and pharmacological concentrations is still unknown (Petterborg *et al.*, 1991; Sack *et al.*, 1991), exogenous MT may have clinical potential as a circadian synchronizer to treat or

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entrain circadian rhythm disorders including sleep disorders (Arendt *et al.*, 1988; Cramer *et al.*, 1974; Dahlitz *et al.*, 1991), jet lag (Petrie *et al.*, 1989), shift work syndrome, and seasonal affective diseases (Waldhauser *et al.*, 1986; Rosenthal *et al.*, 1984) in human subjects.

Sustained release dosage forms which possess a longer biologically effective half life may be of interest to evaluate clinical potential of MT by offsetting rapid brain turnover or mimicking sustained nocturnal secretion of MT (Le Bars *et al.*, 1991; Strassman *et al.*, 1987). However, lack of clinically useful dosage forms may hinder evaluating the full clinical potential of MT. The usual physiological nocturnal secretion of MT over 8 hours coupled with the short half-life of MT prompted us to develop a controlled release delivery system for MT which minics endogenous plasma MT concentration-time profiles.

Application of aqueous polymeric film coatings to drug loaded nonpareils (sugar spheres, NF) has become an increasingly popular method of developing controlled release dosage forms (Jackson *et al.*, 1990; Rekhi *et al.*, 1989). Aqueous polymeric ethylcellulose suspension (Aquacoat®) has replaced the conventional organic solvent-based coating methods because of the potential toxicity and high cost associated with organic solvents (Ghebresellassie *et al.*, 1988). Aquacoat® is an ethylcellulose dispersion stabilized by sodium lauryl sulfate and cetyl alcohol.

The fluid bed process is increasingly popular for coating of drug-loaded particles such as granules, beads, and sugar spheres to control drug release rates (Mehta *et al.*, 1986; Mehta, 1988). The fluidized bed process using bottom-spraying with a Wurster air-suspension column provides the best conditions for coalescence of small polymeric particles, coating efficiency, homogenous drug disposition, and reproducible release characteristics (Rekhi *et al.*, 1989; Mehta, 1988; Hosssain and Ayres, 1990). Polymer coating technology has been coupled with pharmacokinetic simulation to design an oral controlled release dosage form (Hossain and Ayres, 1992).

The purpose of this study was to determine whether or not an oral controlled release drug delivery system for MT could produce plasma MT concentration-time profiles similar to endogenously produced MT profiles in human subjects. Release rate of MT from coated beads were investigated by varying sugar bead size and amount of coating to obtain coated beads with zero-order release over 8 hours. Chemical stability of MT and release rate of MT from coated beads during storage condition was also determined. Computer simulation program was used to determine the dosing regimen of drug and predict plasma concentration-time profiles. Correlation between plasma concentrations of MT and urinary excretion rate of 6-sulphatoxymelatonin (6-STMT), a major metabolite of MT, was also investigated.

2. MATERIALS AND METHODS

2-1. Materials

Melatonin (MT) was purchased from Regis Chemical Co. (Morton Grove, IL, USA). Core sugar spheres (USP/NF) as a substrate for MT loading were from Paulaur Co. (Robbinsville, NJ, USA). Polyvinylpyrrolidone (average molecular wegith 40,000) and hydroxypropylcellulose (average molecular weight 300,000) were from Aldrich Chemical Co. (Milwaukee, WI, USA). Aquacoat® (polymeric ethylcellulose suspension: Type ECD-30) containing 30 % solids was from FMC Corp. (Philadelphia, Pennsylvania, USA). Sebatic acid dibutyl ester was from Sigma Chem. Co. (St. Louis, MO, USA), and triethyl citrate was from Aldrich Chemical Co. (Milwaukee, WI, USA) as plasticizers, respectively. All other chemicals were of reagent grade and used without further purification.

2-2. Preparation of Coated Beads

The apparatus for MT-loading on sugar spheres and applying the polymeric film coating consists of a laboratory scale spray coater having a Wurster column (2″×7″, ST-REA-1, Aeromatic Inc., Columbia, MD, USA) inside clear plexiglass column mounted on a fluid-bed dryer (Lab-Line/P.R.L. Hi-Speed Fluid Bed Dryer, Lab-Line Instruments Inc., Melrose Park, IL, USA). A cross section of spray coater fully assembled is given in Fig. 1.

In order to produce MT-loaded beads, a mixture of MT (1.2g), and polyvinylpyrrolidone (0.24g) and hydroxypropyl cellulose (0.12g) as binders in 200 m*l* of ethanol was applied to prewarmed 300 grams of sugar beads (retained on a either 18-20 or a 8-10 mesh screen) in a fluid-bed coating chamber at 40℃ with continuous fluidizing air supply. The solution was delivered at 4 m*l*/min using a peristaltic pump.

Dibutyl sebacate (4.5g) and triethyl citrate (4.5g) as plasticizers were combined and added at 30% (15% each, based on the solid content of Aquacoat®) to 100g of Aquacoat® suspension. Aquacoat® suspension was then diluted with deionized water (w/w, 1:1). The resulting coating solutions were stirred for at least 2 hours to ensure that plasticizers were well mixed with the polymeric suspension prior to coating, and were continuously stirred throughout the coating process. Coating solutions were applied to 90g of MT-loaded beads to achieve the desired coatings with continuous air supply at a rate of 1 m*l*/min for 10 minutes and then 4 m*l*/min using a peristaltic pump. 31.4, 62.7, or 125.4g of coating solutions produces a 5, 10 or 20% theoretical coating based on solids content of Aquacoat®. Although some solids of coating solution are lost during process, coatings are designated as 5%, 10% or 20% theoretical coating for convenience. The final coated beads were dried in the chambers for 30 minutes and then further air dried in a hood. Typical processing conditions for MT loading and coating of MT-loaded beads are given in Table I. MT content from beads were assayed by HPLC.

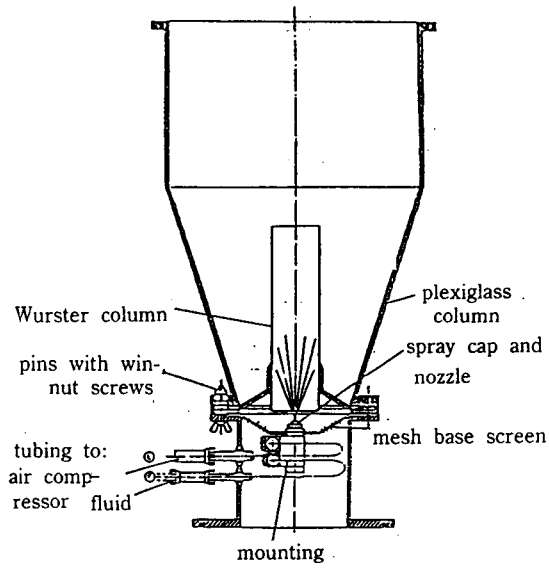

Figure 1—A cross section of spray coater fully assembled.

Table I—Typical Processing Conditions for Coating of MT-loaded Beads (STREA-1, Aeromatic)

Wurster insert	bottom spray
Nozzle size	0.8 mm
Inlet temperature[a]	50℃
Atomization air	12-15 psi
Fluidization air blower	50-70% of full capacity
flow rate[b]	1 m*l*/min and 4 m*l*/min

[a]40℃ was used for the preparation of MT-loaded beads.
[b]Only 4 m*l*/min was used for the preparation of MT-loaded beads.
Peristaltic pump was manually switched between "on" or "off" as necessary to control clumping of beads during the coating process.

2-3. *In vitro* Dissolution

In vitro dissolution of each formulation was performed in triplicate using the USP dissolution apparatus I (Basket method) at 37± 0.5 ℃. The stirring rate was 50 rpm except the effect of the stirring rate on the release rate

of MT was evaluated. Dissolution medium for the first 2 hours was 900 ml of enzyme free simulated gastric fluid (pH 1.4± 0.1) followed by enzyme free simulated intestinal fluid (pH 7.4± 0.1). Dissolution samples were collected at 0.25, 0.5, 0.75, 1.0, 1.5 and 2 hr (in gastric fluid), and 3, 4, 5, 6, 8, 12 and 24 hr (in intestinal fluid) with replacement of equal volume with temperature equilibrated media. Dissolution of MT from 20% coated beads (8-10 mesh) after 6 months storage was compared whether the dissolution profile was the same as that of the initial coated beads. MT concentration in dissolution samples were determined by HPLC. The percentage of MT released is the amount of MT released divided by amount of MT loaded, multiplied by 100.

2-4. Computer Simulation

Plasma MT concentration-time curve were estimated using a computer simulation program (MAXSIM®, version 3.01, Uppsala, Sweden) assuming an one compartment open model. Pharmacokinetic parameters of MT for MAXSIM® computer simulation were selected from literatures (Table II).

2-5. *In Vivo* Study Protocol

Six human volunteers (5 males and 1 female) participated in the study after giving informed consent approved by Oregon Health Science University. All subjects were admitted to the Clinical Research Center, Oregon Health Science University at 10 o'clock, A.M. on the day of the study. Subjects fasted at least 2 hours before the study began. Each gelatin capsule contained 0.1 mg of immediate release MT on uncoated beads and 0.4 mg of controlled release MT on 20% Aquacoat® coated beads designated as low dose (0.5 mg of total dose of MT). Two subjects were also given two capsules of this formulation designated as high dose (1.0 mg total dose of MT).

Blood samples were collected through an indwelling intravenous catheter at 0 (10:30 A.M.), 0.5, 1, 1.5, 2, 3, 4, 6, and 8 hours and kept in heparinized test tube in the freezer until analysis. Urine was also collected every two hours for the determination of 6-STMT, a major metabolite of MT. Baseline values of MT and 6-STMT at 10:30 A.M. was checked before administration of drug. Plasma MT concentrations were determined by high sensitivity GC/MS (Lewy and Markey, 1978). Urinary 6-STMT concentrations were determined by radioimmunoassay (Aldhous and Arendt, 1988).

3. RESULTS AND DISCUSSION

Dissolution profiles of MT from 18-20 mesh beads coated with Aquacoat® are shown in Fig. 2. Most MT was released in simulated gastric fluid, and the desired controlled release was not achieved. A possible interpretation of MT release from the coated beads is that the coating may be incomplete and porous so that the drug is readily released. The amounts of coating up to 20% theoretical coating was not enough to produce the desired controlled release. On ther other hand, dissolution profiles of MT from 8-10 mesh beads coated with Aquacoat® are shown in Fig. 3. A disired controlled release pattern was observed over 8 hours at 20% coating. At 20% coating of 8-10 mesh beads,

Table II—Selected Pharmacokinetic Parameters of MT for Computer Simulation in Human Subjects

K_d (hr^{-1})	K_a (hr^{-1})	V_c (1)	V_d (1)	F	References
0.90	–	13	35.2	–	Iguchi *et al.*, (1982)
–	1.74	–	–	–	Waldhauser *et al.*, (1984)
–	–	–	–	0.1	Lane *et al.*, (1985)

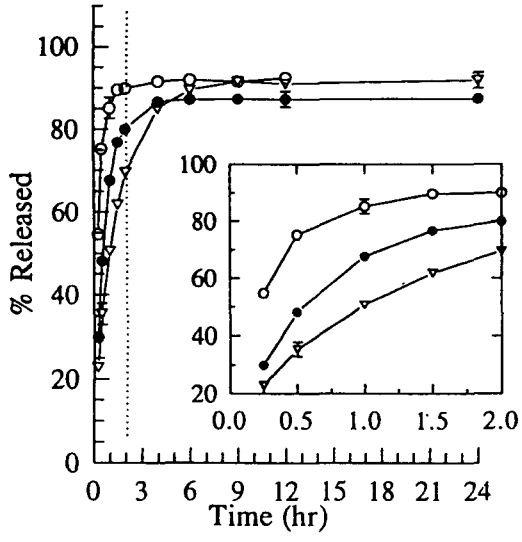

Figure 2—Dissolution profiles of MT from 18-20 mesh beads coated with Aquacoat®.
Coatings: ○; 5%, ●; 10%, and ▽; 20%. Each point represents the mean± standard deviation except where the standard deviation is too small to show.

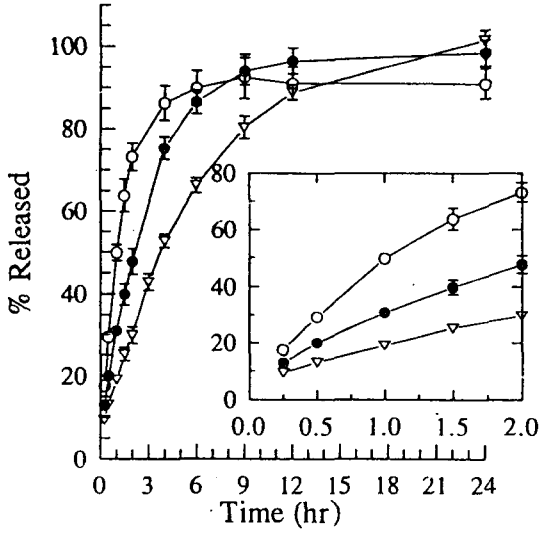

Figure 3—Dissolution profiles of MT from 8-10 mesh beads coated with Aquacoat®.
Coatings: ○; 5%, ●; 10%, and ▽; 20%. Each point represents the mean± standard deviation except where the standard deviation is too small to show.

drug release may by predominantly governed by diffusion through the film barrier. $T_{50\%}$ (time to release 50% of drug release) of MT

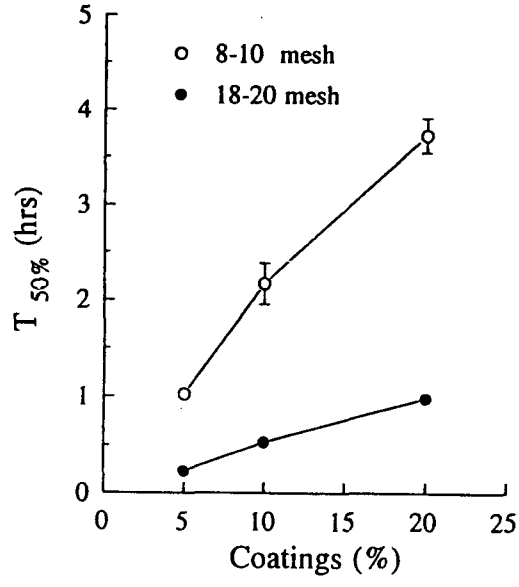

Figure 4—Time for 50% MT dissolution ($T_{50\%}$) as a function of coatings in coated beads. Each point represents the mean± standard deviation except where the standard deviation is too small to show.

from 18-20 mesh and 8-10 mesh coated beads is compared in Fig. 4. As coating amounts are increased, $T_{50\%}$ of MT is increased, as expected. $T_{50\%}$ was about 4 hours for the 20% coating of 8-10 mesh beads which provided the controlled release over 8 hours.

MT was degraded relatively slowly during storage condition (<5%). However, dissolution of MT from the 20% Aquacoat® coated beads (8-10 mesh) after 6 months' storage produced a release of MT from stored beads which was similar to the originally coated beads drug release (Fig. 5). These data suggest that although MT in coated beads was slowly degraded, the wall coat integrity was unchanged under the storage conditions. Beads (8-10 mesh) coated with 20% Aquacoat® was utilized for human clinical study.

Before administering the oral controlled release delivery system to human subjects, plasma MT concentrations were predicted from dissolution data of dosage form and population pharmacokinetic parameters of MT (Table II) using computer simulation (MAX-

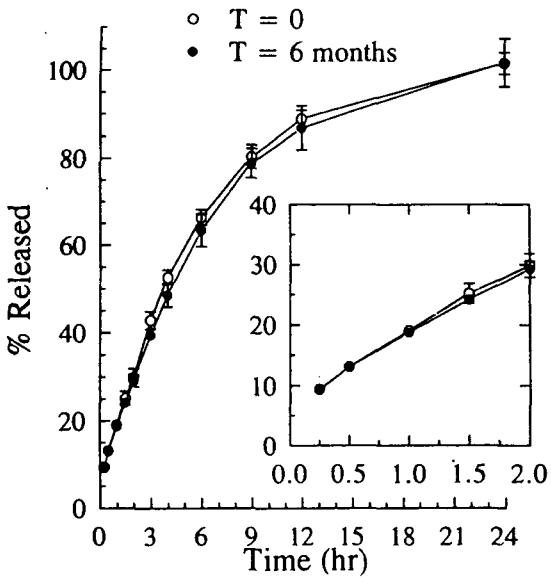

Figure 5—Comparison of dissolution profiles of MT from 8-10 mesh coated beads with 20% coatings after 6 months' storage at room temperature. Each point represents the mean± standard deviation except where the standard deviation is too small to show.

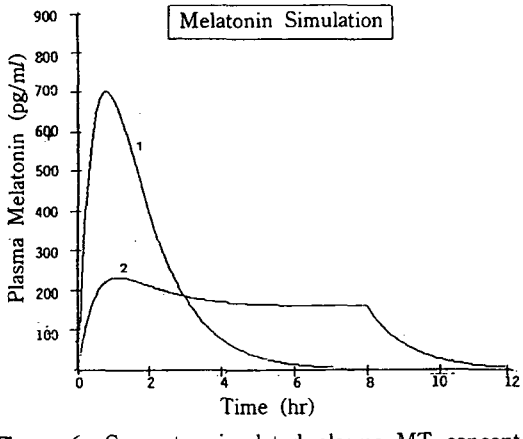

Figure 6—Computer-simulated plasma MT concentration-time curve in human subjects. 1. 0.5 mg immediate release MT only, 2. 0.1 mg immediate release MT and 0.4 mg controlled releast MT in 20% coated beads with Aquacoat®.

SIM®). An ideal dosage form would deliver MT to mimic the endogenous circadian pattern of MT. Computer-simulated plasma MT concentration-time profile in human subjects

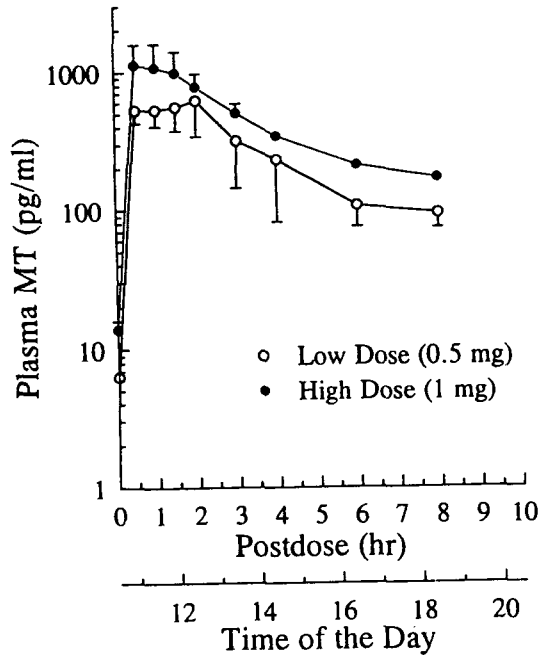

Figure 7—Mean plasma MT concentration-time profiles after administration of oral controlled release system to six human subjects at two different doses. Values are expressed as mean± standard deviation (n=4 for low dose, n=2 for high dose).

for the new dosage form is shown in Fig. 6. A conventional (immediate release) MT capsule (curve 1) dose not produce the desired sustained drug concentration because MT has a short half life of MT (Iguchi *et al.*, 1982; Walhauser *et al.*, 1984). Curve 2 shows that a mixture of uncoated beads containing 0.1 mg MT for immediate release and beads coated with 20% Aquacoat® containing 0.4 mg MT for controlled release are predicted to produce a plasma MT concentration versus time profile which mimics the profile known to be produced by endogenous release of MT during the night. Observed mean plasma MT concentration-time profile after administration of the oral controlled release delivery system to human subjects is shown in Fig. 7. When low dose of MT was administered to four subjects, average peak plasma MT concentration was reached at

about 600 pg/ml and maintained around 100 pg/ml over 8 hors. Peak plasma MT reached about 600 pg/ml as a result of immediate release of MT and extended MT concentrations over 8 hours result from the coated beads. When high dose of MT was administered to two subjects, average peak plasma MT concentrations were doubled, as expected and terminal slope was parallel with low dose. The shape of the plasma MT concentration-time profile was similar for the two different doses.

For further evaluation of plasma data of MT, noncompartmental pharmacokinetic parameters was obtained using RSTRIP II program. The new oral dosage form produced a MRT of 5 times longer compared to the intravenous injection of MT from a different study (Iguchi *et al.*, 1982). Observed MRT for the new oral dosage form ranges from 2.4 to 5 hours. Estimated bioavailability obtained was about 19% and 18% for low and high MT dose, respectively. A low bioavailability may result from extensive first pass metabolism and remaining amounts of MT from controlled beads. Extensive first pass metabolism of MT may be involved in the apparent low fraction of dose absorbed. This may suggest that plasma MT profiles are greatly influenced by the liver function.

Base on these results, observed plasma MT concentration-time profile was compared with computer simulated curve obtained previously (Fig. 8). The delivery system produces a plasma MT concentration-time profile which is in shape similar to that produced at night as a result of endogenous MT release (Waldhauser and Dietzel, 1985). However, maximal plasma MT concentrations produced were three times higher than MT concentrations predicted by computer simulation. These differences may primarily result from high MT dosing, improper ratio of immediate release MT and controlled release MT, and the difference of fraction of bioavailability (F=0.1) selected for computer simulation (Table II) due to intersubject variation

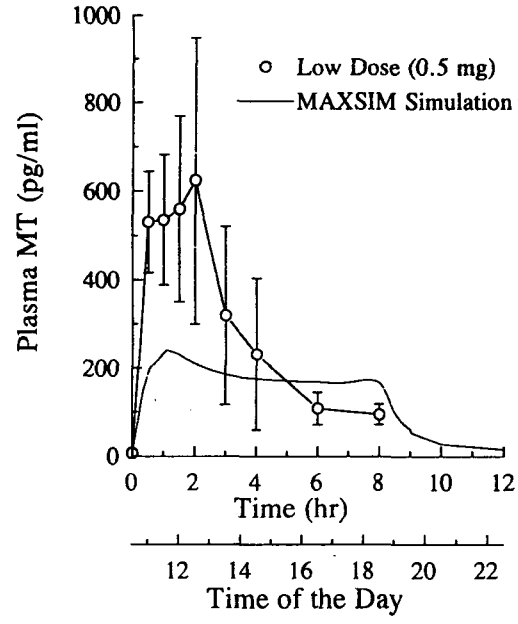

Figure 8—Comparison of the profiles of observed plasma MT concentrations (mean± standard deviation, n=4) and computer-simulated plasma MT concentration at low dose of MT.

of intrinsic metabolic function among subjects. Computer simulation curves with appropriate dosing adjustments are regenerated in Fig. 9. Previously, 10% bioavailability of MT was assumed for both immediate release MT and controlled release MT (Table II). Observed plasma MT concentrations (○) in this study at low dose MT were fitted when F =

0.3 for immediate release MT and F=0.1 for controlled release MT for computer simulation were utilized (Curve 2). Total dose of MT administered and ratio of immediate release MT and controlled release MT were varied. This simulation suggests that a total 0.2 mg MT dose (Curve 5) containing 10% immediate release MT is desirable to approximate a nighttime endogenous plasma MT concentration-time profile. Inherent is the assumption that F is larger for immediate release MT than controlled release MT due to a decreased first pass effect when the drug is rapidly absorbed. This assumption certainly needs confirmation prior to accep-

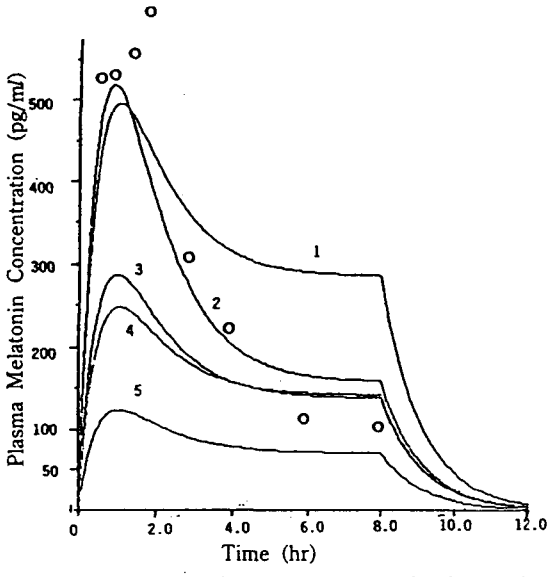

Figure 9—Regeneration of computer simulated plasma MT concentration-time profiles. Total dose and percentage of immediate release portion of MT are as follows: 1, 0.8 mg, 10%; 2, 0.5 mg, 20%; 3, 0.4 mg, 12.5%; 4, 0.4 mg, 10%; 5, 0.2 mg, 10%. Observed plasma MT concentrations at low dose MT studied (○). K_d=0.9 hr^{-1}, K_a=1.74 hr^{-1}, V_d=35.2 *l*, F=0.3 for immediate release MT, and F=0.1 for controlled release MT were used for regeneration of curves.

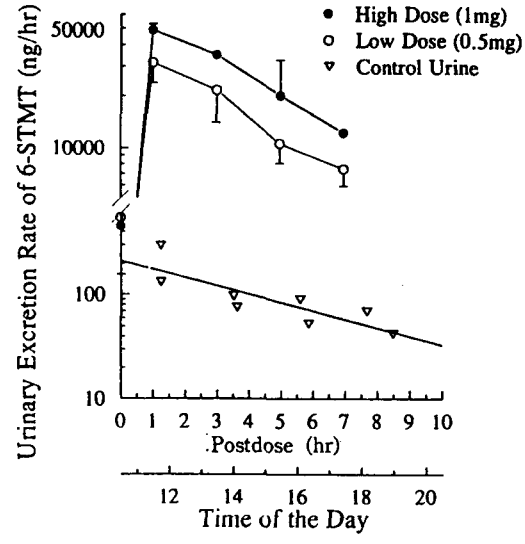

Figure 10—Urinary excretion rate (μg/hr) of 6-STMT determined at the midpoints of each urine collection interval after administration of the oral controlled release delivery system to six human subjects. Values are expressed as mean± standard deviation (n=4 for low dose, n=2 for high dose).

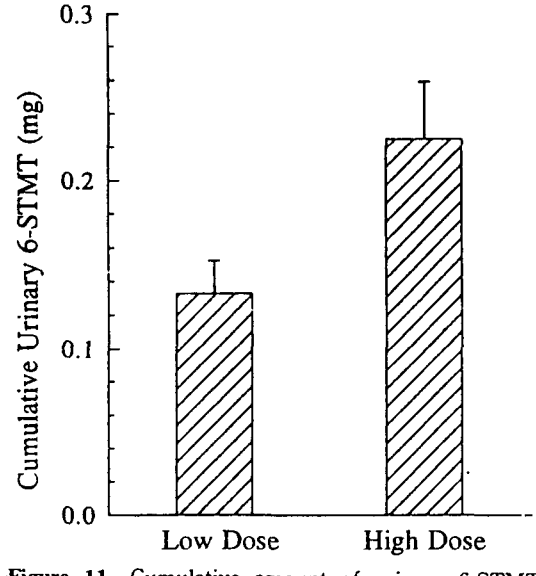

Figure 11—Cumulative amount of urinary 6-STMT (mg) for 6 hours at two different doses of MT. Values are expressed as mean± standard deviation (n=4 for low dose, n=2 for high dose).

tance. Another evaluation of an oral controlled release delivery system in both young and elderly people is under investigation.

It is interesting to correlate urinary excretion rate of 6-STMT with plasma MT because MT is extensively metabolized by the liver and excreted as 6-STMT. Urinary excretion rate of 6-STMT was plotted as a function of both postdose and time of the day (Fig. 10). Urinary excretion rates of 6-STMT during the daytime in subjects not receiving MT was very low (<200 μg/hr). When the oral controlled release delivery system of MT was administered, the urinary excretion rate of 6-STMT greatly increased compared to the control urine. The terminal slopes of urinary excretion rate profiles of 6-STMT were parallel for the two different doses. A correlation between plasma MT and urinary 6-STMT has been reported elsewhere (Brown *et al.*, 1991; Nowak *et al.*, 1987). The profiles of plasma MT and urinary excretion

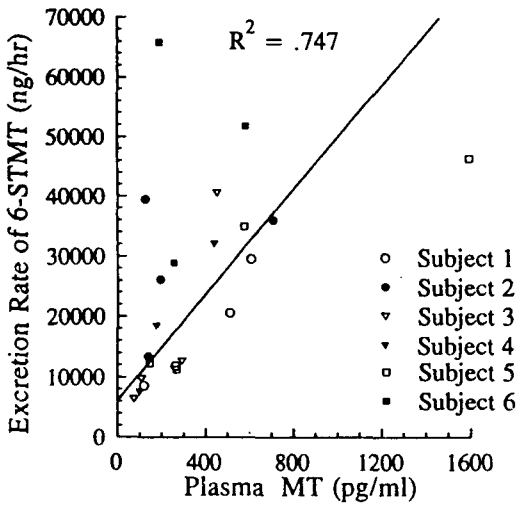

Figure 12—Relationship between plasma MT concentrations and urinary excretion rates of 6-STMT determined at the midpoints of each urine collection interval in six human subjects.

rates of 6-STMT are parallel at each dose. As expected, as plasma MT concentration increased, urinary excretion rate of 6-STMT also increased concomitantly. Urinary 6-STMT appears to reflect behavior of plasma MT concentration. Cumulative amounts of urinary 6-STMT for 6 hours were compared at two different doses of MT (Fig. 11). As MT dose was doubled, urinary 6-STMT was also twice increased. The difference was statistically significant. The relationship between urinary excretion rate of 6-STMT and plasma MT concentration in six human subjects is given in Fig. 12. The linear relationship between urinary excretion rate of 6-STMT and plasma MT concentration was highly significant ($F_{1,18}=2.28$, $r^2=0.56$, $p<0.001$). Furthermore, the linear relationship was much greater when only past peak plasma MT concentrations were correlated ($r=0.838$). These results suggest that urinary 6-STMT can be used as an non-invasive method to assess circadian rhythms of MT in humans.

4. CONCLUSION

A desired controlled release over 8 hours was achieved with 20% coating of 8-10 mesh beads with Aquacoat®. Dissolution profile of 20% coated beads was unchanged over 6 months' periods. An oral preparation designed to release 0.1 mg MT immediately from uncoated beads, and 0.4 mg MT from coated beads in a controlled release fashion over 8 hours produced about 600 pg/m*l* average peak plasma MT concentration, and then maintained MT concentrations at about 100 pg/m*l*. Pharmacokinetic analysis revealed that less than 20% of the MT dose administered was absorbed. A low bioavailability observed may result from extensive first pass metabolism and incomplete absorption from coated beads. Plasma MT concentration-time profile was very similar in shape to the computer-simulated profile. However, maximal plasma MT concentrations were about three times greater than predicted. Base on these results, computer simulation was performed after adjusting MT dose, ratio of immediate and controlled release MT, and fraction of bioavailability to closely mimic endogenous MT concentration-time curve. Urinary excretion rate of 6-STMT profile was statistically correlated with plasma MT concentration. Urinary 6-STMT may be used as non-invasive method to assess pineal gland activity of MT in humans.

REFERENCES

1) Aldhous, M.E. and Arendt, J. Radioimmunoassay for 6-sulphatoxymelatonin in urine using an iodinated tracer. *Ann. Clin. Biochem.*, **25**, 298-303 (1988).
2) Arendt, J., Aldhous, M. and Wright, J. Synchronization of a disturbed sleep-wake cycle in a blind man by melatonin treatment. *Lancet*, April 2, 773 (1988).
3) Brown, G.M., Bar-Or, A., Grossi, D., Kashur, S., Johannson, E. and Yie, S.M. Urinary 6-sulphatoxymelatonin, an index of pineal function in the rat. *J. Pineal Res.*, **10**, 141-147 (1991).
4) Cramer, H., Rudolph, J., Consbruch, U. and Kendel, K. On the effects of melatonin on sleep and behavior in man. *Adv. in Biochem.*

Psychopharmacol., **11**, 187-191 (1974).
5) Dahlitz, M., Alarez, B., Vignau, J., English, J., Arendt, J. and Parkes, J.D. Delayed sleep phase syndrome response to melatonin. *Lancet*, **337**, 1121-1124 (1991).
6) Ghebre-sellassie, I., Iyer, U., Kubert, D. and Fawzi, M.B. Characterization of a new water-based coating for modified release preparations, *Pharm. Tech.*, Sep. 96-106 (1988).
7) Hossain, M. and Ayres, J.W. Pharmacokinetics and pharmacodynamics in the design of controlled-release beads with acetaminophen as model drug. *J. Pharm. Sci.*, **81**(5), 444-448 (1992).
8) Iguchi, H., Kato, K.-I. and Ibayashi, H. Melatonin serum levels and metabolic clearance rate in patients with liver cirrhosis. *J. Clin. Endocrinol. Metab.*, **54**(5), 1025-1027 (1982).
9) Jackson, I.M., Robert, S., Timmins, P. and Sen, H. Comparison of laboratory-scale processing techniques in the production of coated pellets. *Pharm. Tech.*, Feb. 50-56 (1990).
10) Lane, E.A. and Moss, H.B. Pharmacokinetics of melatonin in man: First pass hepatic metabolism. *J. Clin. Endocrinol. Metab.*, **61**, 1214-1216 (1985).
11) Le Bars, D., Thivolle, P., Vitte, P.A., Bojkowski, C., Chazot, G., Arendt, J., Frackowiak, R.S. J., and Claustrat, B. PET and plasma pharmacokinetic studies after bolus intravenous administration of [^{11}C] melatonin in humans. *Nucl. Med. Biol.*, **18**(3), 357-362 (1991).
12) Lerner, A.B., Case, J.D., Heinzelman, R.V. Structure of melatonin. *J. Am. Chem. Soc.*, **81**, 6084-6085 (1959).
13) Lewy, A.J. and Markey, S.P. Analysis of melatonin in human plasma by gas chromatography negative chemical ionization mass spectroscopy. *Science*, **201**(25), 741-743 (1978).
14) Lewy, A.J. and Newsome, D.A. Different types of melatonin circadian secretory rhythms in some blind subjects. *J. Clin. Endocrinol. Metab.*, **56**, 1103-1107 (1983).
15) Mehta, A.M. Scale-up considerations in the fluid-bed process for controlled release products, *Pharm. Tech.*, Feb. 46-52 (1988).
16) Mehta, A.M., Valazza, M.J. and Abele, S.E. Evaluation of fluid-bed processes for enteric coating systems. *Pharm. Tech.*, April. 46-56 (1986).
17) Nowak, R., Mcmillen, I.C., Redman, J. and Short, R.V. The correlation between serum and salivary melatonin concentration and urinary 6-hydroxymelatonin sulphate excretion rate: Two non-invasive techniques for monitoring human circadian rhythmicity. *Clin. Endocrinol.*, **27**, 445-452 (1987).
18) Petrie, K., Conaglen, J.V. Thompson, L. and Chamberlain, K. Effects of melatonin on jet lag after long haul flight. *Br. Med. J.*, **298**, 705-707 (1989).
19) Petterborg, L.J., Thalen, B.E., Kjellman, B.F. and Wetterberg, L. Effects of melatonin replacement on serum hormone rhythm in a patient lacking endogenous melatonin. *Brain Res. Bull.*, **27**(2), 181-185 (1991).
20) Rekhi, G.S., Mendes, R.W., Porter, S.C. and Jambhekar, S.S. Aqueous polymeric dispersions for controlled drug delivery-Wurster process. *Pharm. Tech.*, March 112-125 (1989).
21) Rosenthal, N.E., Sack, D.S., Gillin, J.C., Lewy, A.J. Goodwin, F.K., Davenport, Y., Mueller, P. S., *et al.*, Seasonal affective disorder. A description of the syndrome and preliminary findings with light therapy. *Arch. Gen. Psychiatry*, **41**, 71-80 (1984).
22) Sack, R.L., Lewy, A.J., Blood, M.L., Stevenson, J. and Keith, L.D. Melatonin administration to blind people: Phase advances and entrainment. *J. Biol. Rhythm*, **6**(3), 249-262 (1991).
23) Strassman, R.J., Peake, G.T., Qualls, C.R., and Lisansky, E.J. A model for the study of the acute effects of melatonin in man. *J. Clin. Endocrinol. Metab.*, **65**, 847-852 (1987).
24) Waldhauser, F., Dietzel, M. Daily and annual rhythms in human melatonin secretion: Role in puberty. *Ann., New York Acad. Sci.*, **453**, 205-214 (1985).
25) Waldhauser, F., Vierhapper, H. and Oirich, K. Abnormal circadian melatonin secretion in night-shift workers. *The New Eng. J. Med.*, **7**, 441-446 (1986).
26) Waldhauser, F., Waldhauser, M., Lieberman, H.R., Deng, M.-H., Lynch, H.J., and Wurtman, R.J. Bioavailability of oral melatonin in humans. *Neuroendocrinol.*, **39**, 307-313 (1984).