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Modified release bi-layered tablet of melatonin using β-cyclodextrin

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A modified release bi-layered tablet of melatonin incorporating a fast release fraction consisting of melatonin-β-cyclodextrin inclusion complex and a slow release fraction containing melatonin in HPMC K15M and Carbopol® 971 P matrices was prepared. The formulation developed showed an initial burst followed by a near zero order release pattern for a period of 8 h. The drug content, physical characteristics and the release profile were unaffected after 3 months of an accelerated stability study at 40 °C and 75% relative humidity.

1. Introduction

Melatonin (MT) is a neurohormone secreted by the pineal gland in a circadian fashion. MT plasma concentration is low during the day (<10 pg/ml). It then rises in the late evening and is maintained at 25–120 pg/ml during the night (over 8 h) until it returns to the day time baseline [1]. Dosage forms which mimic the physiologically produced endogenous MT plasma concentration versus time profile will be valuable to fully evaluate the clinical potential of MT in the treatment of sleep disorder syndrome, jet-lag, seasonal affective disease, shift work syndrome and other circadian disorders [2–4].

MT is a poorly soluble hormone (aqueous solubility – 0.96 mg/L) [5] with slow dissolution characteristics. This may be one of the factors responsible for large inter-individual variations in MT plasma concentration observed after oral ingestion [6]. Cyclodextrins (CDs) have been used in pharmaceutical formulations to enhance the solubility, dissolution rate, stability and bioavailability of drugs [7]. The property of enhancing the solubility may be explained by the hollow truncated, cone like structure of CDs which encapsulates the hydrophobic drug molecules in the apolar interior. In contrast, the outer hydrophilic region enables solubilization through interaction with water [8, 9].

In this study, a novel approach was followed for preparing a modified release tablet of melatonin whereby a bi-layered tablet of melatonin was formulated containing a fast release fraction and a slow release fraction. Complexation between MT and β-CD was used to increase the solubility and dissolution rate of MT in the fast release layer while hydroxypropyl methyl cellulose (HPMC – K15M) and Carbopol 971 P matrix were used for the slow release portion to provide an appropriate sustained release.

2. Investigations and results

The phase solubility diagram for MT-β-CD complex system in water is shown in Fig. 1. Solubility of MT increased linearly with increasing concentration of β-CD, thus showing a typical A_L-type phase solubility curve [10], characteristic of complexation with a stoichiometric ratio of 1 : 1. The apparent 1 : 1 stability constant, Kc, was found to be 50.11 M^{-1}.

Evaluation of the prepared inclusion complexes of MT-β-CD by DCS, FTIR and SEM showed that complex formation took place in case of both the kneading method as well as the solid dispersion method. However, the degree of complex formation was better in case of the kneading method. The DSC thermograms of the complex prepared by the kneading method showed the disappearance of the endothermic peaks of MT and β-CD at 120 °C and 140 °C, respectively, which were present in the complex prepared by the solid dispersion method, although in reduced intensity. Similarly, in the FTIR spectra of the complex prepared by kneading, the peaks characteristic of MT were greatly reduced whereas there was very slight reduction in case of the complex prepared by the solid dispersion technique. SEM photographs of the kneaded as well as solid dispersion product showed the absence of MT crystals.

The release profile of MT from tablets prepared with uncomplexed MT and MT-β-CD systems is shown in Fig. 2.

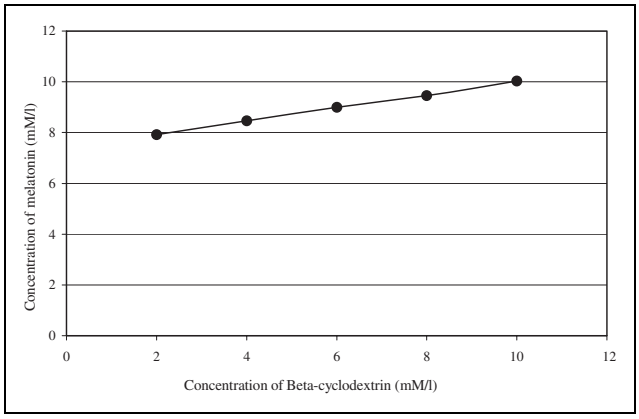

Fig. 1: Phase solubility diagram for the melatonin-β-cyclodextrin system

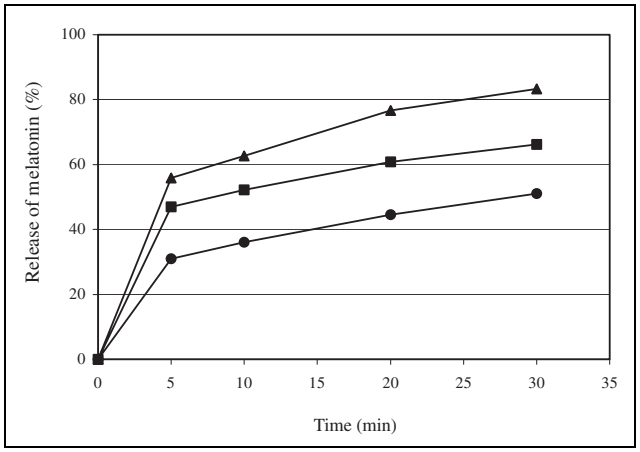

Fig. 2: Release profile of melatonin from fast-release tablets
—●— Uncomplexed melatonin; —■— 1 : 1 complex by solid dispersion; —▲— 1 : 1 complex by kneading

Tablets containing the kneaded MT-β-CD system showed the maximum release (84% after 30 min) whereas tablets containing MT-β-CD complex prepared by solid dispersion showed a release of 66%. In comparison, the tablets containing uncomplexed MT showed a release of only 51% after 30 min. Thus, it could be concluded that complex formation between MT and β-CD resulted in the improvement in the dissolution rate of MT. Since the dissolution was better in case of the kneaded product, tablets prepared with the kneaded MT-β-CD system were selected as fast-release portion for the bi-layered tablet.

Preliminary studies with slow release tablets prepared from HPMC alone showed that although HPMC was able to sustain the release of MT for 8 h, there was an initial high release because of erosion of the tablet matrix. On the other hand, tablets prepared with CP-971P alone were unable to retard the release of MT for more than 5 h. However, CP-971P formed a soft matrix and there was no erosion of the tablets. Hence, it was decided to use a combination of HPMC and CP-971P for sustaining the release of MT.

Table 1 gives the composition of the different formulations prepared and Fig. 3 shows the release profile of MT from these formulations. Formulation F-I could not sustain the drug release for 8 h and showed complete release of the drug within 6 h. Formulation F-III showed a release of only 83% after 8 h. Formulation F-II sustained the drug release for 8 h and showed almost complete release at 8 h.

Table 1: Formulation composition of slow-release melatonin tablets

Components	Formulations (quantity per tablet in mg)		
	F1	F2	F3
Melatonin	2	2	2
HPMC K15M	40	60	80
Carbopol 971P	20	30	40
Avicel PH 102	134	104	74
Magnesium stearate	2	2	2
Purified talc	2	2	2

Table 2: Composition of the final bi-layered tablet of melatonin

Components	Quantity (in mg per tablet)
Fast-release portion	
Melatonin-β-cyclodextrin complex	5.8
Avicel PH 102	127.2
Maize starch	15.0
Purified talc	2.0
Slow-release portion	
Melatonin	2.0
HPMC K15M	60.0
Carbopol 971P	30.0
Avicel PH 102	104.0
Magnesium stearate	2.0
Purified talc	2.0

Hence, it was selected as the optimized formulation for incorporation into the slow-release portion of the bi-layered tablet. All the three formulations exhibited a firm, rigid matrix throughout the dissolution study and did not show any erosion.

Table 2 gives the formula for the optimized bi-layered tablet of MT and Fig. 4 shows the drug release profile from the optimized tablet. As is evident from the Fig., about 36% of the total drug was released from the tablet within the first 30 min. This amount of drug would be available for immediate absorption in the body resulting in peak plasma levels. The rest of the drug released slowly with near zero-order kinetics and would be responsible for maintaining the plasma MT level achieved by the fast-release portion over a period of 8 h.

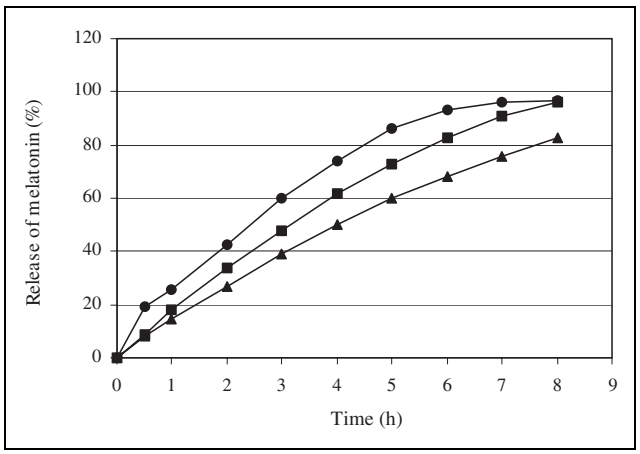

Fig. 3: Release profile of melatonin from slow release tablets
—●— Formulation F-1; —■— Formulation F-2 —▲— Formulation F-3

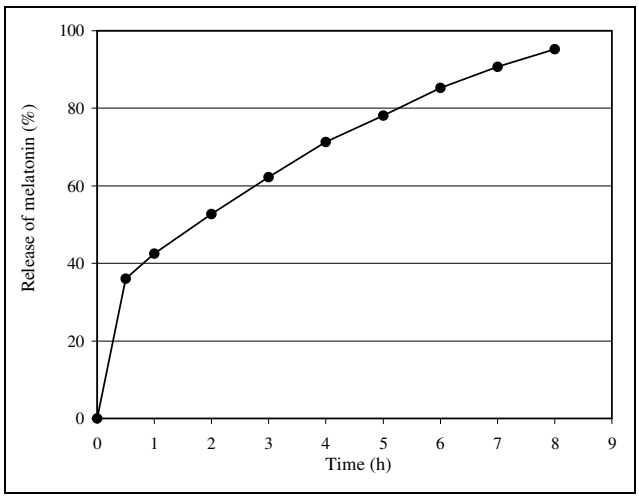

Fig. 4: Release profile of melatonin from final bi-layered tablets

Table 3: Stability study of the final bi-layered tablets of melatonin

Time (d)	Description	% Drug content	Average weight (mg)	Hardness (kg cm^{-2})
0	Off-white, bilayered tablets	99.0	349.3	9.6
30	Off-white, bilayered tablets	98.2	352.7	9.4
60	Off-white, bilayered tablets	97.6	353.8	9.4
90	Off-white, bilayered tablets	97.4	354.9	9.2

Table 3 shows the results of the stability study carried out on the final bi-layered tablet. The percentage drug content after 3 months storage at 40 °C and 75% RH was found to be 97.4% and other physical parameters of the tablets like appearance and hardness remained unchanged throughout the study period. Although the tablets gained some weight during the stability study, the release profile remained unchanged.

3. Discussion

The present study was an attempt to develop a bi-layered tablet consisting of a fast release portion and a slow release portion of MT. The bi-layered formulation for MT was selected in order to mimick the circadian rhythm of MT. The prepared tablet showed a desirable drug release profile with the fast-release fraction giving an initial burst effect followed by a sustained release effect from the slow-release fraction. Complexation of MT with β-CD was successfully employed for improving the dissolution rate of the drug in the fast-release portion. A combination of 30% HPMC K15 and 15% CP-971P in the slow-release portion could sustain the drug release for 8 h with a near zero-order kinetics. The final bi-layered tablet remained unaffected after storage at 40 °C and 75% RH for 3 months. The developed formulation thus has a potential for commercial application for persons whose sleep pattern is disturbed due to shift change, seasonal change or traveling through various time zones. The *in-vivo* bioavailability of the drug from the developed formulation is to be determined.

4. Experimental

4.1. Materials

MT was obtained as a gift sample from Dabur Research Foundation, India. β-CD (American Maize Products Co., USA), Carbopol 971 P (CP-971P) (BF Goodrich Co., USA), HPMC (Methocel K15M) (Colorcon Ltd. UK) and Avicel PH 102 (FMC Corporation, USA) were used as received. All other chemicals and reagents were of AR grade.

4.2. Phase solubility study on MT-β-CD system

Feasibility of complex formation between MT and β-CD and stoichiometry of complex was studied by the phase solubility method of Higuchi and Connors [10]. An excess amount of the drug was added to the solution of β-CD in water (pH 6.8) at various concentrations (2–10 mML^{-1}). The flasks were sealed and magnetically stirred for 72 h at 25 ± 1 °C. After equilibrium was attained, the samples were filtered (0.45 μm membrane filter), diluted suitably and the absorbance recorded at 2 nm using Spectronic-21 UV spectrophotometer (Bausch & Lomb).

4.3. Preparation of inclusion complexes

Complexes of MT with β-CD were prepared in molar ratio of 1 : 1 by the techniques of solid dispersion as well as kneading. Solid dispersions were prepared by dissolving the required quantities of MT and β-CD in ethanol (50%) and evaporating the solution to dryness in a hot air oven at 60 °C. In the kneading technique, equimolar quantities of MT and β-CD were mixed in a mortar, wetted with purified water and kneaded until the product started drying on the wall of the mortar. Further drying was carried out in a hot air oven at 60 °C. The dried complexes were powdered and sieved through 180 μm mesh to give the final product.

4.4. Evaluation of solid inclusion complexes

The prepared solid inclusion complexes were evaluated by differential scanning calorimetry, fourier transform infrared spectroscopy and scanning electron microscopy, as described previously [11].

4.5. Preparation of MT tablets

For initial optimization studies, the fast-release and slow-release portions were compressed separately and evaluated. The optimized formulations of the two layers were combined to give the final modified-release bi-layered tablet of MT.

4.5.1. Preparation of MT fast release tablets

The fast-release layer was prepared by directly compressing the MT-β-CD complex along with excipients on a IR hydraulic press using a 13 mm diameter die and a pressure of 2 t for 5 s. Directly compressible microcrystalline cellulose (Avicel PH 102) was used as the diluent. 10% maize starch acted as the disintegrant while 1% purified talc were used as lubricant. Tablet weight was kept at 150 mg and the MT content was 1.0 mg per tablet.

4.5.2. Preparation of MT slow release tablets

The slow-release layer was also prepared by directly compressing MT along with varying quantities of HPMC and CP-971P and other excipients on IR hydraulic press using a 13 mm diameter die and a pressure of 2 t for 5 s. Directly compressible microcrystalline cellulose (Avicel PH 102) was used as the diluent. 10% Maize starch acted as the disintegrant while 1% magnesium stearate and 1% purified talc were used as lubricants. Tablet weight was kept at 200 mg and the MT content was 2.0 mg per tablet.

4.5.3. Preparation of modified release bi-layered tablets of MT

The bi-layered tablet was prepared by first lightly compressing the slow-release portion on the hydraulic press and then adding the material of the fast-release portion directly over the slow-release layer in the die. The tablet was then compressed at a pressure of 2 t applied for 5 s.

4.6. Drug release studies

Drug release studies were carried out in a specially designed dissolution apparatus comprising a vertical glass cylinder of 200 ml capacity. This was kept in a jacketed vessel of water maintained at 37 ± 1 °C. This vessel was kept under stirring by means of a paddle manufactured for this purpose on a dissolution apparatus. Stirring speed for all the release studies was kept at 50 rpm.
For the fast-release tablets, USP simulated gastric fluid without enzymes (SGF) was used as the dissolution medium while for the slow-release tablets, the dissolution medium employed was USP simulated intestinal fluid without enzymes (SIF). Release studies for the bi-layered tablet were carried out in SGF for the first 2 h followed by SIF for the next 6 h. Samples were withdrawn at regular intervals, filtered and analyzed by HPLC method described by Lee and Min [2].

4.7. Stability studies

Accelerated stability testing as per WHO guidelines was carried out on the final bi-layered tablets of MT in order to determine the physicochemical stability of the formulation.
The tablets were stored in tightly closed glass bottles having rubber stoppers and metallic caps. These were placed in humidity chambers maintained at 40 ± 2 °C and a relative humidity of 75%. Samples were withdrawn at 0, 30, 60 and 90 days and were analyzed for active drug content, hardness, weight gain/loss and drug release profile.

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