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Solubility and Stability of Melatonin in Propylene glycol and 2-Hydroxypropyl-β-cyclodextrin Vehicles

Beom-Jin Lee[1], Han-Gon Choi[2], Chong-Kook Kim[2], Keith A. Parrott[3], James W. Ayres[3] and Robert L. Sack[4]

[1]Biological Rhythm and Controlled Release Lab., College of Pharmacy, Kangwon National University, Chuncheon 200-701, Korea, [2]Department of Physical Pharmacy, College of Pharmacy, Seoul National University, Seoul 151, Korea, [3]College of Pharmacy, Oregon State University, Corvallis, OR, [4]School of Medicine, Oregon Health Science University, Portland, OR

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The physicochemical properties of melatonin (MT) in propylene glycol (PG) and 2-hydroxypropyl-β-cyclodextrin (2-HPβCD) vehicles were characterized. MT was endothermally decomposed as determined by differential scanning calorimetry (DSC). Melting point and heat of fusion obtained were 116.9±0.24°C and 7249±217 cal/mol, respectively. MT as received from a manufacture was very pure, at least 99.9%. The solubility of MT in PG solution increased slowly until reaching 40% PG and then steeply increased. Solubility of MT increased linearly as concentration of 2-HPβCD without PG increased (R^2=0.993). MT solubility in the mixtures of PG and 2-HPβCD also increased linearly but was less than the sum of its solubility in 2-HPβCD and PG individually. The MT solubility was low in water, simulated gastric or intestinal fluid but the highest in the mixture of PG (40 v/v %) and 2-HPβCD (30 w/v %) although efficiency of MT solubilization in 2-HPβCD decreased as the concentration of PG increased. MT was degraded in a fashion of the first order kinetics (r^2>0.90). MT was unstable in strong acidic solution (HCl-NaCl buffer, pH 1.4) but relatively stable in other pH values of 4~10 at 70°C. In HCl-NaCl buffer, MT in 10% PG was more quickly degraded and then slowed down at a higher concentration. However, the degradation rate constant of MT in 2-HPβCD was not changed significantly when compared to the water. The current studies can be applied to the dosage formulations for the purpose of enhancing percutaneous absorption or bioavailability of MT.

Key words : Melatonin, Solubility, Stability, Propylene glycol, 2-Hydroxypropyl-β-cyclodextrin

INTRODUCTION

Melatonin (MT) is an indole amide neurohormone (Lerner *et al.*, 1959). It is primarily secreted by the pineal gland in a circadian rhythm (Waldhauser and Dietzel, 1985). Exogenous MT has been shown to be effective in resetting disordered circadian rhythms of MT in humans and may be useful in the treatment of disordered sleep syndrome, jet lag, shift work syndrome and seasonal affective diseases in humans. Controlled drug delivery systems of MT for oral (Lee *et al.*, 1995, Lee *et al.*, 1996; Lee and Min, 1996), transdermal (Lee *et al.*, 1994, Konsil *et al.*, 1995) and transmucosal route (Benes *et al.*, 1993) have been widely investigated to deliver MT in a physiological fashion. We previously investigated that MT in PG vehicle could be delivered transdermally (Lee *et al.*, 1994) but the percutaneous absorption was so low due to its low water solubility (Lewy, 1983). The very variable oral bioavailability of MT, possibly resulting from extensive first-pass metabolism and variable absorption was also reported (Waldhauser *et al.*, 1984; Lane and Moss, 1985). Therefore, it is interesting to investigate the solubilization and stability of MT using commonly used vehicles. Currently, the physicochemical properties of MT is not widely studied.

Cosolvents or complexing agents are commonly used to overcome solubility and stability problems of drugs in the pharmaceutical industry. PG is among the most useful cosolvents for drugs because of its ability to increase aqueous solubility. It is inexpensive, stable and nontoxic, and hence used widely in commercial preparations (Yalkowsky and Rubino, 1985). Another approach is to use cyclodextrins which complex with lipophilic drugs. The highly water soluble 2-HPβCD, a commercially useful complexing agent used to en-

Correspondence to: Beom-Jin Lee, Ph.D., Biological Rhythm and Controlled Release Lab., College of Pharmacy, Kangwon National University, Chuncheon 200-701, Korea

capsulate numerous compounds at the molecular level, is commonly used by formulators to increase aqueous solubility and chemical stability of various substances (Yoshida *et al.*, 1988; Loftsson *et al.*, 1989; Duchene and Wouessidjewe, 1990).

The purpose of the present research was to characterize the thermal behavior and purity of MT using differential scanning calorimetry (DSC). Aqueous solubility and stability of MT in the presence of PG and/or 2-HPβCD vehicles were also investigated.

MATERIALS AND METHODS

Materials

MT was purchased from Regis Chemical Co. (Morton Grove, IL, USA). 2-HPβCD with average molecular weight (1542) and average degree of molar substitution (6-7 moles of hydroxypropy per mole of β-cyclodextrin) was provided by courtesy of American Maize-Product Company (Hammond, ID, USA). PG was purchased from J.T. Baker Inc. (Phillipsburg, NJ, USA). All other chemicals used were of reagent grade. Deionized water was used throughout the study.

DSC analysis of MT

Perkin-Elmer differential scanning calorimetry (DSC) was used to characterize thermal properties of MT, including the melting point, heat of fusion, and purity of MT. The DSC apparatus consists of a Thermal Analysis Data Station system, Interface, System 4 Microprocessor Controller, DSC-4 Analyzer with sampling provision, and a recorder. Dried nitrogen gas continuously purged the DSC sample holder. Temperature was scanned from 50 to 150°C at a rate of 10°C/min. A pure indium sample was used for temperature calibration.

Solubility study

Solubility studies were carried out according to the method of Higuchi and Connors (1965). Excess amounts of MT were added to water, simulated gastric (pH 1.4) and intestinal (pH 7.4) fluid (Lee and Lee, 1995), and 0.05M phosphate buffer (pH 6.1) containing various concentrations of PG and/or 2-HPβCD. Parafilm was used to cover the top to prevent evaporation. Equilibrium was reached within 24 h in a 25.0±0.1°C constant-temperature water bath. Samples were centrifuged at 10,000g for 15 min and the clear supernatant was filtered and then diluted with phosphate buffer for HPLC assay. The concentration of MT was determined using HPLC as mentioned previously (Lee and Min, 1996)

Stability study

Stability of MT was investigated under conditions of varying pH and varying concentrations of PG and 2-HPβCD. The simulated gastric HCl-NaCl Buffer (pH 1.4), acetate buffer (pH 4.7), simulated intestinal phosphate buffer (pH 7.4), and carbonate buffer (pH 10) were used to study the effect of pH on stability of MT. Known concentrations of MT solution at different pH were stored in an incubator at 70±2°C in the dark over 9 months. The degradation rate of MT with varying concentrations of PG and 2-HPβCD was determined only in the acidic NaCl-HCl buffer (pH 1.4). The concentration of MT was determined using HPLC as mentioned previously (Lee and Min, 1996).

RESULTS AND DISCUSSION

DSC analysis of MT

DSC evaluation was undertaken to determine the purity of MT as received from the supplier to decide whether or not this product was suitable for evaluation in solubility and stability study, as well as for a proposed in vivo administration to people as an investigational new drug. MT was endothermally melted as identified by a DSC tracing. Melting point, heat of fusion, and purity of compounds obtained were 116.8± 0.25°C, 7249±217 cal/mol, and 99.97±0.05%, respectively. The commercially available MT is sufficiently pure to be used for *in vivo* human studies.

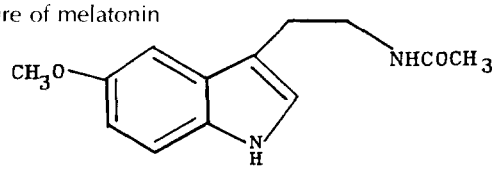
Solubility study

A group contribution theory based on the thermodynamic partition of each structural moiety is often useful to calculate solubility parameters, which express the cohesion forces between molecular moieties (Barton, 1983). The calculated solubility parameter of MT was 11.6 $(cal/cm^3)^{1/2}$ (Table I). The solubility of MT in water, simulated gastric (pH 1.4) and intestinal fluid (pH 7.4) was very low and independent on pH. MT solubility was changed when PG as a cosolvent was added. The solubility of MT in PG solution increased slowly until reaching 40% PG and then steeply increased (Fig. 1). There was a linear tendency between the log solubility of MT and the volume fraction (concentration) of PG (data not shown). The solubility of MT as a function of 2-HPβCD concentration in the absence or presence of PG is shown in Figure 2. Solubility of MT increased linearly as the concentration of 2-HPβCD without PG increased (R^2=0.993). MT solubility in the mixtures of PG and 2-HPβCD also increased linearly as 2-HPβCD increased. In the mixtures of PG and 2-HPβCD, observed MT solubility must be equivalent to theoretical MT solubility (sum of MT solubility in PG and 2-HPβCD individually) if PG and 2-HPβCD behave independently as solubilizers. How-

Table I. Solubility parameters of MT[a] calculated by group contribution method

Atoms or Group	Number	ΔU_i (KJ/mol)[b]	ΔV_i (cm^3/mol)[b]
CH_3	2	4.71×2=9.42	33.5×2=67.0
CH2	2	4.94×2=9.88	16.1×2=32.2
CH	4	4.31×4=17.24	13.5×4=54.0
O	1	3.35	3.8
C=O	1	17.40	10.8
NH	2	8.40×2=16.80	4.5×2=9.0
C	4	4.31×4=17.24	-5.5×4=22.0
Ring closure	2	1.05×2=2.10	16.0×2=32.0
Conjugation	4	1.67×4=6.68	-2.2×4=-8.8
Total		$\Sigma\Delta U_i$=100.11	$\Sigma\Delta V_i$=178
Solubility parameter=$(\Sigma\Delta U_i/\Sigma\Delta V_i)^{1/2}$=11.6 (cal/$cm^3$)$^{1/2}$			

[a]Structure of melatonin



[b]ΔU_i and ΔV_i are the additive atomic and group contribution for the energy of vaporization and molar volume, respectively. Note the unit conversion to calculate solubility parameter

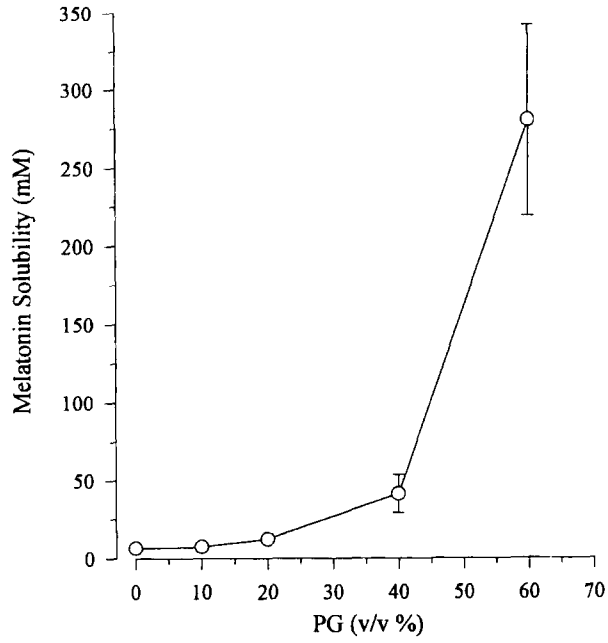


Fig. 1. Melatonin solubility (mM) as a function of PG concentration (v/v%) at 25°C in 0.05 M phosphate buffer (pH 6.1)/PG solutions.

ever, observed MT solubility was less than theoretical MT solubility. These results suggest that interactions between PG, 2-HPβCD, and/or MT may exist. MT solubility in the mixtures of PG and 2-HPβCD was not additive.

The initial linear portion of a solubility curve can be useful to examine the efficiency of complexation since the slope reflects the solubility increase as a

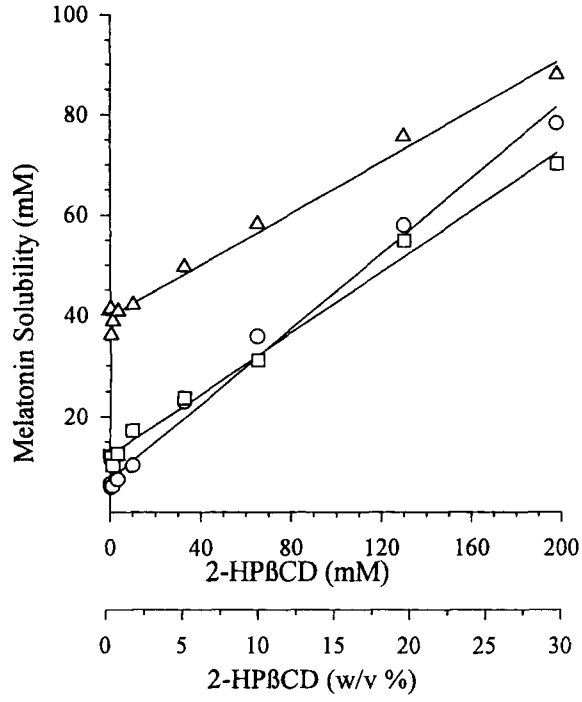


Fig. 2. Melatonin solubility (mM) as a function of 2-HPβCD concentration in the presence of various PG concentrations at 25°C in 0.05 M phosphate buffer (pH 6.1). 0% (○); 20% (□); 40 % (△).

function of 2-HPβCD concentration. The steeper the slope, the more efficient the complexation. Fig. 2 provides that the initial slopes obtained in buffer, PG 20%, and PG 40% as a function of 2-HPβCD concentration are 0.38, 0.31, and 0.26, respectively. Complexation efficiency between MT and 2-HPβCD was not high. However, the slope is often useful to calculate the stability constant (or complex formation) on the basis of a 1:1 stoichiometric ratio between drug and complexing agent. Although more than one drug molecule can be complexed with 2-HPβCD, an apparent stability constant, calculated with the assumption of only 1:1 stoichiometry is often used to describe the system (Higuchi and Connor, 1965). The equation used is as follows:

$$\text{Stability constant } (K_{1:1}) = \frac{\text{slope}}{S_o\,(1\text{-slope})}$$

where S_o refers to the solubility of drug in water without PG and 2-HPβCD. The stability constant (M^{-1}) and the slope of the solubility isotherm are given in Fig. 3. The stability constant and the slope of the solubility isotherm were decreased without deviating from linearity as the concentration of PG was increased. Combination of 2-HPβCD with PG reduced the solubilization capacity of 2-HPβCD so that solubilization of MT was less efficient. In the mixtures of PG and 2-HPβCD, PG may have competitively displaced MT

molecules from complexed cavity of 2-HPβCD. Because 2-HPβCD complexes MT in the inner ring closure by noncovalent bonding or hydrophobic properties, PG may influence surrounding forces such as a hydrogen bridge between 2-HPβCD and MT by changing water structure and entropy inside the hydrophobic ring (Muller and Albers, 1991). The overall MT solubility in various vehicles is compared in Fig. 4. The MT solubility was low in water, simulated gastric or intestinal fluid. The use of PG or 2- HPβCD was useful to enhance the MT solubility. Apparent MT solubility was the highest in the mixture of PG (40 v/v %) and 2-HPβCD (30 w/v %) although efficiency of MT solubilization in 2-HPβCD decreased as the concentration of PG increased as mentioned previously.

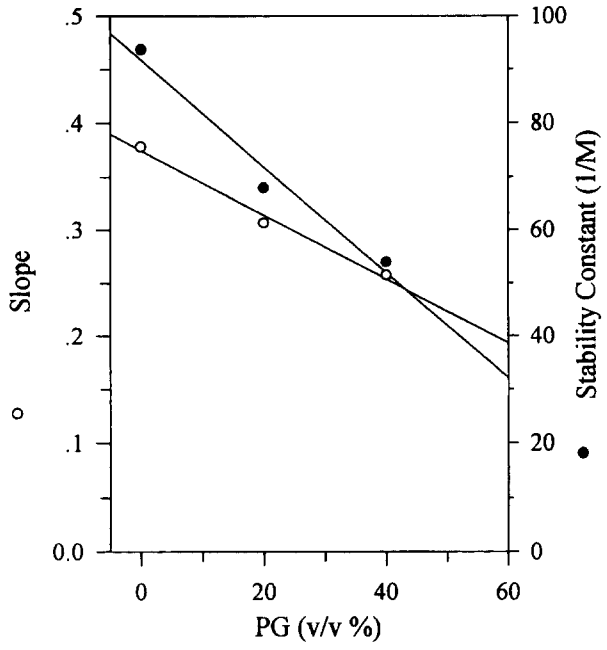


Fig. 3. Effect of concentrations of PG on the stability constant of melatonin (M^{-1}) and the slope calculated from the linear portion of melatonin solubility versus 2-HPβCD concentration (mM) curves.

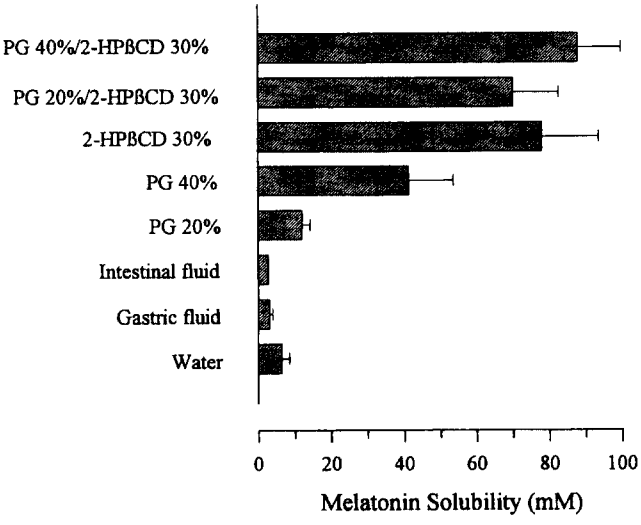


Fig. 4. Overall comparison of melatonin solubility in various vehicles.

Stability study

The pH-stability profiles of MT in various buffer solutions at 70°C is given in Fig. 5. MT was degraded in a fashion of the first order kinetics (r^2>0.90). MT was unstable in strong acidic solution (HCl-NaCl buffer, pH 1.4) but relatively stable in other pH values of 4~10 at 70°C. An amide linkage appears to be sensitive to acid catalyzed hydrolysis in low pH solution (Dilbeck *et al.*, 1978; Connors *et al.*, 1979).

The stability of MT in PG or 2-HPβCD solution at 70°C in NaCl-HCl buffer solution (pH 1.4) was also investigated (Fig. 6). The use of mixed solvents to achieve greater aqueous solubility may result in altering the stability of drugs (Marcus and Taraszka, 1959). In HCl-NaCl buffer, MT in the presence of PG was more unstable as compared to its stability in water, resulting in accelerated degradation of MT. MT in 10% PG solution was degraded 85 times more quickly than in aqueous solution without PG at 70°C. Acid catalyzed degradation of MT slowed down at a higher concentration. Degradation rate may be dependent on water content and solvent polarity or dielectric constant (Marcus and Taraszka, 1959; Connors *et al.*, 1979). In 10% PG, a glycolated proton would be a

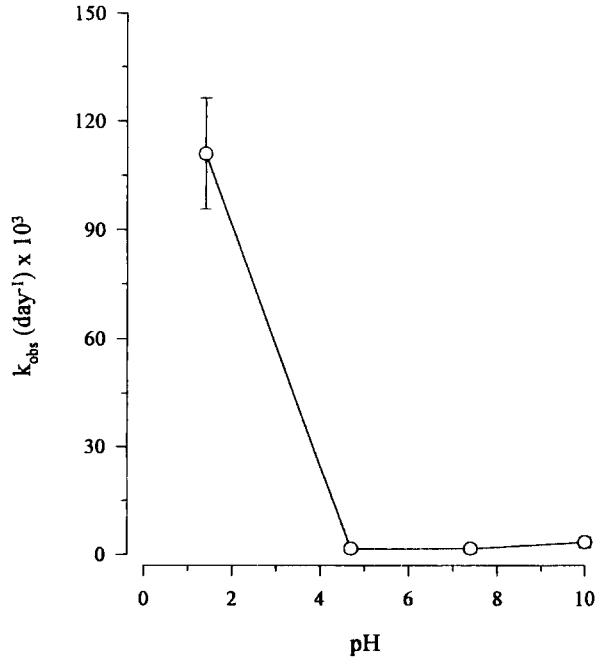


Fig. 5. The pH-stability profiles of melatonin in various buffer solutions.

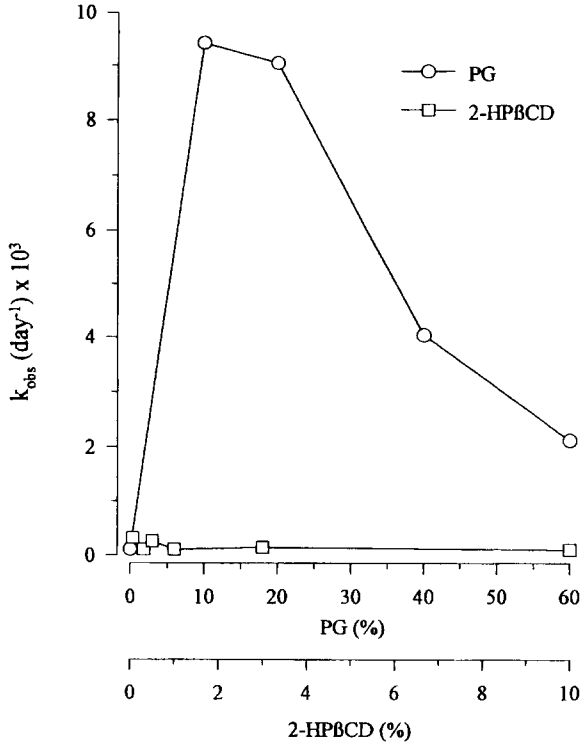


Fig. 6. The stability of melatonin in PG or 2-HPβCD vehicles in NaCl-HCl buffer solution (pH 1.4) at 70°C.

stronger acid than the hydronium ions, thus achieving a greater catalytic effect. However, as the concentration of PG increased, the "water" reaction significantly decreased because water content decreased, resulting in slowly decreased acid catalyzed hydrolysis. On the other hand, the degradation rate constant of MT in 2-HPβCD was not changed significantly when compared to the water. The amide moiety of MT may be less sensitive to protonation or acid catalyzed degradation in 2-HPβCD solutions when compared to PG solutions. The detailed degradation mechanism of MT and complexation phenomena between MT and 2-HPβCD needs to be further investigated.

In conclusion, the solubility of MT was enhanced in a mixture of PG or 2-HPβCD. The stability of MT was dependent on pH of buffer solution and the type of vehicle selected. The current studies can be applied to the dosage formulations for the purpose of enhancing percutaneous absorption or bioavailability of MT.

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