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Title: Development of a Transdermal Delivery System for Melatonin

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Melatonin is an neurohormone secreted by the pineal gland. Several therapeutic possibilities for melatonin administration have been suggested. However, clinical studies with melatonin have been limited by lack of a delivery system capable of mimicking the physiological release pattern of melatonin. There is an increasing recognition that the skin can serve as a port of administration for systemically active drug. The stability, solubility, skin-vehicle partition coefficient, and in vitro percutaneous penetration of melatonin in a series of buffered propylene glycol-water mixtures (10, 20, 40, and 80%; v/v) over a pH range of 3.3-8.7 were examined. Stability, solubility, and partition coefficient studies were conducted at 25°C while diffusion analysis was performed at 30°C. Melatonin is more stable in acidic solution than in alkaline solution. Higher percent of propylene glycol does not affect the melatonin stability at pH 3.3 but could stabilize melatonin at pH 8.7. The solubility of melatonin in propylene glycol-water mixtures is minimally affected by pH and ranged from 2.2 mg/ml for 10%

propylene glycol to over 25 mg/ml for 80% propylene glycol. Hairless mouse skin was used to determine the skin-vehicle partition coefficient, and for percutaneous penetration studies. Melatonin was able to move across hairless mouse skin in these studies (donor phase, PG-water mixtures; receptor phase, normal saline). Flux from PG-water solutions ranged from 6.08 to 0.56 μg/cm/hr while flux from gel preparations ranged from 0.23 to 0.07 μg/cm/hr. Percutaneous delivery of melatonin is feasible and the existence of a transdermal delivery system should facilitate clinical studies in human subjects.

DEVELOPMENT OF A TRANSDERMAL DELIVERY SYSTEM FOR MELATONIN

by

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INTRODUCTION

MELATONIN

Melatonin (N-acetyl-5-methoxytryptamine) is an indole amide neurohormone secreted primarily from the pineal gland (1,2). Melatonin has also been found in the retina, the Harderian gland, and the gut (2,3). Active production of melatonin occurs only at night, in the dark. The onset of production (typically beginning at about 9 P.M.) produces a 10-20 fold increase in blood concentrations which remain fairly constant until about 9 A.M. When production ceases, concentrations fall to low daytime levels (4-7). This hormone, through an action in the brain, appears to be involved in the regulation of various neural and endocrine processes that are cued by the daily change in photoperiod (3). These include the regulation of seasonal effects on reproduction, body weight, metabolism, and coat color in photoperiodic mammals such as sheep and hamsters, the control of circadian rhythms in birds and reptiles, and the modulation of retinal physiology (2,8,9).

There is evidence to suggest that melatonin may be effective in synchronizing disturbed circadian rhythms in

mammals, including humans (10-12). Administration of melatonin at scheduled times is effective in entraining and synchronizing circadian rhythms regulated primarily by the hypothalamic suprachiasmatic nuclei (2,10,11,13). In addition, pineal melatonin appears to regulate several neuroendocrine functions such as sleep (14,15), thyroid hormone secretion (16), and growth hormone release (17) in humans. Sleep disturbances caused by jet lag (which results from rapid flight across several time zones) and the subsequent desynchronization of circadian rhythms are being treated by administration of melatonin at scheduled times (12,13). Changes in melatonin levels have also been shown at different stages of the menstrual cycle, with different seasons (18,19), and also with age (15,20). Melatonin has also been shown to lower intraocular pressure and may be useful in the treatment of glaucoma (21).

In the synthesis of melatonin, tryptophan is hydroxylated by tryptophan hydroxylase into 5-hydroxytryptophan. This 5-hydroxytryptophan is decarboxylated to serotonin by L-aromatic acid decarboxylase. N-acetyltransferase (NAT) converts serotonin to N-acetylserotonin, which is methylated to melatonin by hydroxyindole-O-methyltransferase (HIOMT) (2,3). Melatonin is metabolized in the liver and brain

(2,3,22). The major pathway for the metabolism of melatonin is hydroxylation at position 6, followed by conjugation with sulfate or glucuronic acid in the liver. A small fraction of melatonin is enzymatically cleaved at position 1 of the indole ring and metabolized to N-acetyl-5-methoxykynurenamine as the final product in the brain (22).

While there are an increasing number of reports which involve oral administration to man of pineal indole melatonin, data on the pharmacokinetics of melatonin are scant. Some pharmacokinetic parameters were reported by Waldauser et al (23), Iguchi et al (22) and Lane et al (24). Melatonin clearance from blood showed a biphasic pattern of first-order kinetics. The half-life in minutes and disappearance rate constant (per minute) in healthy subjects were $t_1 1/2 = 5.6 \pm 0.5$, $K_1 = -0.055$, $t_2 1/2 = 43.6 \pm 1.5$, and $K_2 = -0.0071$ (22). Normal melatonin production was 28.8 μg/day, while the production rate for cirrhotic patients was 12.3 μg/day. The mechanism for a decreased melatonin production rate in patients with cirrhosis may involve a negative feedback effect of high plasma concentration on biosynthesis in the pineal gland. The clearance of melatonin for normal subjects and cirrhotic patients is 631 and 127 ml/min, respectively. The oral bioavailability for melatonin is 0.03-0.76 (24). The

volume of distribution, calculated from clearance and K_2, is 88.87 l.

TRANSDERMAL DELIVERY SYSTEM

Traditionally, drugs are administered in the form of tablets, capsules, and injectables into the body. However, these routes of administration usually produce large fluctuations of drug concentration in the blood stream and tissues. Consequently, potentially unfavorable patterns of efficacy and toxicity develop (25).

Alternative methods of administration have been attempted, with the topical route certainly amongst those tried, given its accessibility and extensiveness. Because melatonin has properties which lend itself to be administered transdermally, a review of transdermal dosage forms is presented. Drugs were apparently used topically by early Egyptian and other Mediterranean civilizations not only to treat surface wounds and the local manifestations of disease, but also to cure general illnesses (26). Poultices, cataplasms, and plasters were still in wide use as cold remedies and for other human afflictions at the dawn of the 20th century. However, only in the last 20 years has it proven possible to unequivocally demonstrate that systemic therapy by the topical route is indeed feasible (26). A plasma

concentration that is effective but not toxic can be maintained for a desired time using the transdermal route.

Since introduction of a transdermal delivery system for scopolamine (27), there has been a proliferation of interest in this route of drug administration. Transdermal drug delivery can be defined as a technique or method in which active chemicals are made available to pass through intact skin at a rate and duration designed to accomplish an intended effect (25,28). A definition perhaps more acceptable to the chemist and engineer may be the permeation-moderated transfer of an active material from a reservoir to a target surface to maintain a predetermined concentration or emission level for a specified period of time (25). Transdermal delivery can increase the therapeutic value of many drugs by obviating specific problems associated with the drug. Such problems might include, gastrointestinal irritation, low absorption, decomposition due to hepatic "first pass" effect, formation of metabolites that cause side effects, and short half-life necessitating frequent dosing (25). In transdermal medication, the above problems can be eliminated because the drug diffuses over a prolonged period of time directly into the bloodstream. An excellent example is that of nitroglycerin used in angina

pectoris patients as a vasodilator. Nitroglycerin has a 90% hepatic "first pass" effect, so it is not used orally to prevent angina pectoris attacks. Its main use has been as a sublingual tablet to abort an attack after it occurred. With the advent of transdermal medication, nitroglycerin is now used as a patch to prevent angina pectoris attacks (25).

Though the advantages of transdermal medication are impressive, the merits of each application have to be examined individually. Only a small percentage of drugs can be delivered transdermally due to three limitations, difficulty of permeation through human skin, skin irritation, and clinical need (25,27). A major limitation to permeation is the lipophilicity of the drug. For a drug to reach systemic circulation, it needs to be hydrophilic to pass the epidermis and lipophilic to pass the stratum corneum (29). The most ideal drugs are the ones with solubility properties for environments in oil and water. Thus, the partition coefficient of a drug is one of several important considerations.

The major parts of a transdermal system are the vehicle (polymer matrix or matrices) that regulate release of drug, the drug, and enhancers and other excipients. The applicability of transdermal delivery system is most limited at the present time by the

necessity that the drug be extremely potent. It is possible that higher doses of drugs can be delivered through the skin in the presence of penetration enhancers. There are potential problems associated with the incorporation of these adjuvants into a transdermal formulation. Because an enhancer must act upon the skin in order for delivery to be improved, additional materials (e.g. the promoter itself or cosolvents) will gain access to the systemic circulation and to the local viable cutaneous tissue. There is an increased possibility, therefore, of irritant responses and other unwanted side-effects (25).

Permeation via the transepidermal route begins with a solution-diffusion process in the stratum corneum. There appear to be two distinguishable conduit regimes (25). The first is formed directly from the acutely flattened cellular building blocks of the horny layer. The cell units are 30 to 40 micrometers in diameter and 1/4 to 1 micrometer thick; they are filled with a semicrystalline, polar biopolymer (keratin) (25). A considerable portion of the keratin is found as tightly compacted bundles of fibrils. The bulk of the lipid of the stratum corneum resides in seams which lie between and separate the cellular units and may act to cement the cell layers together. This intercellular region

constitutes the second permeation regime in the stratum corneum. This lipid region may account for 20% or more of the total dry weight of the horny layer. After traversing the stratum corneum, a permeant must diffuse across the living epidermis, a 100 micrometer or more avascular wedge of living cells. Few molecules have difficulty in passively negotiating this zone. Molecules too hydrophobic to dissolve in the watery cytosol of the cells may find this and the underlying dermal zone difficult to pass due to their limited solubility in the bulk of the tissue. Large and extremely polar molecules like vidarabine also have difficulty in crossing the living cellular region; such molecules have problems crossing individual cell membranes. However, the living epidermal resistance encountered by solutes of extreme polarity is far less than the resistance of the intact stratum corneum. Therefore, for polar drugs, the resistance of the living epidermis only becomes important on denuded skin. Diffusion across the layer of the dermis above the actively functioning vascular appears to be through the watery, gelatinous ground substance which fills the interstitial space between dermal collagen and elastin fibers (25). While diffusional resistance of the dermis will usually be nominal, the diffusional resistance could conceivably be significant if diffusive

penetration to great depth was necessary to reach the circulation (25,30).

OBJECTIVES AND ARRANGEMENT OF THIS STUDY

The overall goal of this research is to develop a transdermal delivery system for melatonin which can mimic the timing and concentration of melatonin blood levels. Transdermal administration is promising because the targeted plasma concentration are low (50-100 pg/ml) (22) and potentially achievable by this route; also the physio-chemical properties of melatonin (lipid solubility, etc.) suggest that it will diffuse through the skin if the proper formulation is developed.

This research took place in three parts. Part I was the development of an assay method for melatonin. Part II involved measurement of melatonin's stability and solubility in selected propylene glycol-buffer mixtures and its partition coefficient between the vehicle and skin. These data were used to predict desirable formulations for a topical melatonin dosage form. In Part III of the study, three liquid vehicles and three gel formulations were tested to determine drug diffusion through hairless mouse skin (which has been shown to be a good model for the diffusion of drugs through human skin).

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Chapter I Determination of Melatonin by High-Performance Liquid Chromatography with Ultra-Violet Detection

INTRODUCTION

Many analytical methods have been developed for quantitation of melatonin including bioassay (1), fluorimetry (2), gas-chromatography-mass spectrometry (3-5), radioimmunoassay (6-9), high-performance liquid chromatography (HPLC) with electrochemical detection (10) and high-performance liquid chromatography (HPLC) with ultraviolet and fluorometric dual detection (11). All these methods have some merits and demerits in respect of sensitivity, selectivity, specificity or convenience. A simple and rapid technique for determination of melatonin by high-performance liquid chromatography (HPLC) with UV detection has been developed in our laboratory.

EXPERIMENTAL

INSTRUMENTATION

A high-performance liquid chromatograph equipped with an M-45 solvent delivery system, WISP 710B injector, reversed-phase C18 (5u) radial compression column and Model 441 absorbance detector with a 229-nm light source (all from Waters Associates) was used. Detector output was recorded with a C-R3A integrator (Shimadzu corporation, Kyoto, Japan).

CHEMICALS AND REAGENTS

Glacial Acetic acid (J.T. Baker, Phillipsburg, U.S.A.) and sodium acetate (EM Science, NJ, U.S.A.) were reagent grade. Methylparaben was analytical grade. Methanol was HPLC grade (EM Science NJ). Melatonin was obtained from Regis Chemical Co. (Morton Grove, IL, U.S.A.). All water was deionized before use.

MOBILE PHASE

The mobile phase was 0.2 M acetate buffer (0.2 M sodium acetate and 0.2 M acetic acid)-methanol (65:35), pH 5.4 (12). After filtration and sonification for at least 15 minutes, the degassed mobile phase was pumped through the column at a flow rate of 1 ml/min.

ASSAY STANDARDS

Three stock solutions of melatonin in water (stock solution A: 21.48 μg/ml, stock solution B: 21.12 μg/ml and stock solution C: 28.64 μg/ml) were prepared. For assay work the stock solution was diluted with water into different concentrations. Both stock solution and dilution were prepared fresh weekly and stored at 4^{0}C. The internal standard, methylparaben, was also prepared in water. A concentration of approximately 50 μg/ml of methylparaben (internal standard) was also prepared weekly and stored at 4^{0}C.

ANALYTICAL METHOD

Each stock solution was diluted into six different concentrations (1.056-8.592 μg/ml). One hundred ul of each diluted melatonin solution was mixed with 100 ul of internal standard. An aliquot (20 ul) of the mixture was injected onto the C_{18} radial compression column. All injections were done in duplicate. The peak area ratio (or the peak height ratio) of melatonin to methylparaben (I.S.) was plotted vs melatonin concentration to obtain a standard calibration curve for melatonin. All experiments were performed at ambient temperature. Linear regression analysis was used to calculate the slope, intercept and correlation coefficient. Mean percent of theory for each

curve was calculated using the technique of inverse estimation (13).

RESULTS AND DISCUSSION

Nine standard curves (six points) were run over a period of three weeks. The slope, intercept and correlation coefficient for these nine curves are shown in Table I.1. Melatonin and the internal standard (methylparaben) were clearly separated and eluted within 12 minutes (Figure I.1). Mean retention times for melatonin and methylparaben were 7.3 and 10.4 min, respectively. Mean percent of theory for the nine curves was 100.09% with a mean coefficient of variation of 2.07%, see Table I.2. Day-to-day accuracy and precision of the assay are shown by the data in Table I.3, I.4 and I.5. Mean percent of theory for stock solution A, B and C is 100.20, 100.51 and 99.88% with a mean coefficient of variation of 0.98, 1.20 and 1.85%, respectively. A typical standard curve for melatonin is shown in Figure I.2. It was possible to decrease the lower limit of the assay to 0.1 μg/ml by increasing the injection volume to 100 ul and changing sensitivity of the UV spectrophotometer or integrator.

CONCLUSIONS

According to the assay data, this rapid and simple HPLC assay for melatonin is suitable for various preformulation studies of melatonin. However, the assay method is not sufficiently sensitive for analysis of melatonin plasma where concentrations are typically 4-100 pg/ml (14).

TABLE I.1 STANDARD CURVE DATA FOR MELATONIN IN WATER

Curve*	Correlation coefficient (r)	Slope	Intercept
1	0.999	0.2362	-0.0417
2	0.999	0.2373	-0.0272
3	0.999	0.2662	-0.0893
4	0.999	0.2371	-0.0303
5	0.999	0.2316	-0.0124
6	0.999	0.2365	-0.0355
7	0.999	0.2450	-0.0341
8	0.999	0.1918	-0.0596
9	0.999	0.2019	-0.0614

* Six points per curve, range 1.1-8.6 μg/ml

TABLE I.2 STANDARD CURVE DATA FOR ASSAY OF MELATONIN IN WATER

Curve*	Mean percent of theory	Coefficient of variation (%)
1	100.42	1.93
2	99.98	1.23
3	100.59	2.49
4	100.04	1.95
5	99.59	1.76
6	99.69	1.94
7	99.47	2.48
8	100.79	3.62
9	100.24	1.22
Mean±S.D.	100.09±0.432	2.07±0.693

* Six points per curve, range 1.1-8.6 μg/ml.

TABLE I.3 PRECISION AND ACCURACY FOR ASSAY OF MELATONIN IN WATER USING STOCK SOLUTION A

Actual concentration (μg/ml)	Mean experimental concentration* (μg/ml)	Mean percent of theory	Coeff. of Var. (%)
1.074	1.090	101.45	2.89
1.611	1.624	100.81	0.11
2.148	2.143	99.76	0.93
3.222	3.222	99.99	1.26
4.296	4.239	98.67	0.61
6.444	6.478	100.53	0.08
Mean ± S.D.		100.20±.98	0.88±.95

* Two determinations

TABLE I.4 PRECISION AND ACCURACY FOR ASSAY OF MELATONIN IN WATER USING STOCK SOLUTION B

Actual concentration (μg/ml)	Mean experimental concentration (μg/ml)	Mean percent of theory	Coeff. of var. (%)
1.056	1.098	103.96	2.95
1.584	1.625	102.58	1.04
2.112	2.097	99.28	1.67
3.168	3.112	98.23	1.02
4.224	4.132	97.83	0.15
6.336	6.412	101.20	0.34
Mean ± S.D.		100.51±2.26	1.20±.93

* Two determinations

TABLE I.5 PRECISION AND ACCURACY FOR ASSAY OF MELATONIN IN WATER USING STOCK SOLUTION C

Actual concentration (μg/ml)	Mean experimental concentration* (μg/ml)	Mean percent of theory	Coeff. of var. (%)
1.432	1.410	98.44	3.54
2.148	2.143	99.76	1.03
2.864	2.885	100.73	1.60
4.296	4.325	100.68	2.13
5.728	5.717	99.70	1.92
8.592	8.587	99.94	0.85
Mean ± S.D.		99.88±.76	1.845±.88

* Five determinations.

Fig. I.1 Chromatogram for melatonin and methylparaben

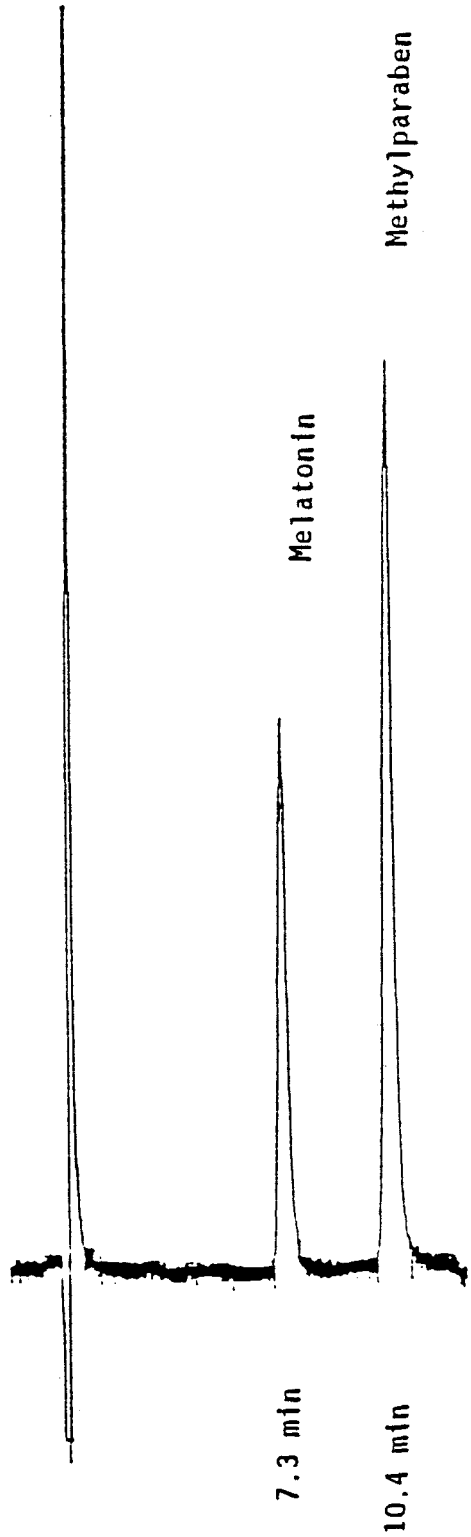
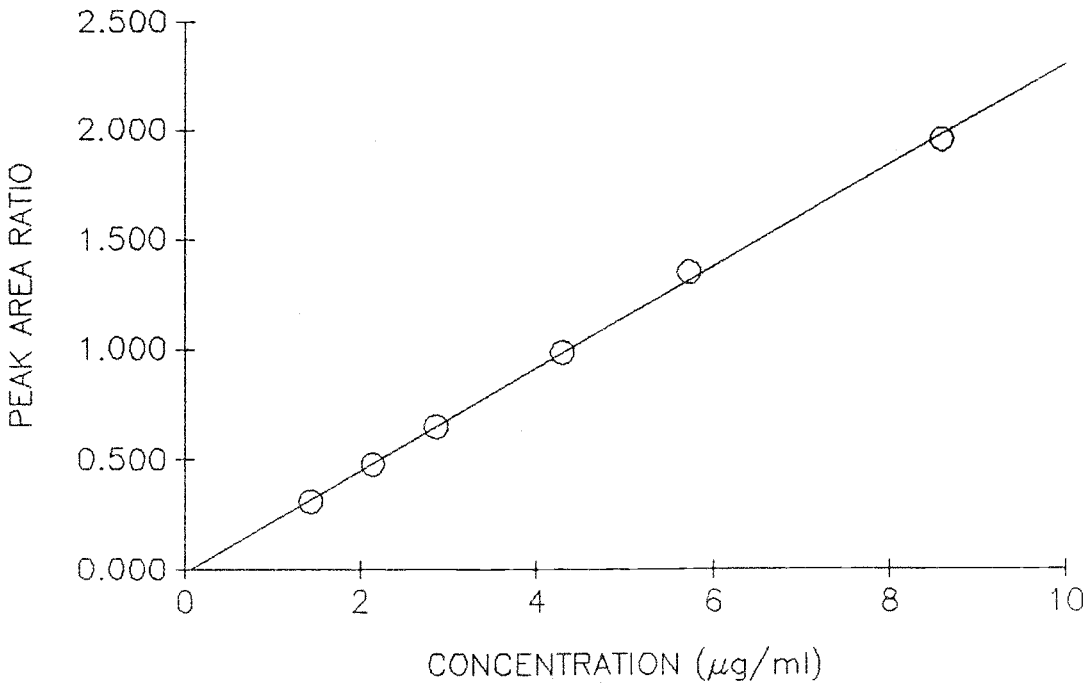


Fig. I.2 A typical standard curve for melatonin.



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Chapter II Preformulation Studies for Melatonin: Determination of Melatonin Stability, Solubility, and Partition Coefficient

INTRODUCTION

While melatonin has potential value in clinical medicine, no melatonin dosage forms are currently commercially available, and clinical studies with melatonin have been limited by lack of a delivery system capable of mimicking the physiological release pattern of melatonin. Most recently, there is an increasing recognition that the skin can serve as a port of administration for systemically active drug. A plasma concentration that is effective but not toxic may be maintained for the desired time through transdermal administration (1).

The form of the equation often used in evaluating a transdermal delivery system is: $J = (D)(PC)(C)/h$ where J equals the rate of penetration of drug through the skin (flux), D equals the diffusion coefficient of the drug in skin, PC equals the partition coefficient of the drug between the skin and vehicle, C equals the concentration of diffusible drug in the vehicle, and h is the thickness of the skin barrier (2-4). PC and C are dependent on the vehicle and thus may be modified by vehicle composition to maximize flux. The stability, solubility, and skin-vehicle partition coefficient of melatonin in a series of buffered propylene glycol-water mixtures (10, 20, 40, and

80%; v/v) over a pH range of 3.3-8.7 were examined. The purpose of these studies is to determine the vehicle composition (of those tested) which will result in maximum flux.

MATERIALS AND METHODS

BUFFER SOLUTIONS

Two buffer systems were used in this study (5). One was an acid phthalate buffer system; the other was a phosphate buffer system. The pH 3.3 buffer solution was made by dissolving 2.042 g of potassium biphthalate in 50 ml of water which was added to 10.4 ml of 0.2 M HCl; then the above solution was diluted to 200 ml, and the resulting solution was adjusted with 0.2 M HCl to a pH of 3.3. The pH 6.1 buffer solution was made by dissolving 1.3609 g of monobasic potassium phosphate in 50 ml of water which was added to 5.6 ml of 0.2 M NaOH; then the above solution was diluted to 200 ml, and the resulting solution was adjusted with 0.2 M NaOH to a pH of 6.1. The pH 8.7 buffer solution was made by dissolving 1.3609 g of monobasic potassium phosphate in 50 ml of water which was added to 46.1 ml of 0.2 M NaOH; then the above solution was diluted to 200 ml, and the resulting solution was adjusted with 0.2 M NaOH to a pH of 8.7.

VEHICLE PREPARATION

Propylene glycol/buffer solutions of 10%, 20%, 40%, and 80% (v/v) were prepared at the following pH's: 3.3, 6.1 and 8.7. These vehicles were used throughout the study and were prepared fresh for each part of the study.

STABILITY STUDY

Known amounts of melatonin (see Table II.1) were dissolved in 75 ml of each propylene glycol-buffer solution. Two ml of solution was placed in each of 30 polyethylene tubes which were stored in an incubator at 25°C in the dark. Samples were taken in triplicate at the following intervals: 0, 1, 5, 12, 24 hours, day 2, day 4, day 5, and day 7. and stored at -40°C until analysis. Analysis of melatonin content was via high pressure liquid chromatography (HPLC) using the technique described in chapter I. All stability studies were performed at 25°C with results expressed in terms of percent drug remaining.

SOLUBILITY STUDY

The solubility of melatonin in the different percentage propylene glycol/buffer solutions was determined in duplicate by agitating an excess amount of melatonin in 10 ml of the solvent until equilibrium was achieved. The study was conducted using a Franz Diffusion

Cell (see Figure II.1) at a controlled temperature of 25°C. Parafilm was used to cover the top to prevent evaporation. Samples were collected at the following times: 15, 30 min, 1.5, 3, 6, 10, 14, 18, 21, and 24 hours. Samples were centrifuged and stored at -40°C until analysis by HPLC. Excess melatonin was not placed in the 80% propylene glycol/buffer solutions due to its great solubility in this solvent.

APPARENT PARTITION COEFFICIENT

Seven to ten weeks old male hairless mice (HRS/J strain) were sacrificed by exposure to a CO_2 atmosphere about one hour before the study started, and the abdominal skin surgically removed. The skin was then allowed to soak in the propylene glycol-buffer solutions for 30 minutes prior to the start of the study.

Hairless mouse skin/vehicle (see vehicles preparation, page 31) apparent partition coefficients (aPC) were determined in duplicate at 25°C using the technique described by Scheuplein (4) and later modified by Blank and McAuliff (6). Abdominal skin of known weight (0.641-1.569 g, see Table II.2) was agitated with known volumes (10 ml each) of solution containing known amounts of melatonin (see Table II.2) for 36 hours at controlled temperature of 25°C. Samples of solution were collected at the following intervals: 0, 12, 18, and 24 hours.

Samples were stored at -40°C until analysis. Control solutions were prepared as above but without mouse skin and sampled in parallel with the experimental solution.

The skin/vehicle solution apparent partition coefficient was calculated using the following equation (4,6):

$$aPC = \frac{(Co - Ct)/\text{Weight of skin}}{Ct/\text{Volume of solution}}$$

Where Co is the initial concentration of melatonin in the solution and Ct is the concentration of melatonin in solution at 24 hours. The difference between Co and Ct represents the amount of melatonin absorbed by skin.

RESULTS AND DISCUSSION

STABILITY STUDY

The stability of melatonin in different propylene glycol-buffer solutions is shown in Table II.3 and Figure II.2.3.4. The linearity of the plots (logarithm % drug remaining vs time) indicate that degradation of melatonin follows first-order kinetics (7). Melatonin is more stable in acidic solution than in alkaline solution. The higher percent of propylene glycol did not affect the stability of melatonin much at pH 3.3 but stabilized the melatonin at pH 8.7. Stability is sufficient to develop test formulations, but may be a problem in actual product development. Thus, initial formulations of melatonin may require refrigeration but additional stability studies are needed to determine appropriate storage conditions.

SOLUBILITY STUDY

Solubility data for melatonin in different solvents are shown in Table II.4 and Figures II.5.6.7. The solubility of melatonin in propylene glycol-buffer mixtures increases as the percent of propylene glycol increases. The solubilities of melatonin in the same percentage of propylene glycol-buffer solutions are almost the same, independent of pH change. Thus, the

influence of pH on solubility is not significant.

APPARENT PARTITION COEFFICIENT

The real partition coefficient measurement for a drug that can be exactly related to its rate of diffusion through skin is the value determined for equilibrium distribution of drug between vehicle and skin (8). Because of the limited stability of melatonin and possible development of bacterial contamination in the solvent (due to skin microorganisms), we conducted this study for only 24 hours and called this parameter an "apparent partition coefficient" at time t (aPCt). Linear regression analysis was used to calculate the slope and intercept for data points of drug concentration in vehicle vs time plots and the technique of inverse estimation (9) used to calculate Ct at t = 24 hours (Figures II.8-19 and Table II.5). In the control solutions Co-Ct was not significant indicating that the change in melatonin concentration at 24 hours was not due to degradation or adsorption to the container. The apparent partition coefficient was calculated using the equation mentioned in the methods section, and is shown in Table II.6. The apparent partition coefficient increased as the percentage of propylene glycol decreased, except for the 10% propylene glycol-buffer solution.

CONCLUSIONS

According to the flux equation, J = (D)(PC)(C)/h, the rate of penetration (flux J) through the skin is proportional to the product of the partition coefficient (PC) and solubility (C). In this study the apparent partition coefficient between vehicle and skin generally increased as the solubility of the drug in vehicle decreased. This is in agreement with data in the literature for other drugs. A large aPC value indicates that the vehicle has poor affinity for the drug. A low aPC value reflects the tendency of the drug to remain in the vehicle. Hence, the release of a substance will be favored by selection of vehicles having a low affinity for the drug or those in which the drug is least soluble (8). For melatonin, profiles of solubilities and partition coefficients are shown in Figures II.20.21.22. Products of the apparent partition coefficients and solubilities were calculated (Table II.7). The 40% propylene glycol-buffer solution at pH 6.1 had the largest product of the apparent partition coefficient and solubility. Although 80% propylene glycol/buffer solutions would be expected to have a large product of apparent partition coefficient and solubility due to their very large melatonin solubility, the release of melatonin would be expected to be minimal as discussed

above. Thus, maximum flux would be expected when the 40% propylene glycol-buffer at pH 6.1 solution is used as a vehicle for melatonin.

TABLE II.1 AMOUNTS OF MELATONIN USED FOR EACH 75 ML VEHICLE IN STABILITY STUDY

Vehicles	Amount of melatonin dissolved in 75 ml of vehicle
10% P.G. pH 3.3*	0.25 mg
10% P.G. pH 6.1	0.30 mg
10% P.G. pH 8.7	0.30 mg
20% P.G. pH 3.3	0.32 mg
20% P.G. pH 6.1	0.30 mg
20% P.G. pH 8.7	0.28 mg
40% P.G. pH 3.3	0.31 mg
40% P.G. pH 6.1	0.35 mg
40% P.G. pH 8.7	0.38 mg
80% P.G. pH 3.3	0.31 mg
80% P.G. pH 6.1	0.30 mg
80% P.G. pH 8.7	0.33 mg

* 10% P.G. pH 3.3 is 10% propylene glycol/ 90% pH 3.3 buffer solution

TABLE II.2 INITIAL SKIN WEIGHT AND MELATONIN CONCENTRATION USED TO DETERMINE PARTITION COEFFICIENT

Vehicles	Skin Weight (g)	Concentration of melatonin (μg/ml)
10%P.G. pH 3.3(A)*	1.347	9.047
(B)*	1.058	9.047
10%P.G. pH 6.1(A)	1.569	9.047
(B)	1.479	9.047
10%P.G. pH 8.7(A)	1.295	9.047
(B)	1.173	9.047
20%P.G. pH 3.3(A)	0.758	7.896
(B)	0.575	7.896
20%P.G. pH 6.1(A)	0.694	7.896
(B)	1.169	7.896
20%P.G. pH 8.7(A)	1.107	7.896
(B)	1.284	7.896
40%P.G. pH 3.3(A)	0.818	8.192
(B)	0.960	8.192
40%P.G. pH 6.1(A)	0.750	8.192
(B)	0.874	8.192
40%P.G. pH 8.7(A)	1.102	8.192
(B)	0.933	8.192
80%P.G. pH 3.3(A)	0.724	9.083
(B)	0.641	9.083
80%P.G. pH 6.1(A)	0.935	9.083
(B)	0.976	9.083
80%P.G. pH 8.7(A)	0.881	9.083
(B)	0.912	9.083

* A and B are duplicate samples for each vehicle.

TABLE II.3 STABILITY DATA FOR MELATONIN

Vehicle *	Degradation rate (10^{-4}/hr)	% remaining at 24 hr	% remaining at day 7	Time to 90% drug remaining(hr)
1	5.94	96.77	79.49	77.10
2	5.55	96.98	80.67	82.40
3	24.27	87.45	39.11	18.86
4	8.33	95.50	72.44	54.90
5	7.94	95.70	73.54	57.60
6	23.35	87.89	40.52	19.59
7	10.68	94.27	66.17	42.86
8	15.31	91.89	55.32	29.90
9	19.80	89.64	46.49	23.11
10	6.36	96.54	78.18	71.91
11	11.33	93.93	64.52	40.39
12	9.03	95.13	70.51	50.65

* Vehicle 1 is 10% PG/pH 3.3 buffer solution.
2 is 10% " pH 6.1 "
3 is 10% " pH 8.7 "
4 is 20% " pH 3.3 "
5 is 20% " pH 6.1 "
6 is 20% " pH 8.7 "
7 is 40% " pH 3.3 "
8 is 40% " pH 6.1 "
9 is 40% " pH 8.7 "
10 is 80% " pH 3.3 "
11 is 80% " pH 6.1 "
12 is 80% " pH 8.7 "

TABLE II.4 SOLUBILITY DATA FOR MELATONIN

Vehicles	Solubility (mg/ml)
10% P.G. pH 3.3*	2.441 ± 0.323
10% P.G. pH 6.1	2.173 ± 0.299
10% P.G. pH 8.7	2.078 ± 0.311
20% P.G. pH 3.3	4.046 ± 0.055
20% P.G. pH 6.1	3.084 ± 0.189
20% P.G. pH 8.7	2.716 ± 0.072
40% P.G. pH 3.3	11.458 ± 0.578
40% P.G. pH 6.1	10.610 ± 0.470
40% P.G. pH 8.7	10.084 ± 0.603
80% P.G. pH 3.3	>25
80% P.G. pH 6.1	>25
80% P.G. pH 8.7	>25

* 10% P.G. pH 3.3 is 10% propylene glycol/ pH 3.3 buffer solution.

TABLE II.5 AMOUNTS OF MELATONIN ABSORBED BY SKIN IN APPARENT PARTITION COEFFICIENT STUDY

Vehicle	Co[a] (μg/ml)	Ct[b] (μg/ml)	Co-Ct[c] (μg/ml)
10%P.G.pH 3.3(A)*	9.861	7.955	1.906
(B)*	7.898	6.713	1.185
10%P.G.pH 6.1(A)	9.100	8.239	0.861
(B)	8.773	7.645	1.128
10%P.G.pH 8.7(A)	9.580	8.705	0.875
(B)	10.194	9.607	0.587
20%P.G.pH 3.3(A)	7.933	7.052	0.881
(B)	7.923	7.122	0.801
20%P.G.pH 6.1(A)	8.599	7.870	0.729
(B)	7.684	7.080	0.604
20%P.G.pH 8.7(A)	8.035	7.076	0.959
(B)	8.314	7.417	0.897
40%P.G.pH 3.3(A)	8.462	8.071	0.301
(B)	8.301	8.047	0.254
40%P.G.pH 6.1(A)	7.752	7.237	0.515
(B)	8.793	8.072	0.721
40%P.G.pH 8.7(A)	8.417	7.754	0.663
(B)	8.189	7.593	0.596
80%P.G.pH 3.3(A)	8.657	8.541	0.116
(B)	9.114	8.743	0.371
80%P.G.pH 6.1(A)	8.538	8.369	0.169
(B)	9.168	8.788	0.380
80%P.G.pH 8.7(A)	9.303	8.928	0.375
(B)	9.227	8.924	0.303

* A and B are duplicate samples for each vehicle.
a: Co is the initial concentration of melatonin in the solution.
b: Ct is the concentration of melatonin in the solution at 24 hours.
c: Co - Ct represents the amount of melatonin absorbed by skin.

TABLE II.6 APPARENT PARTITION COEFFICIENT DATA FOR MELATONIN

Vehicle	Co-Ct[a] (μg/ml)	Apparent P.C.[b] (10^{-1}ml/g)	Mean of apparent P.C. (10^{-1}ml/g)
10%P.G.pH 3.3(A)[c]	1.906	0.1779	
(B)[c]	1.185	0.1668	0.1723 ± 0.0055
10%P.G.pH 6.1(A)	0.861	0.0666	
(B)	1.128	0.0997	0.0832 ± 0.0166
10%P.G.pH 8.7(A)	0.875	0.0776	
(B)	0.587	0.0521	0.0648 ± 0.0128
20%P.G.pH 3.3(A)	0.881	0.1647	
(B)	0.801	0.1957	0.1802 ± 0.0155
20%P.G.pH 6.1(A)	0.729	0.1335	
(B)	0.604	0.0730	0.1032 ± 0.0303
20%P.G.pH 8.7(A)	0.959	0.1224	
(B)	0.897	0.0942	0.1084 ± 0.0141
40%P.G.pH 3.3(A)	0.391	0.0593	
(B)	0.254	0.0328	0.0460 ± 0.0132
40%P.G.pH 6.1(A)	0.515	0.0948	
(B)	0.721	0.1023	0.0985 ± 0.0037
40%P.G.pH 8.7(A)	0.663	0.0776	
(B)	0.596	0.0841	0.0808 ± 0.0033
80%P.G.pH 3.3(A)	0.116	0.0187	
(B)	0.371	0.0663	0.0425 ± 0.0238
80%P.G.pH 6.1(A)	0.169	0.0215	
(B)	0.380	0.0443	0.0329 ± 0.0114
80%P.G.pH 8.7(A)	0.375	0.0477	
(B)	0.303	0.0373	0.0425 ± 0.0052

a: Co is the initial concentration of melatonin in the solution and Ct is the concentration of melatonin in the solution at 24 hours. Co-Ct represents the amount of melatonin absorbed by skin.

b: Apparent partition coefficient was calculated using the equation: $a.P.C. = \frac{(Co-Ct)/\text{Weight of skin}}{Ct/\text{volume of solution}}$

For example, the a.P.C. for melatonin from 10% P.G. pH 3.3 solution A into skin was

$a.P.C. = \frac{(9.861 - 7.955)/\ 1.347}{7.955} = 0.1779$

The volume of each solution was constant (10 ml) in this study.

c: A and B are duplicate samples for each vehicle.

TABLE II.7 THE PRODUCT OF PARTITION COEFFICIENT AND SOLUBILITY

Vehicle	Solubility (mg/ml)	a.P.C.*	The product of solubility and a.P.C.
10%P.G.pH 3.3	2.441	0.1723	0.421
10%P.G.pH 6.1	2.173	0.0832	0.181
10%P.G.pH 8.7	2.078	0.0648	0.135
20%P.G.pH 3.3	4.046	0.1802	0.729
20%P.G.pH 6.1	3.084	0.1032	0.318
20%P.G.pH 8.7	2.716	0.1083	0.294
40%P.G.pH 3.3	11.458	0.0460	0.528
40%P.G.pH 6.1	10.610	0.0985	1.046
40%P.G.pH 8.7	10.084	0.0808	0.815
80%P.G.pH 3.3	>25	0.0425	--
80%P.G.pH 6.1	>25	0.0329	--
80%P.G.pH 8.7	>25	0.0425	--

__* a.P.C. is apparent partition coefficient.
--: These data were not calculated because the solubilities for 80% propylene glycol/buffer solutions are larger than 25 mg/ml and have not been actually determined.

Fig. II.1 A Franz diffusion cell

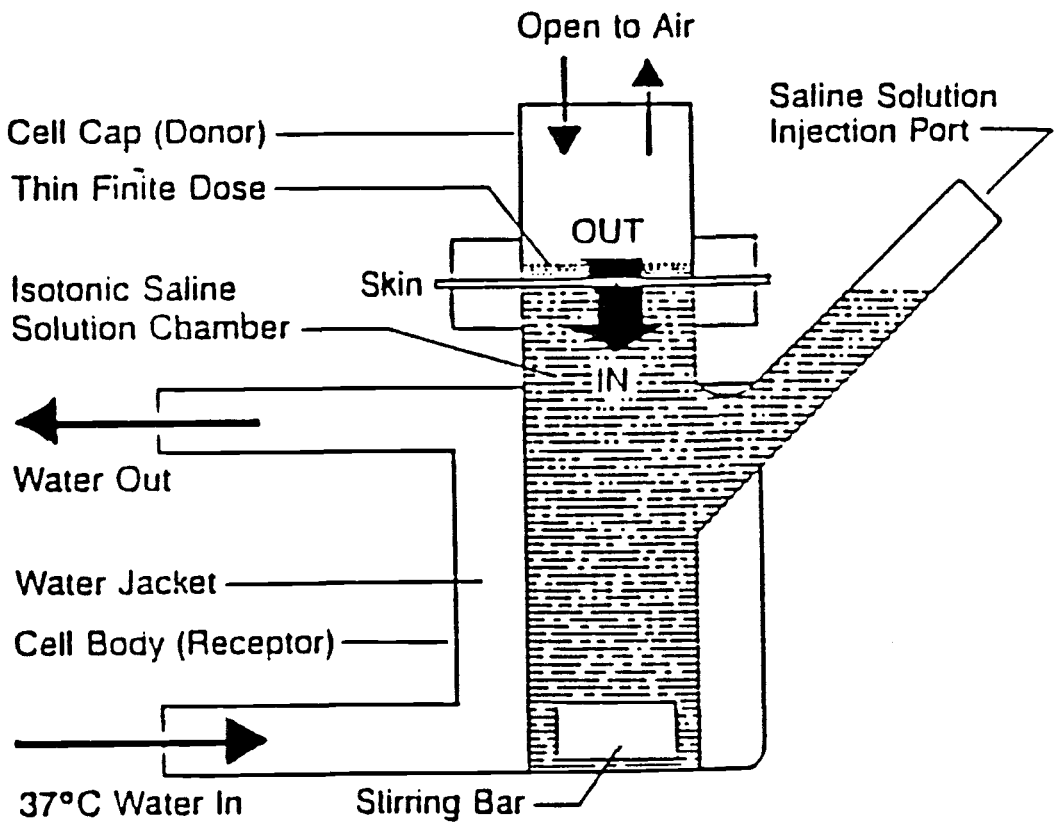


Fig. II.2 Propylene glycol (P.G.) effect on melaton stability at pH 3.3.

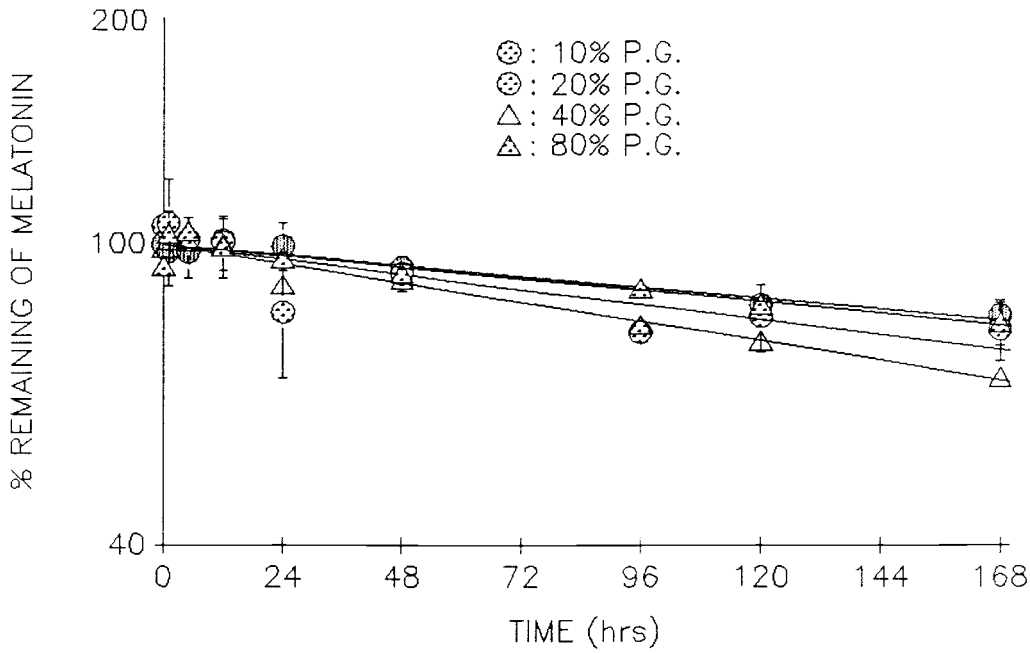


Fig. II.3 Propylene glycol (P.G.) effect on melatonin stability at pH 6.1.

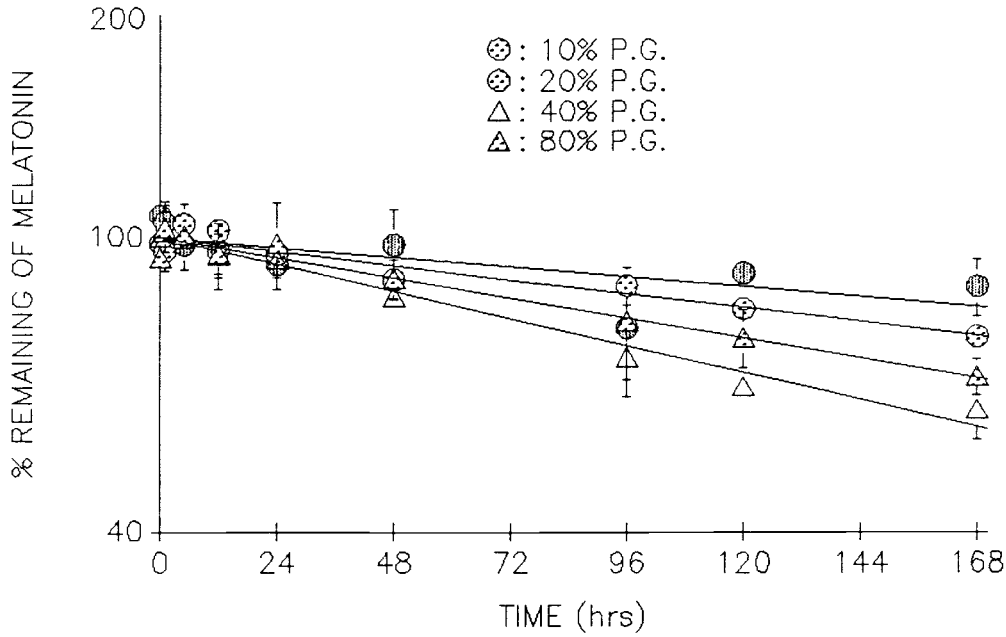


Fig. II.4 Propylene glycol (P.G.) effect on melatonin stability at pH 8.7.

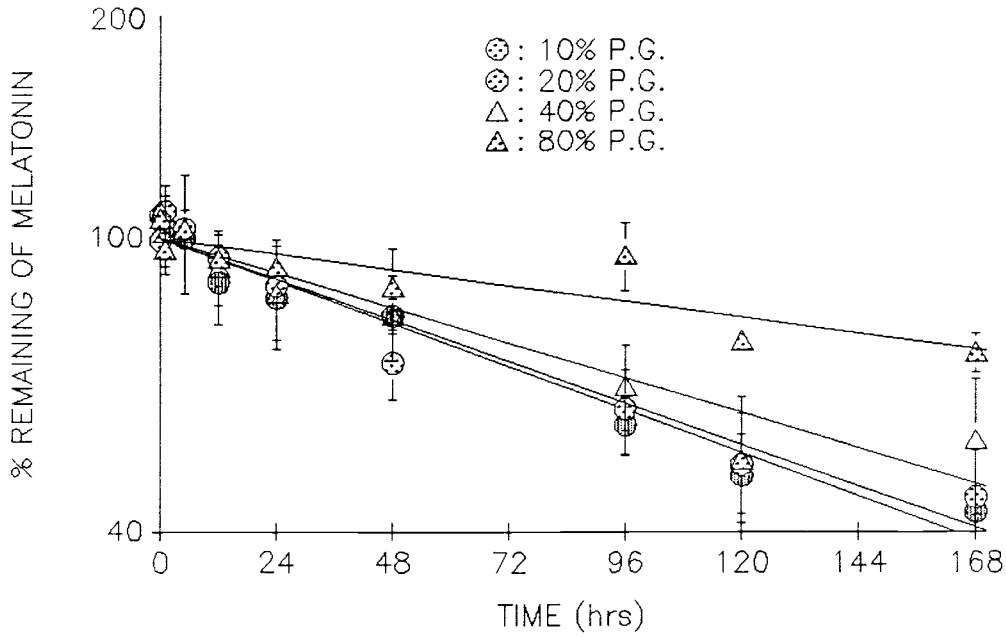


Fig. II.5 Propylene glycol (PG) effect on melatonin solubility at pH 3.3.

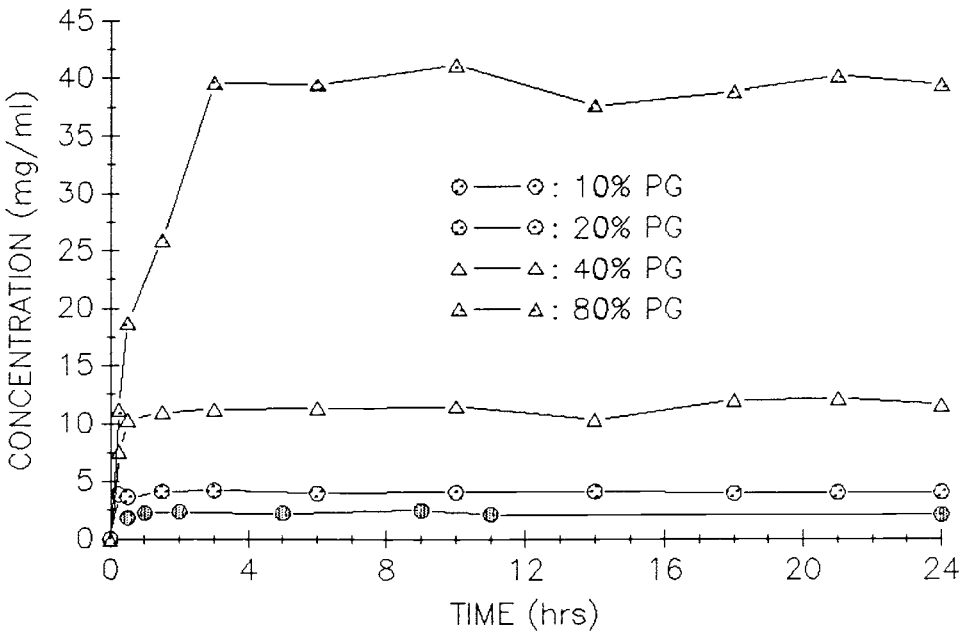


Fig. II.6 Propylene glycol (PG) effect on melatonin solubility at pH 6.1.

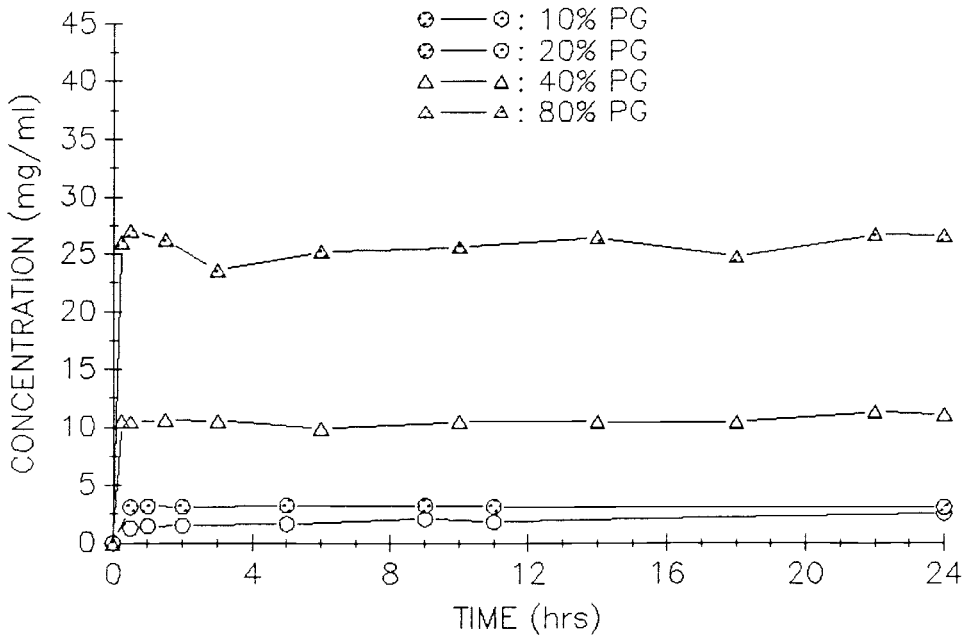


Fig. II.7 Propylene glycol (PG) effect on melatonin solubility at pH 8.7.

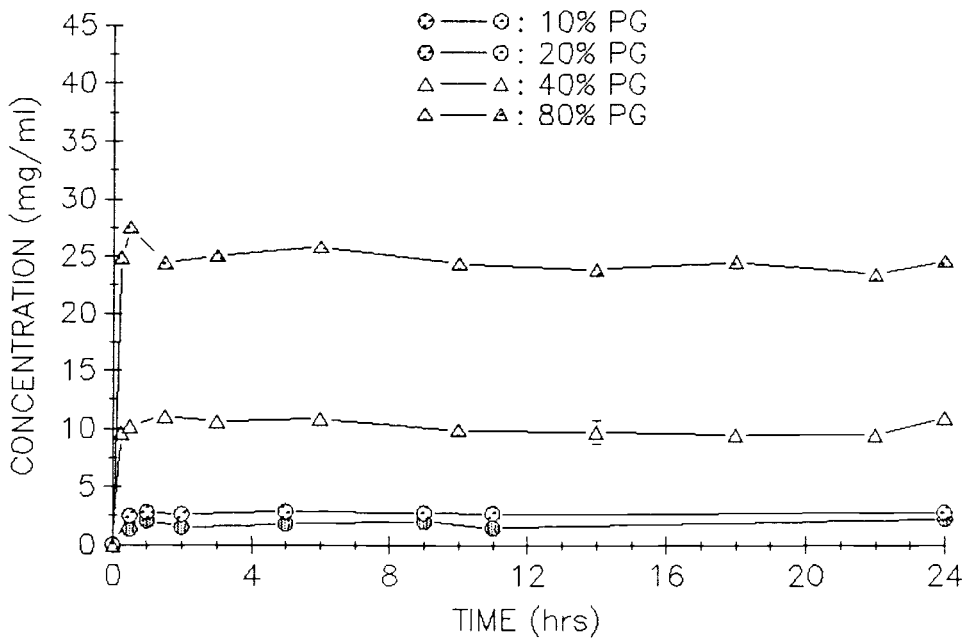


Fig. II.8 Data points of melatonin concentration in 10% propylene glycol/pH 3.3 buffer solution vs. time plots in apparent partition coefficient study. (A and B are duplicate samples)

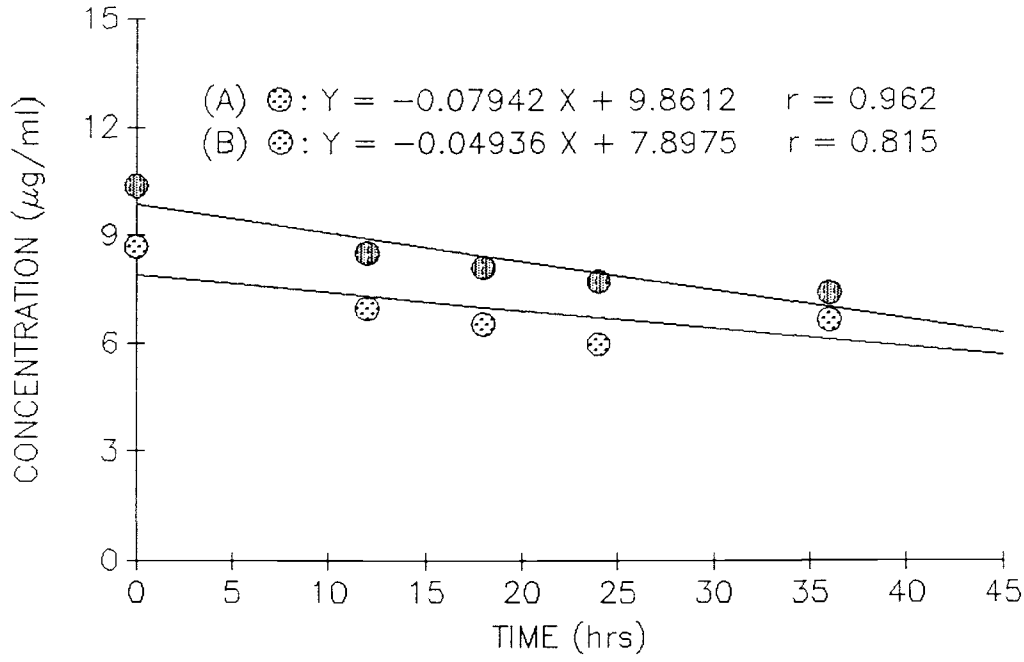


Fig. II.9 Data points of melatonin concentration in 10% propylene glycol/pH 6.1 buffer solution vs. time plots in apparent partition coefficient study. (A and B are duplicate samples)

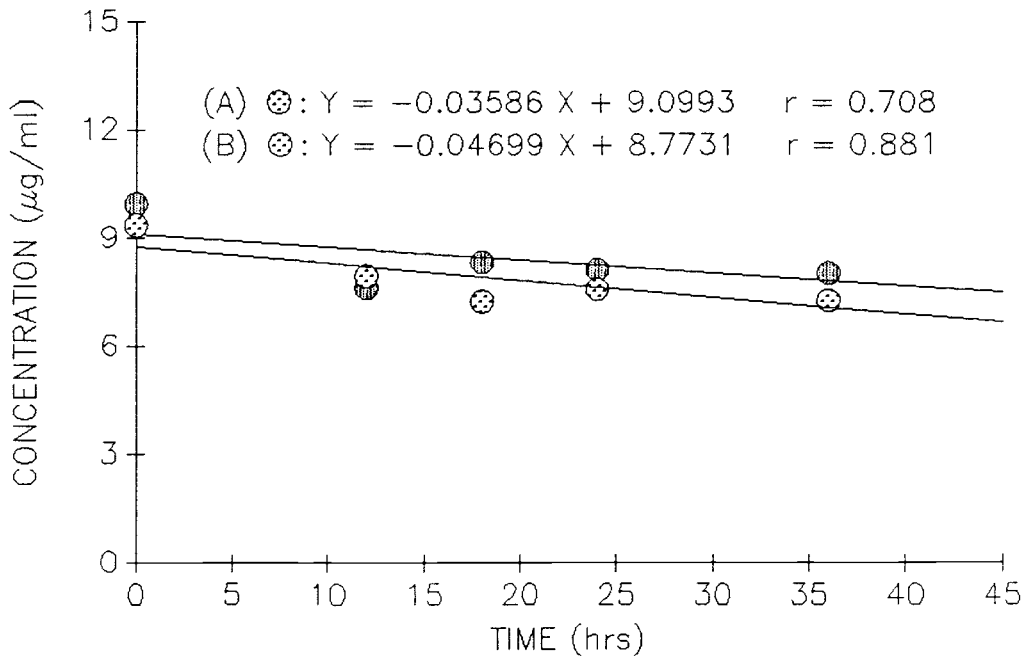


Fig. II.10 Data points of melatonin concentration in 10% propylene glycol/pH 8.7 buffer solution vs. time plots in apparent partition coefficient study. (A and B are duplicate samples)

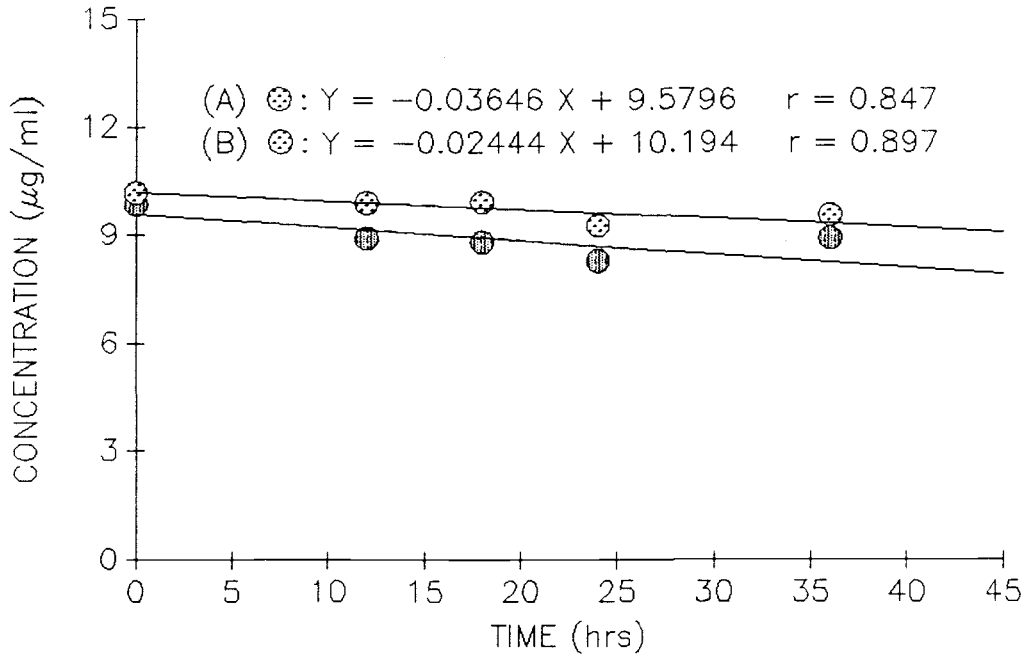


Fig. II.11 Data points of melatonin concentration in 20% propylene glycol/pH 3.3 buffer solution vs. time plots in apparent partition coefficient study. (A and B are duplicate samples)

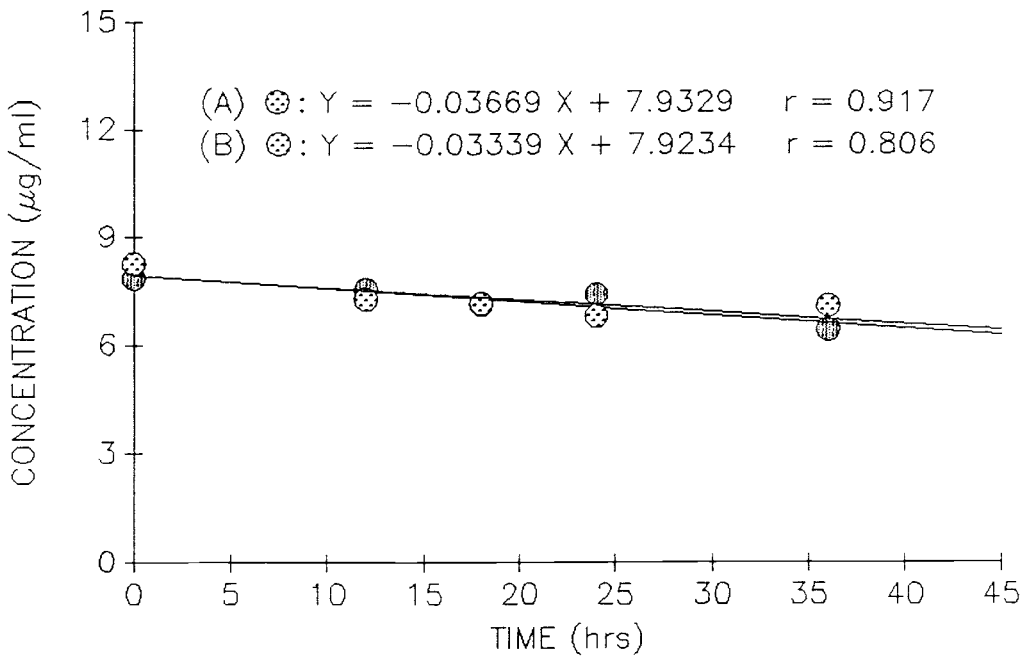


Fig. II.12 Data points of melatonin concentration in 20% propylene glycol/pH 6.1 buffer solution vs. time plots in apparent partition coefficient study. (A and B are duplicate samples)

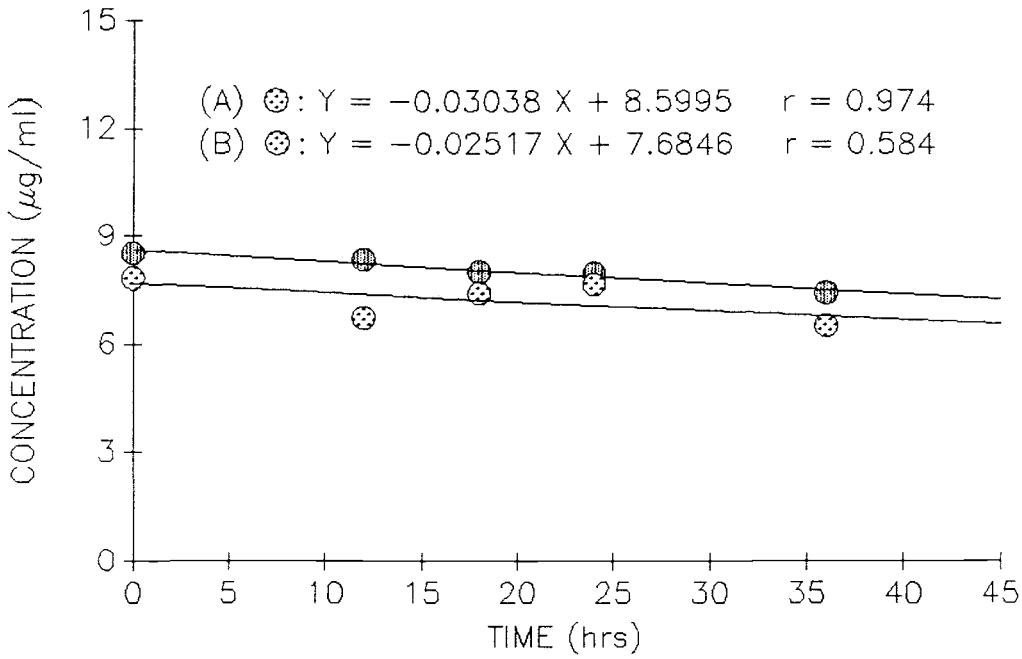


Fig. II.13 Data points of melatonin concentration in 20% propylene glycol/pH 8.7 buffer solution vs. time plots in apparent partition coefficient study. (A and B are duplicate samples)

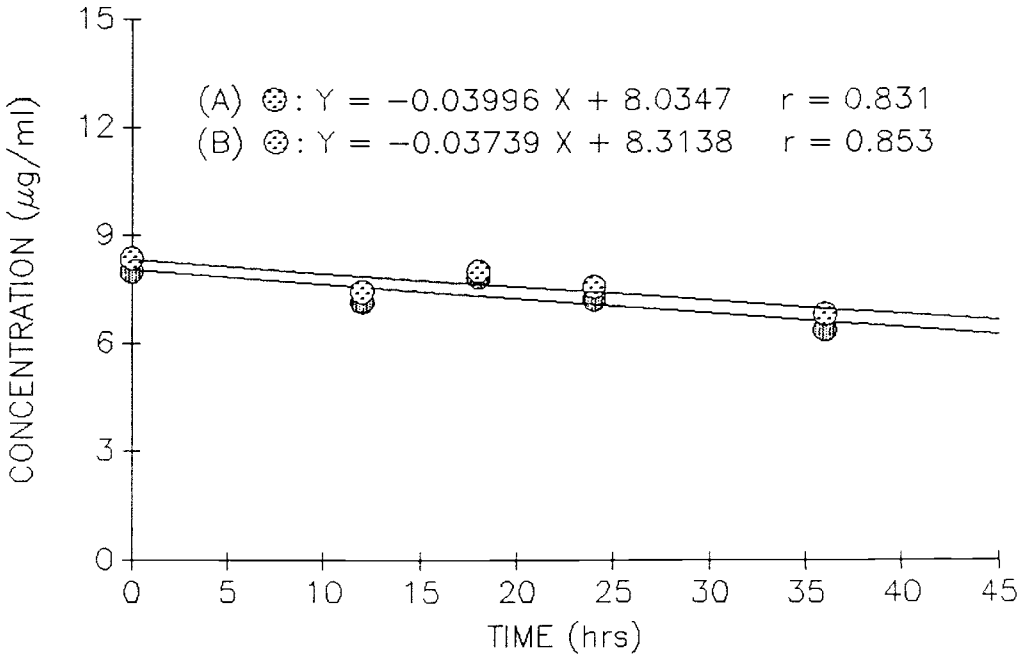


Fig. II.14 Data points of melatonin concentration in 40% propylene glycol/pH 3.3 buffer solution vs. time plots in apparent partition coefficient study. (A and B are duplicate samples)

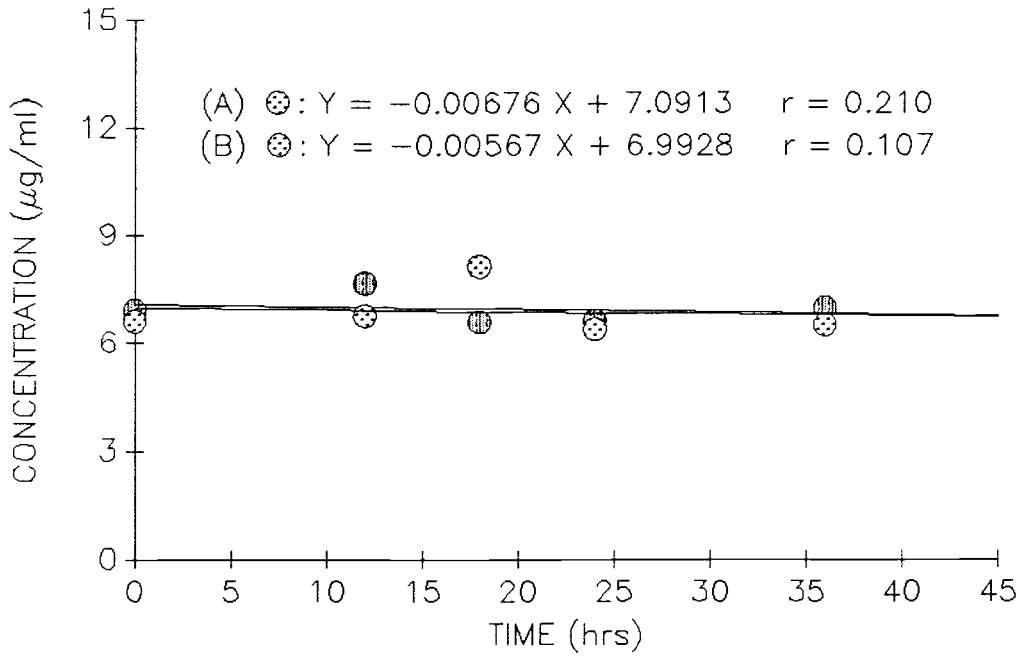


Fig. II.15 Data points of melatonin concentration in 40% propylene glycol/pH 6.1 buffer solution vs. time plots in apparent partition coefficient study. (A and B are duplicate samples)

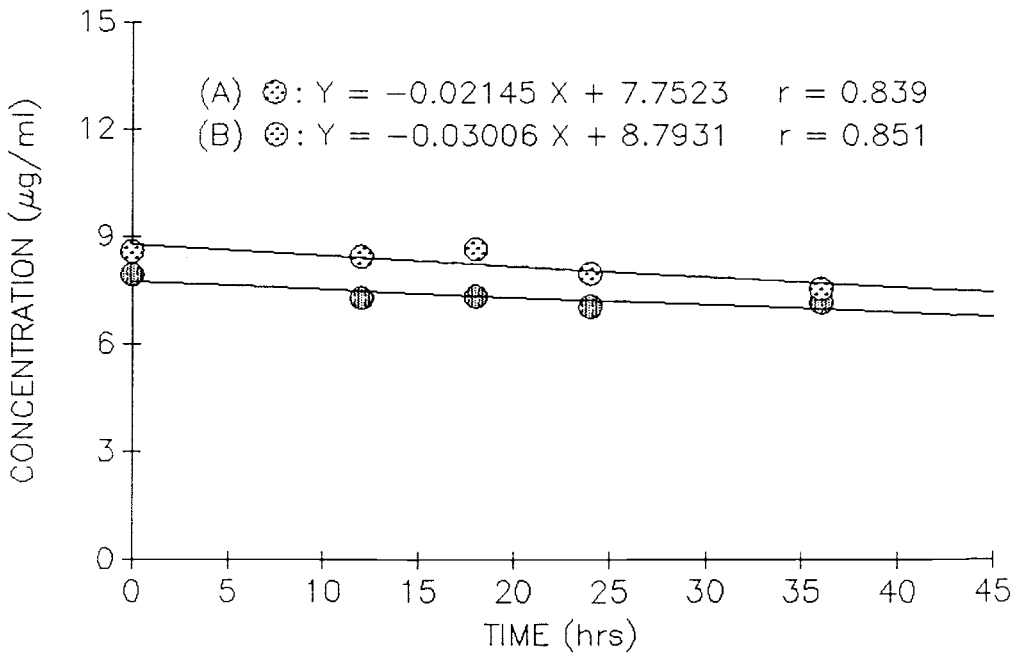


Fig II.16 Data points of melatonin concentration in 40% propylene glycol/pH 8.7 buffer solution vs. time plots in apparent partition coefficient study. (A and B are duplicate samples)

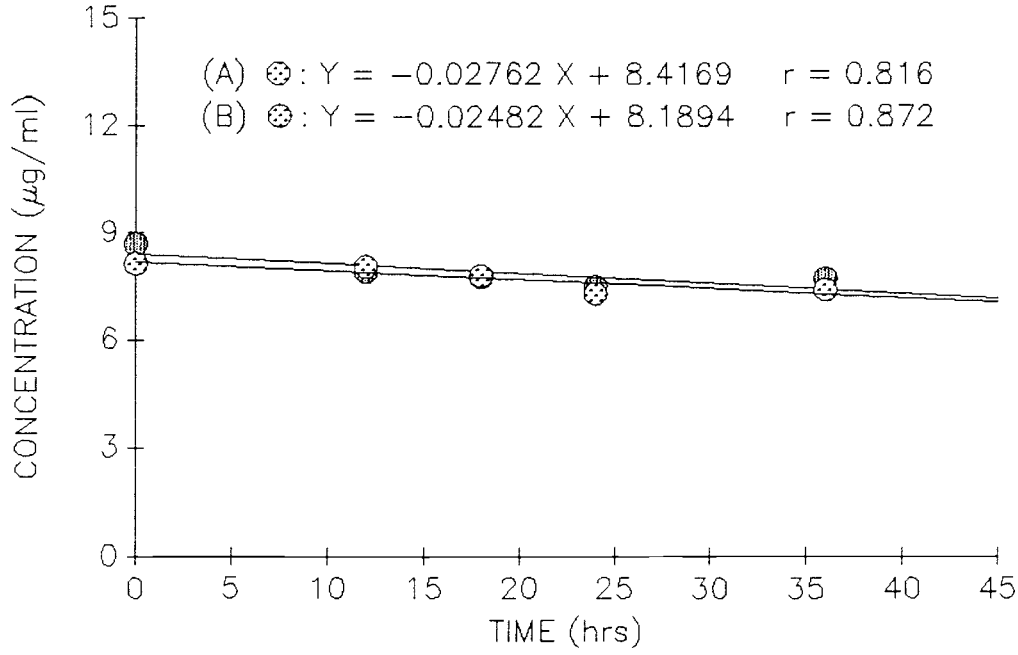


Fig. II.17 Data points of melatonin concentration in 80% propylene glycol/pH 3.3 buffer solution vs. time plots in apparent partition coefficient study. (A and B are duplicate samples)

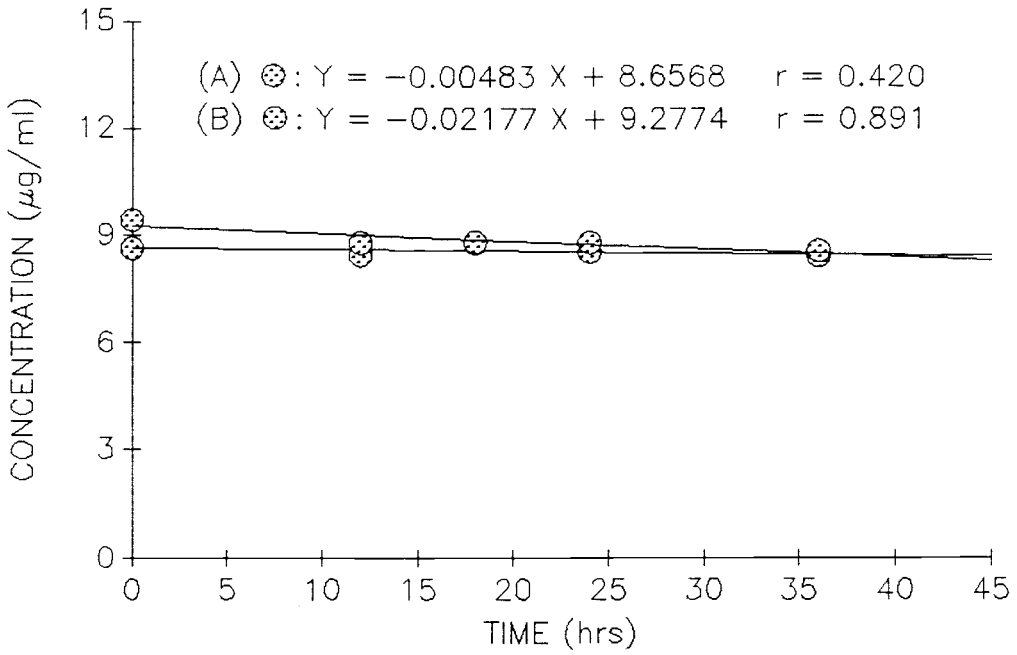


Fig. II.18 Data points of melatonin concentration in 80% propylene glycol/pH 6.1 buffer solution vs. time plots in apparent partition coefficient study. (A and B are duplicate samples)

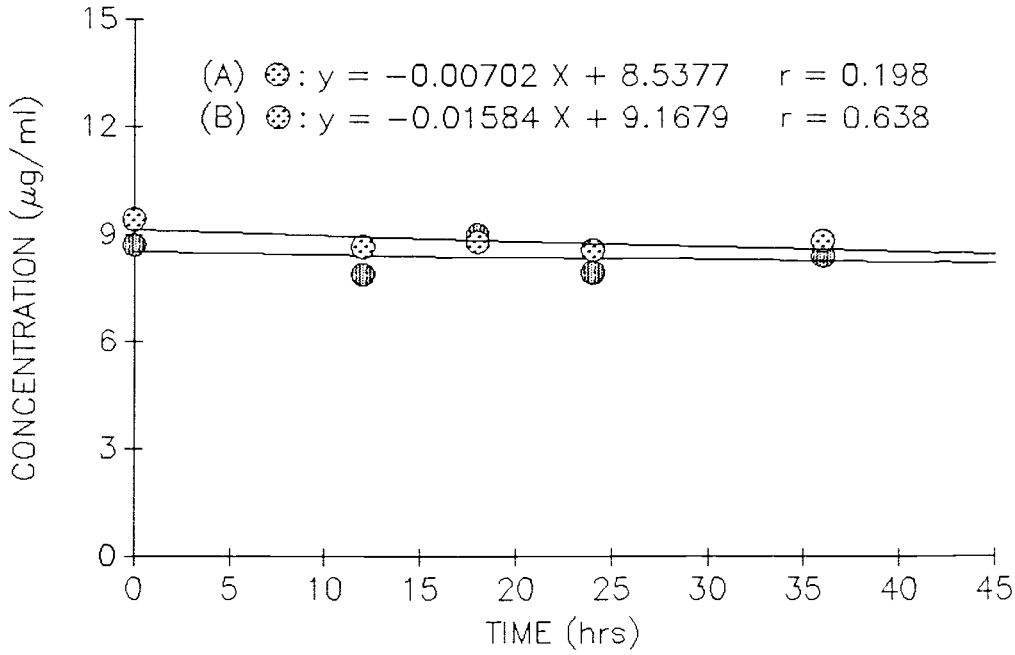


Fig. II.19 Data points of melatonin concentration in 80% propylene glycol/pH 8.7 buffer solution vs. time plots in apparent partition coefficient study. (A and B are duplicate samples)

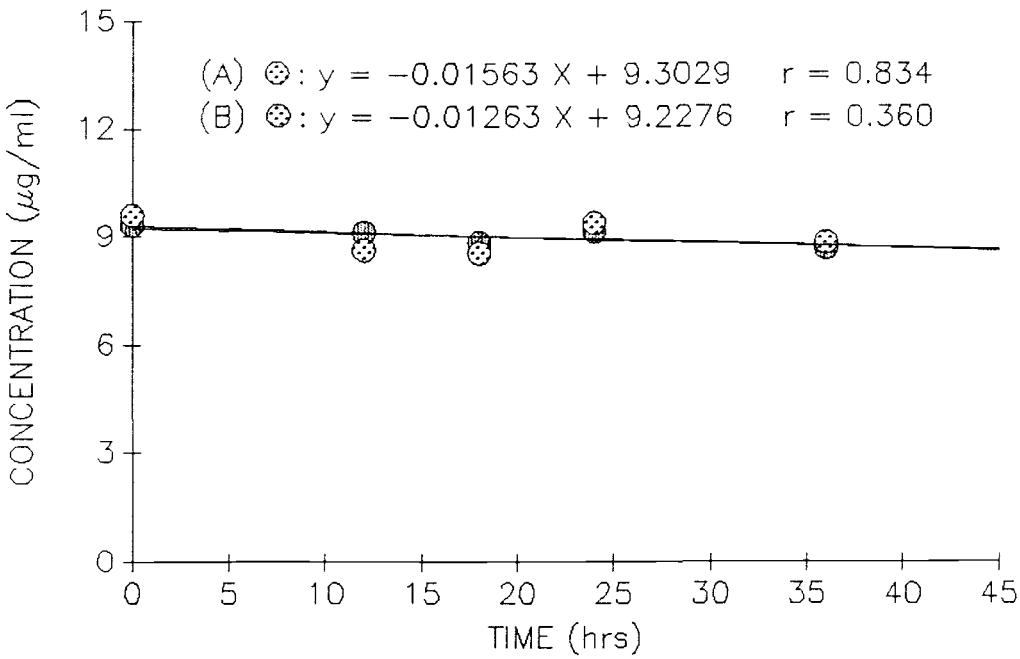


Fig. II.20 The relationship of apparent partition coefficient (a.P.C.) and solubility at pH 3.3.

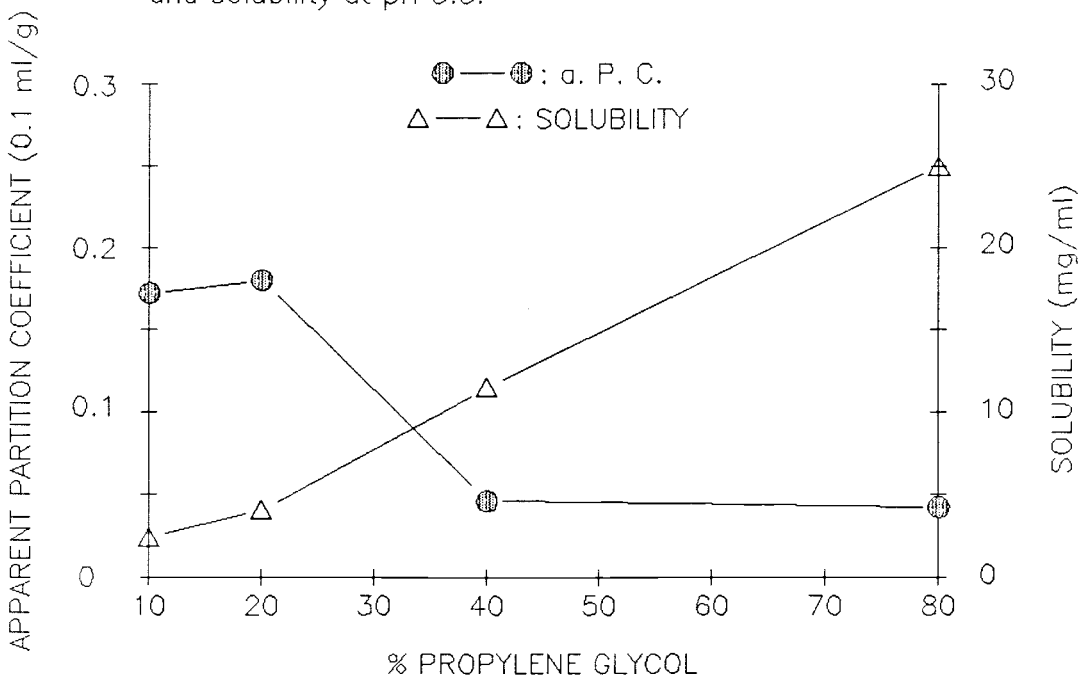


Fig. II.21 The relationship of apparent partition coefficient (a.P.C.) and solubility at pH 6.1.

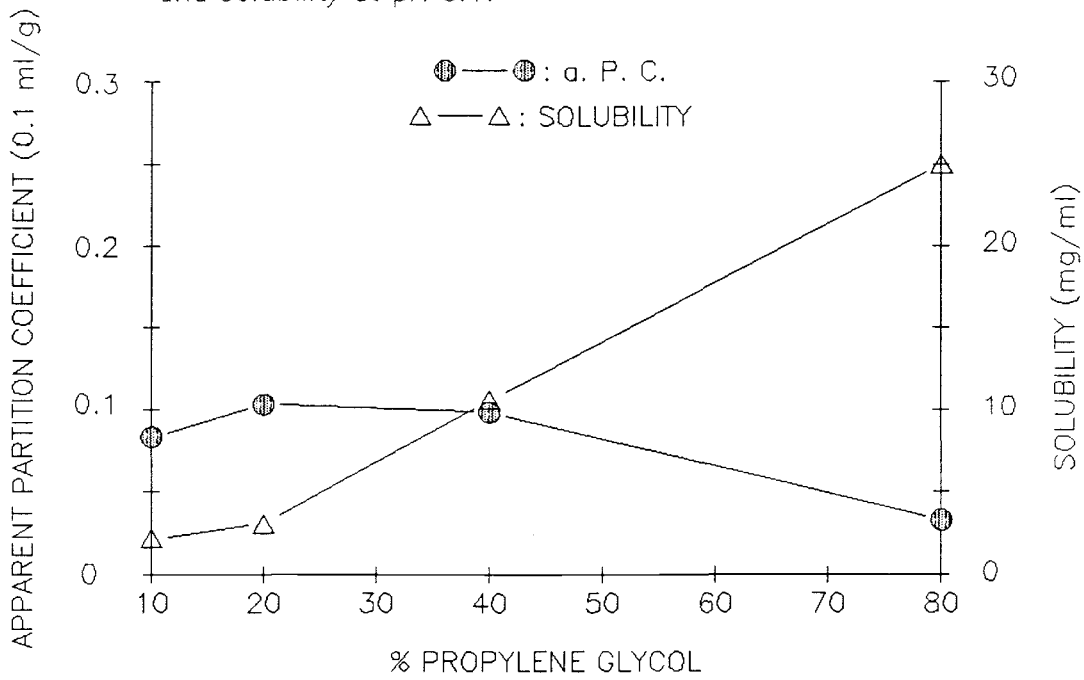
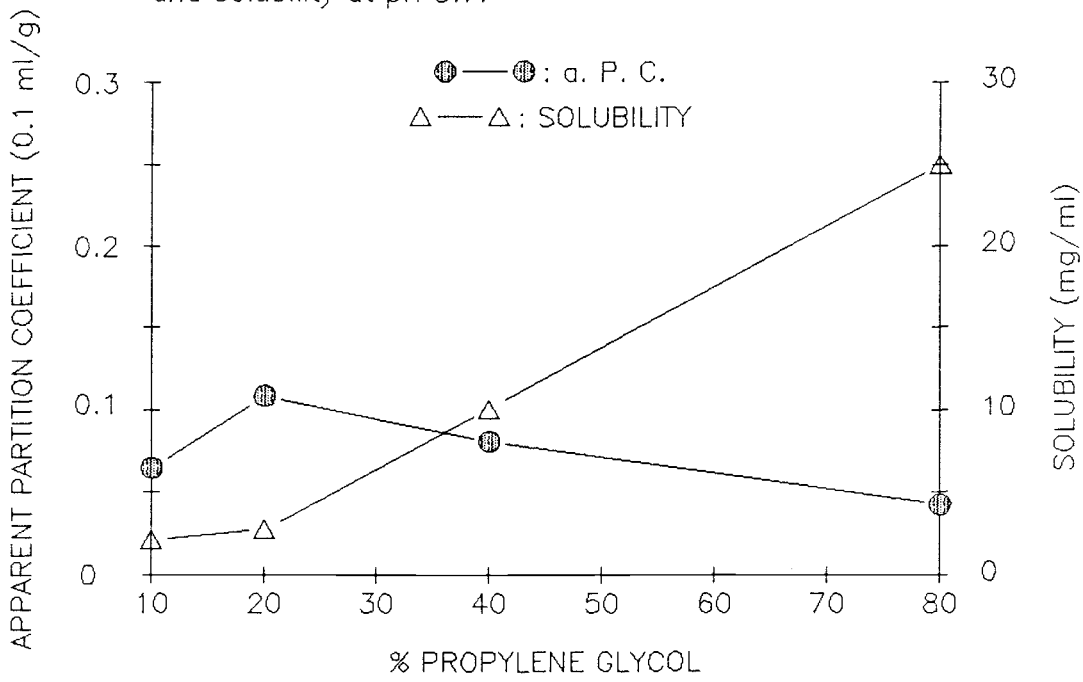


Fig. II.22 The relationship of apparent partition coefficient (a.P.C.) and solubility at pH 8.7.



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Chapter III Diffusion Studies of Melatonin Through Hairless Mouse Skin

INTRODUCTION

In the formulation of vehicles for topical delivery of drugs, the efficacy of such dosage forms is often dependent on the composition of the vehicle (1). Based on results from Chapter II, 40% Propylene-glycol/pH 6.1 buffer solution would be the best vehicle of those investigated to maximize drug flux. Two other vehicles (20% propylene glycol/ pH 6.1 buffer solution and 65% propylene glycol/ pH 6.1 buffer solution) were also chosen for study because the 20% propylene glycol/pH 6.1 buffer solution had a larger apparent partition coefficient and a lower solubility, while the 65% propylene glycol/pH 6.1 buffer solution had a smaller apparent partition coefficient and higher solubility relative to 40% propylene glycol/ pH 6.1 buffer solution. Diffusion of melatonin from each of these vehicles through hairless mouse skin was studied. Three carbopol gels prepared with propylene glycol-buffer mixtures in varying proportions, which contained melatonin, were also evaluated.

MATERIALS AND METHODS

DIFFUSION APPARATUS

The apparatus used in diffusion experiments was a Franz skin permeation diffusion apparatus (Crown Glass Co.), see Figure II.1. Skin (3.1416 cm^2) was mounted between the cell cap (donor) and the cell body (receptor). The dermis was bathed from below by an isotonic saline solution injected through a port provided for that purpose. Temperature was maintained at 30°C by thermostatically controlled water which entered the lower port of the water jacket surrounding the saline solution chamber, and circulated out through the upper port. Water was supplied and removed by two (upper and lower) stainless steel manifolds connected to a constant temperature bath. Homogenous distribution of temperature in the saline bathing solution was accomplished by the agitating motion of a teflon-covered magnetic stirring bar, driven by an external magnet mounted on a timing motor. The cell cap was covered by parafilm to prevent any evaporation from the donor phase.

HAIRLESS MOUSE SKIN

The abdominal skin of male hairless mice (10 weeks old, HRS/J strain) was used for this study. Hairless mouse skin has been shown to be an acceptable model for

the diffusion of drugs through human skin (2). The mice were housed at the College of Pharmacy. The mice were sacrificed by exposure to a CO_2 atmosphere about one hour before the diffusion studies started. The abdominal skin was collected as previously reported (3), and soaked in saline solution before use.

RECEPTOR SOLUTION

Normal saline solution (4) was used as receptor phase in all studies. This solution was prepared fresh for each experiment.

EXPERIMENTAL

DIFFUSION STUDY A: DIFFUSION FROM VEHICLE

After dissolving an appropriate amount to produce a saturated solution (that is solubility in mg/ml times 5 ml for each vehicle) of melatonin in 5 ml of the vehicle (Table III.1), 1.5 ml of the solution was put in the donor portion of the diffusion cell. Fifteen ml of the saline solution was put in the receptor chamber. Skin was then placed between the donor formulation and the receptor phase. Samples of receptor phase (200 ul each time) were removed via sampling port at the following times: 0, 2, 4, 6, 9, 12, 20, and 24 hr. Samples were replaced with an equal volume of saline solution. Melatonin content was determined by HPLC analysis. This study was performed in triplicate for each vehicle.

PREPARATION OF 4.76% (CARBOPOL/VEHICLE; W/W) GELS

An appropriate amount to produce a saturated solution (solubility in mg/ml times 30 ml) of melatonin was dissolved in 30 ml of each of the different vehicles (Table III.2). Then 1.5 g of a long chain polymer (Carbopol 934P) was added into the vehicle slowly, and was stirred until a gel formed.

DIFFUSION STUDY B: DIFFUSION FROM 4.7% (W/W) CARBOPOL GEL

A known amount of gel was placed in the donor part of the diffusion cell (Table III.3). Samples of the receptor phase (200ul) were collected at the following times:0, 2, 4, 6, 12, 20, and 24 hours. Samples were replaced with an equal volume of the receptor phase. This diffusion study was also performed in triplicate for each gel.

RESULTS AND DISCUSSION

DIFFUSION STUDY A

Results were plotted as the cumulative amount of drug penetrated through skin (Y axis) versus time (X axis) (see Figure III.1-9). From this data the amount of melatonin delivered/unit skin surface area /hour (Flux) was calculated (Table III.4). In addition, the lag time (time between application of solution to the skin and its

appearance in the receptor phase) was calculated using linear regression analysis through the last few points where diffusion had achieved a pseudo-steady state (Table III.5) (5,6). Percent release was calculated by the amount of melatonin penetrated divided by the amount of melatonin originally in the vehicle (Table III.5).

Sixty-five percent propylene glycol/buffer solution had a longer lag time, a lower percent released, and a lower flux than 20% and 40% propylene glycol/buffer solution even though it contained a larger percentage of drug. This is consistent with the tendency of this solvent to "hold" the drug due to its greater solubility (see discussion on page 32).

Because of considerable variation in the study with the 20% propylene glycol/buffer solution it is difficult to make comparisons with the 40% propylene glycol preparation. Although the standard deviation of the diffusion data in the duplicate studies for the 20% preparation was large, the release pattern was similar in shape although not amount. Based on the results from Chapter II, greater flux from the 40% preparation compared to the 20% preparation would be anticipated.

While this diffusion study was performed in triplicate for each preparation, one data set from each preparation was not included in the results due to large

bubbles which developed between the skin and receptor phase. Once the bubble developed, contact of the receptor phase with skin was not good and the results were not considered reliable.

DIFFUSION STUDY B

Results for the gel formulations were also plotted as the cumulative amount of drug penetrated through skin (Y axis) versus time (X axis) (Figure III.10-20). Flux was calculated from the plots using the technique of linear regression analysis (Table III.6). Melatonin release from gels began almost immediately (no lag time exists). Percent release was calculated by the amount of melatonin penetrated divided by the amount of melatonin originally in the dosage form. (Table III.7). The 20% propylene glycol/pH 6.1 gel had the largest flux of the three gels even though, as in Study A, it was expected that the 40% propylene glycol gel would have the greatest flux. This may have occurred because of the different viscosity for each gel. The viscosity increased as the percentage of propylene glycol increased. Additional studies are needed to clarify the relationship between flux and gel viscosity. In this study two of the skins which were used for drug released from gels (containing 65% propylene glycol/buffer solutions) had been injured

by mice fighting each other and diffusion through these skins was omitted as values were too large to use.

CONCLUSIONS

As shown in Table III.8, larger amounts of melatonin are released in 24 hours from solutions than from gels. This means melatonin released from solutions would have a larger flux than from gels. Thus, solutions might be the dosage forms which can maximize flux. But, solutions need another device, such as a patch, to contain the solution when applied to human skin. Gels could also be dosage forms for melatonin delivery through the skin, but a larger surface area would be necessary for gels than solutions to achieve the same administration rate. Melatonin would be released immediately from gels and have a shorter lag time which may be an advantage for gels over solutions.

Intravenous infusions of melatonin at 3 μg/hr and 6 μg/hr have produced a mean plasma concentration of 61 pg/ml of melatonin in human volunteers having a mean plasma concentration of approximately 40 pg/ml without infusion (7). Thus, based on the results of Chapter III, either a one square centimeter "solution patch" with the twenty or forty percent preparation or a twenty square

centimeter "gel patch" of either the 20 or 40% preparation could produce plasma concentrations similar to those produced by intravenous infusion of melatonin. Further studies in human volunteers are necessary to determine the dose of topical melatonin necessary to achieve a given plasma concentration, and to determine if the topical dosage form can mimic the physiologic release pattern of melatonin. Percutaneous delivery of melatonin appears to be feasible and the existence of a transdermal delivery system should facilitate clinical studies in human subjects.

TABLE III.1 AMOUNTS OF MELATONIN USED FOR EACH 5 ML VEHICLE IN DIFFUSION STUDY A (DIFFUSION FROM VEHICLE)

Vehicle	Amount of melatonin dissolved in 5 ml of vehicle
20% P.G. pH 6.1	15.57 mg
40% P.G. pH 6.1	53.42 mg
65% P.G. pH 6.1	98.34 mg

TABLE III.2 AMOUNTS OF MELATONIN USED FOR EACH GEL IN DIFFUSION STUDY B (DIFFUSION FROM GEL)

Gel	Amount of melatonin dissolved in 30 ml of vehicle
20% P.G. pH 6.1*	74.42 mg
40% P.G. pH 6.1	318.56 mg
65% P.G. pH 6.1	591.69 mg

* This vehicle was made into gel after melatonin was dissolved in the vehicle.

TABLE III.3 WEIGHTS OF GELS APPLIED TO BE A DONOR PHASE

Vehicle		Weight of Gel (g)
20% P.G. pH 6.1	(A)*	5.254
	(B)*	5.536
	(C)*	5.006
40% P.G. pH 6.1	(A)	4.965
	(B)	5.019
	(C)	5.252
65% P.G. pH 6.1	(A)	5.015
	(B)	4.800
	(C)	4.268

* A, B and C are triplicate samples for each vehicle.

TABLE III.4 DIFFUSION STUDY DATA FROM VEHICLE FOR MELATONIN

Vehicle*	Cumulated amount of drug released (μg) in receptor solution		Flux [g] (μg/cm/hr)	
20%P.G.pH 6.1(A)*	739.39		9.817	
(B)*	161.93	450.66$\pm$288[a]	2.334	6.075$\pm$3.74[b]
40%P.G.pH 6.1(A)	243.88		3.532	
(B)	294.93	269.40$\pm$25[c]	4.892	4.212$\pm$0.68[d]
65%P.G.pH 6.1(A)	7.89		0.154	
(B)	45.00	34.94$\pm$27[e]	0.965	0.560$\pm$0.41[f]

* A and B are duplicate samples for each vehicle.
a, c, and e: Mean and standard deviation of cumulated amount of melatonin released from 20%, 40%, and 65% propylene glycol/buffer solution.
b, d, and f: Mean and standard deviation of flux for 20%, 40%, and 65% propylene glycol/buffer solution
g: Flux is penetration rate which is slope of linear regression through the data points representing pseudo-steady state.

TABLE III.5 THE LAG TIME AND % RELEASE FROM THE VEHICLE

Vehicle	Lag time (hr)		% release from vehicle	
20%P.G.pH 6.1(A)*	0		15.80	
(B)*	0.11	0.06±0.06[a]	3.47	9.63±6.17[d]
40%P.G.pH 6.1(A)	2.81		1.52	
(B)	5.60	4.21±1.40[b]	1.84	1.68±0.16[e]
65%P.G.pH 6.1(A)	6.90		0.03	
(B)	7.95	7.42±0.53[c]	0.15	0.09±0.06[f]

a, b, and c are the mean and standard deviation of lag time for 20%, 40%, and 65% propylene glycol/buffer solution respectively.
d, e, and f are the mean and standard deviation of % released from 20%, 40%, and 65% propylene glycol/buffer solution.
*: A and B are duplicate samples for each vehicle.

TABLE III.6 CUMULATED AMOUNT OF MELATONIN AND FLUX FOR DIFFUSION STUDY B (DIFFUSION FROM GEL)

Gel	Cumulated amount of melatonin released (μg)		Flux (μg/cm/hr)	
A.1[a]	12.82		0.071	
A.2[a]	33.35		0.380	
A.3[a]	19.45	21.87$\pm$8.56[d]	0.246	0.232$\pm$0.127[f]
B.1[b]	8.39		0.053	
B.2[b]	7.73		0.070	
B.3[b]	36.97	17.70$\pm$13.63[e]	0.487	0.203$\pm$0.201[g]
C.1[c]	11.71		0.065	
C.2[c]	164.59*		1.823*	
C.3[c]	124.45*		1.708*	

a: A1, A2, and A3 are the triplicate samples for the gel which contained 20% propylene glycol/buffer solution
b: B1, B2, and B3 are the triplicate samples for the gel which contained 40% propylene glycol/buffer solution.
c: C1, C2, and C3 are the triplicate samples for the gel which contained 65% propylene glycol/buffer solution.
d and e: Mean and standard deviation of cumulated amount of melatonin penetrated from gel which contained 20% and 40% propylene glycol/buffer solution respectively.
f and g: Mean and standard deviation of flux for gel which contained 20% and 40% propylene glycol/buffer solution respectively.
*: The skins which had been used for these studies were injured due to the mice fighting each other. These data were excluded.

TABLE III.7 PER CENT RELEASE OF MELATONIN FROM GEL

Gel		% Released	
20% P.G. pH 6.1	(A)	0.103	
	(B)	0.255	
	(C)	0.164	0.174 ± 0.086[a]
40% P.G. pH 6.1	(A)	0.017	
	(B)	0.015	
	(C)	0.070	0.034 ± 0.026[b]
65% P.G. pH 6.1	(A)	0.012	
	(B)	1.258*	
	(C)	1.052*	

* The % release of melatonin is large due to the skin which has some injury caused by mouse fighting each other.
a: Mean and standard deviation of % release of melatonin from gel which contained 20% propylene glycol/buffer solution.
b: Mean and standard deviation of % release of melatonin from gel which contained 40% propylene glycol/buffer solution.

TABLE III.8 MELATONIN RELEASE CHARACTERISTICS FROM GEL AND SOLUTION PREPARATIONS

	Mean Values (N)			
Prep	Total Released (ug/24 Hrs)	% Released	Flux*	Lag Time, Hrs
20% PG Sol	450.7(2)	9.63(2)	6.08(2)	.1(2)
20% PG Gel	21.9(3)	0.17(3)	0.23(3)	0(3)
40% PG Sol	269.4(2)	1.68(2)	4.21(2)	4(2)
40% PG Gel	17.7(3)	0.03(3)	0.20(3)	0(3)
65% PG Sol	35.0(2)	0.09(2)	0.56(2)	7(2)
65% PG Gel	11.7(1)	0.01(1)	0.07(1)	0(1)

* ug/cm/hr

Fig. III.1 Mean diffusion profile of melatonin through hairless mouse skin from 20% propylene glycol/pH 6.1 buffer solution.

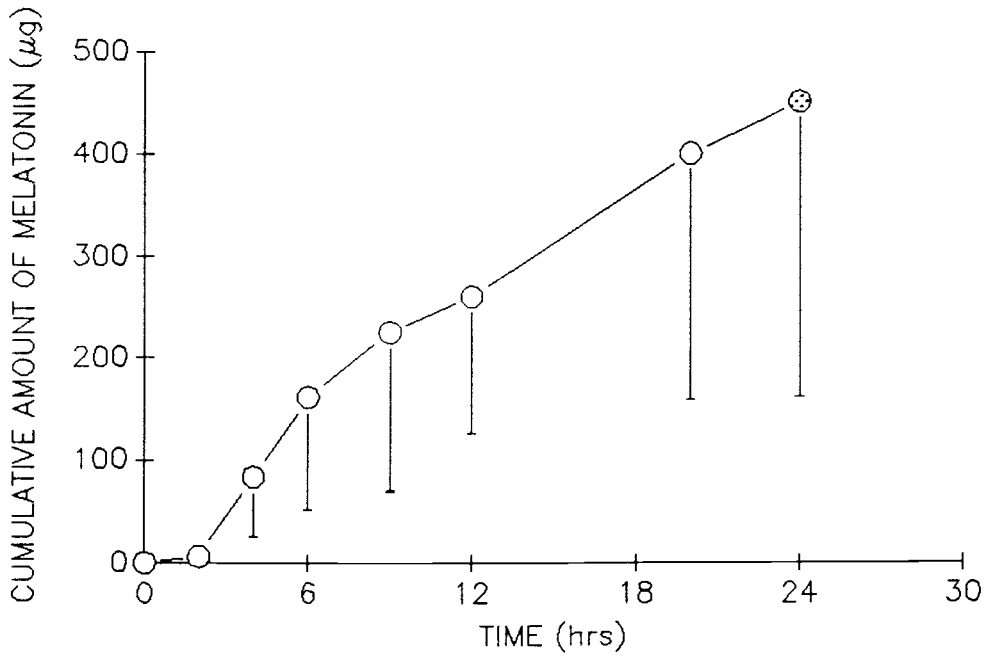


Fig. III.2 Individual diffusion profile of melatonin through hairless mouse skin from 20% propylene glycol/pH 6.1 buffer solution.

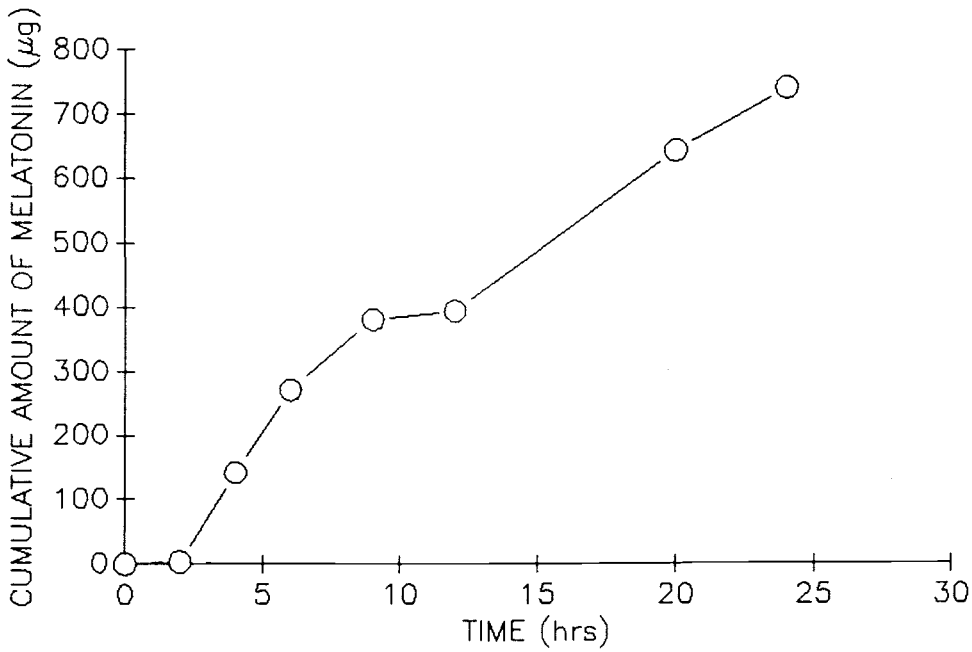


Fig. III.3 Individual diffusion profile of melatonin through hairless mouse skin from 20% propylene glycol/pH 6.1 buffer solution.

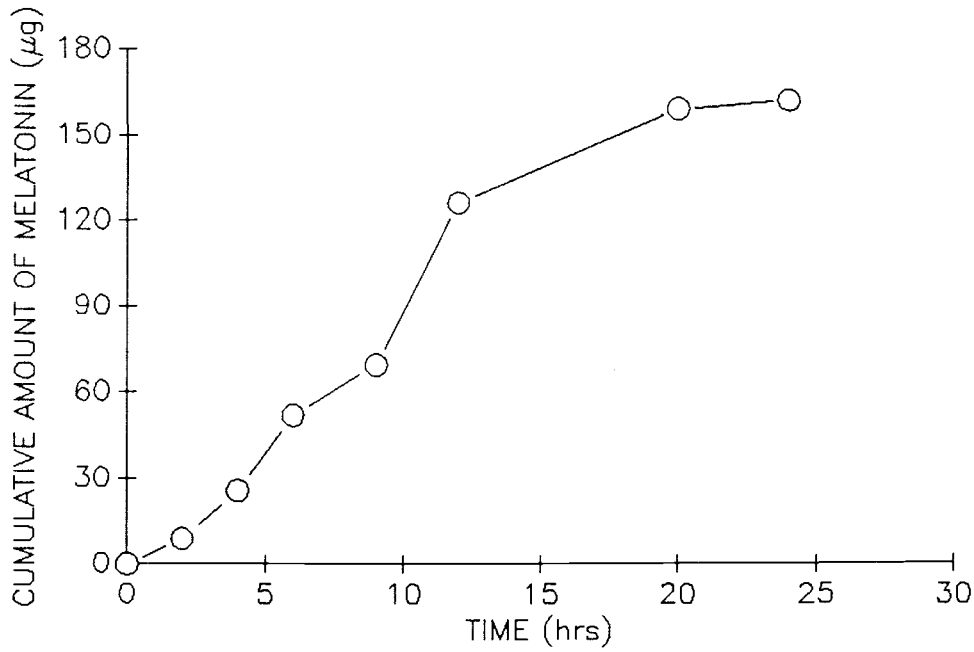


Fig. III.4 Mean diffusion profile of melatonin through hairless mouse skin from 40% propylene glycol/pH 6.1 buffer solution.

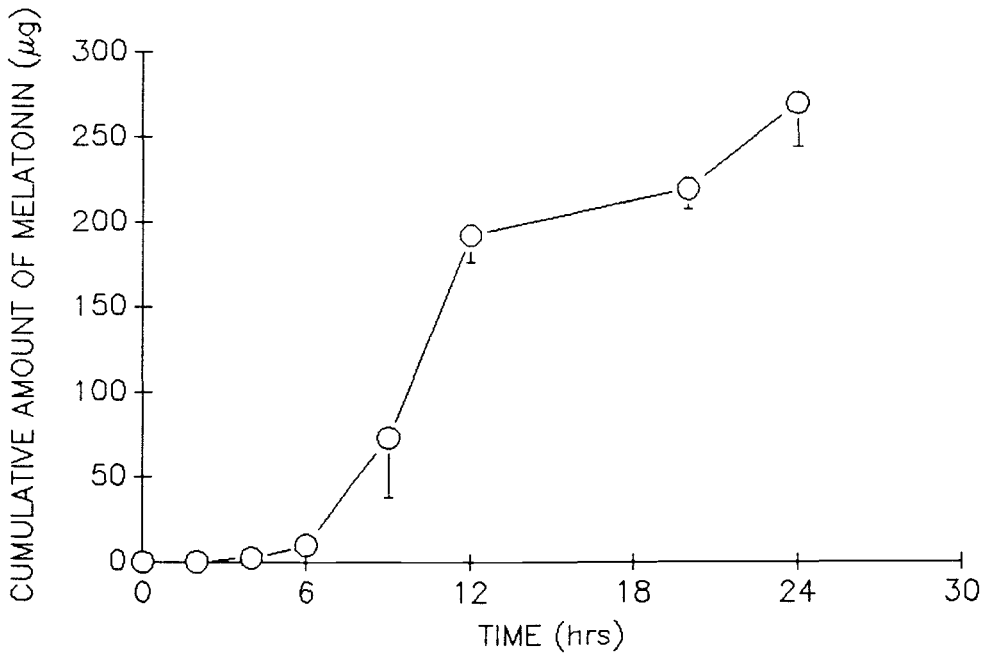


Fig. III.5 Individual diffusion profile of melatonin through hairless mouse skin from 40% propylene glycol/pH 6.1 buffer solution.

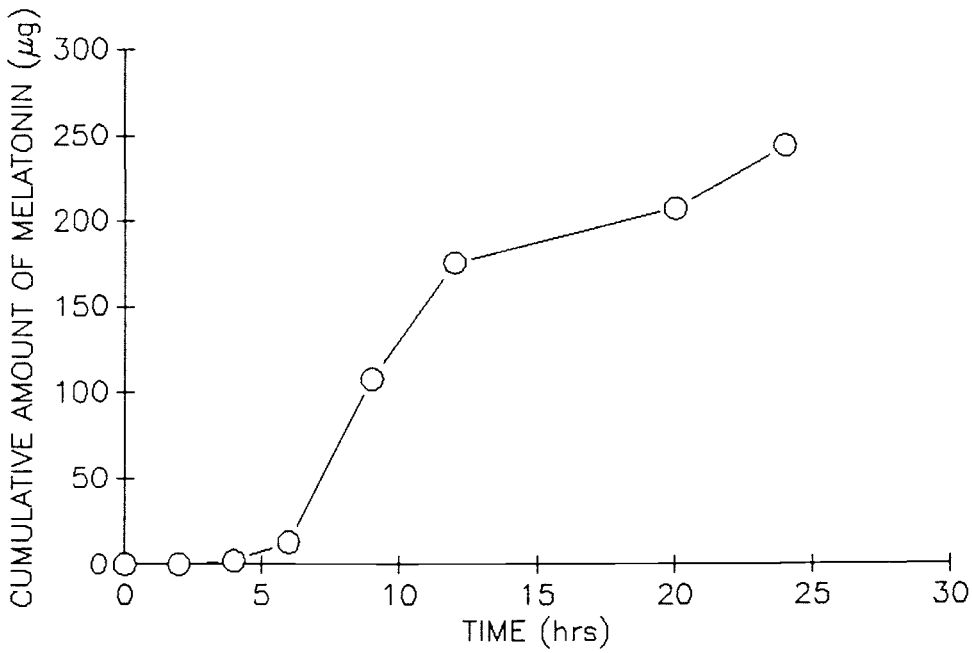


Fig. III.6 Individual diffusion profile of melatonin through hairless mouse skin from 40% propylene glycol/pH 6.1 buffer solution.

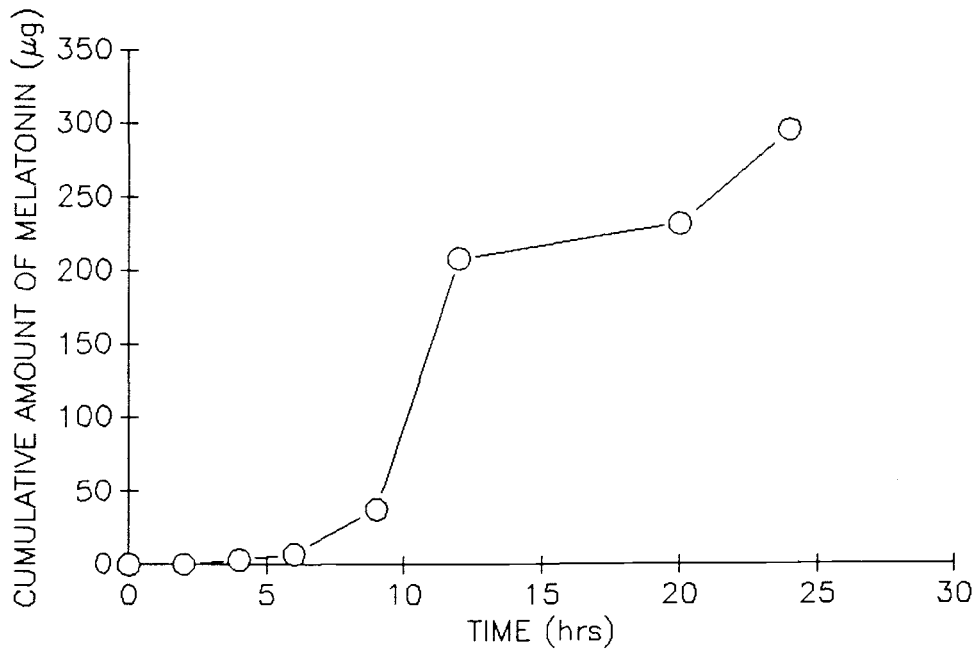


Fig. III.7 Mean diffusion profile of melatonin through hairless mouse skin from 65% propylene glycol/pH 6.1 buffer solution.

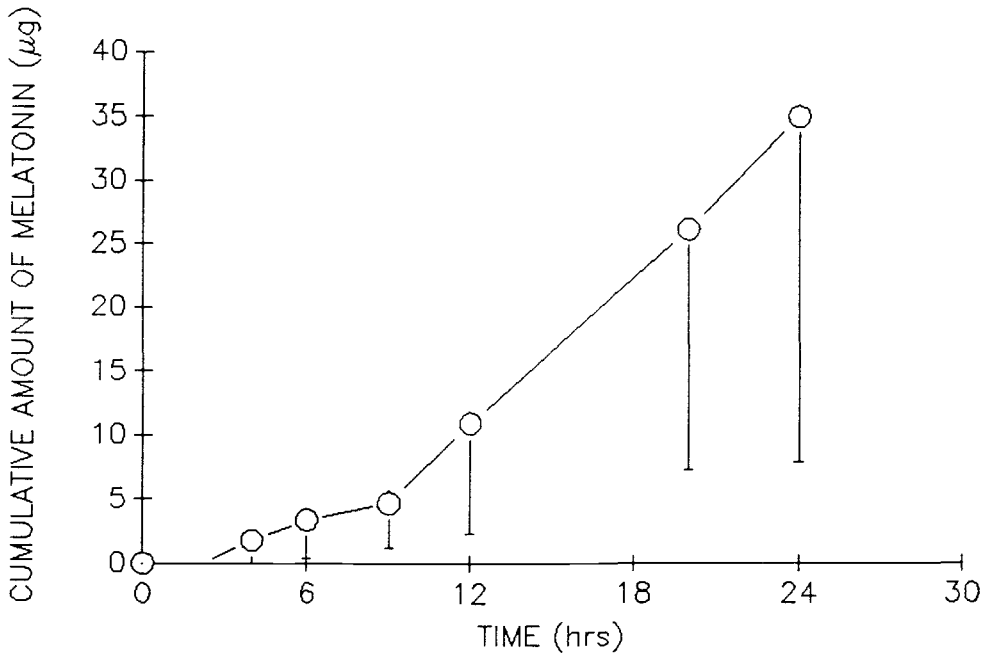


Fig. III.8 Individual diffusion profile of melatonin through hairless mouse skin from 65% propylene glycol/pH 6.1 buffer solution.

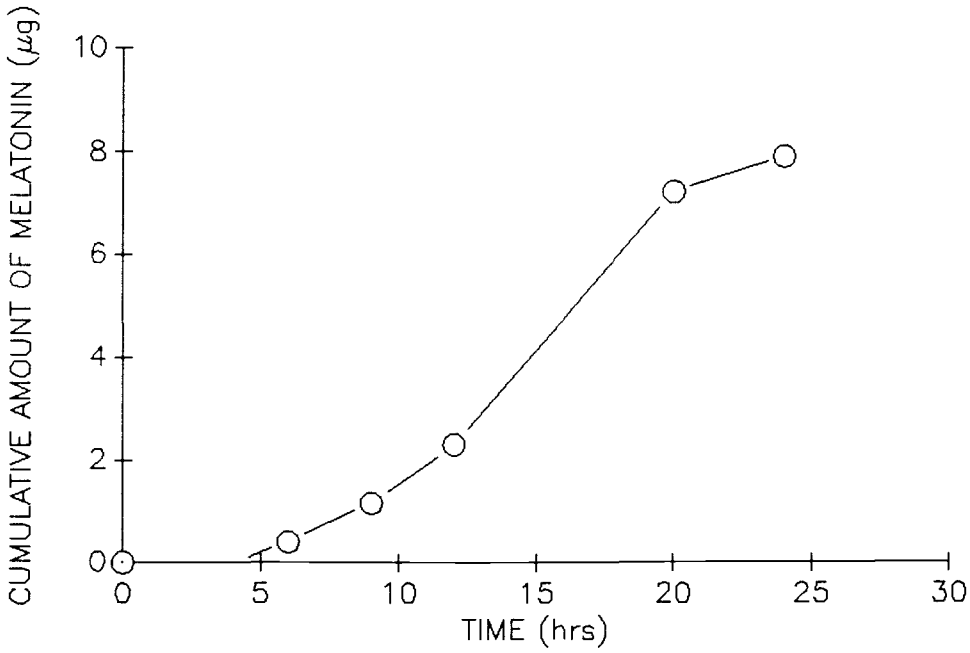


Fig. III.9 Individual diffusion profile of melatonin through hairless mouse skin from 65% propylene glycol/pH 6.1 buffer solution.

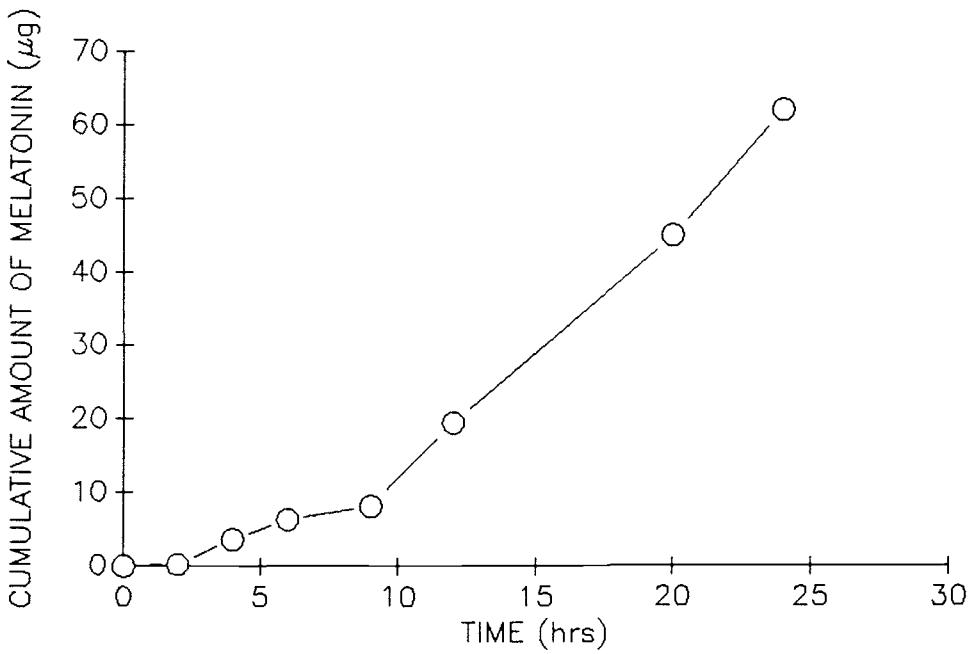


Fig. III.10 Mean diffusion profile of melatonin through hairless mouse skin from gel prepared with 20% propylene glycol/pH 6.1 buffer solution.

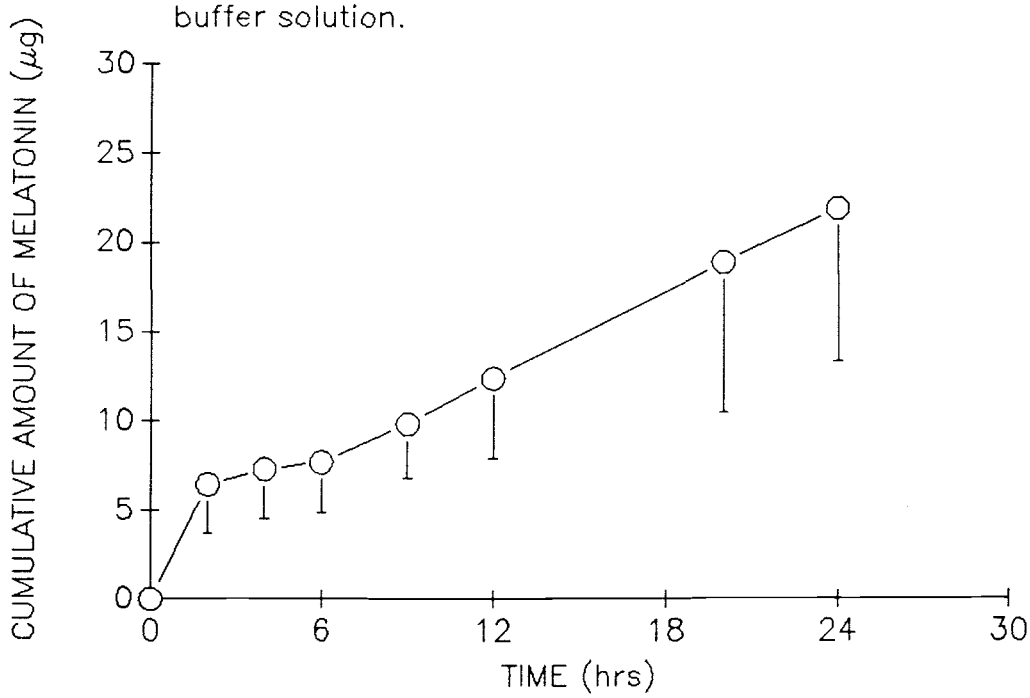


Fig. III.11 Individual diffusion profile of melatonin through hairless mouse skin from gel prepared with 20% propylene glycol/pH 6.1 buffer solution.

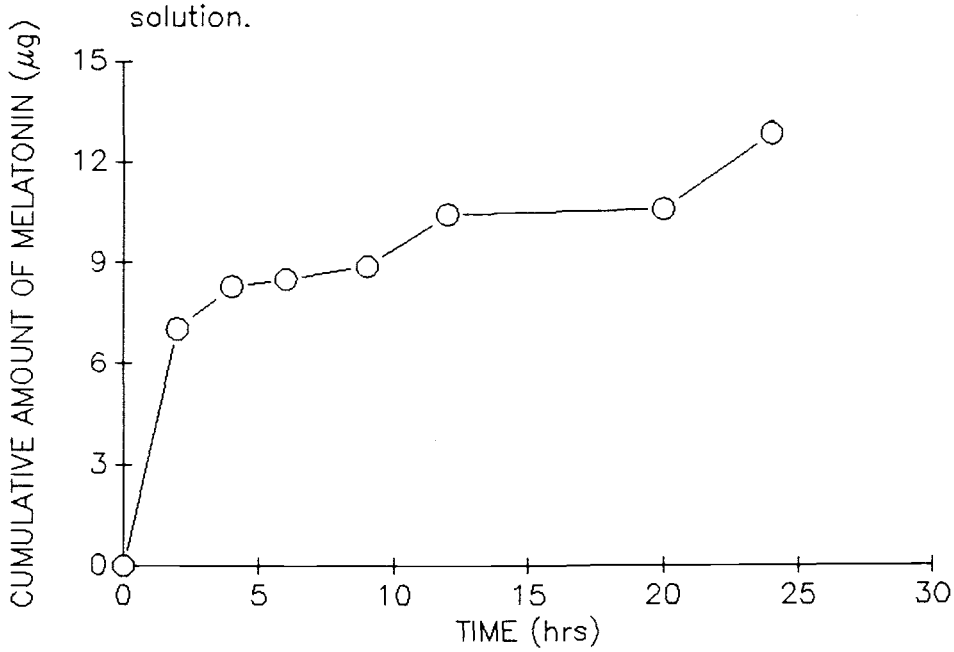


Fig. III.12 Individual diffusion profile of melatonin through hairless mouse skin from gel prepared with 20% propylene glycol/pH 6.1 buffer solution.

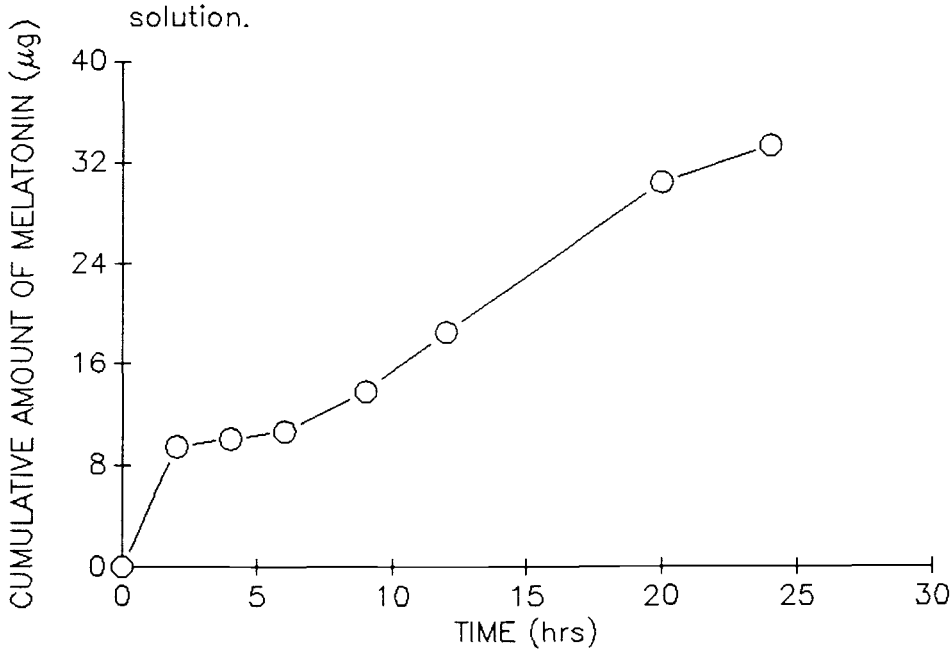


Fig. III.13 individual diffusion profile of melatonin through hairless mouse skin from gel prepared with 20% propylene glycol/pH 6.1 buffer solution.

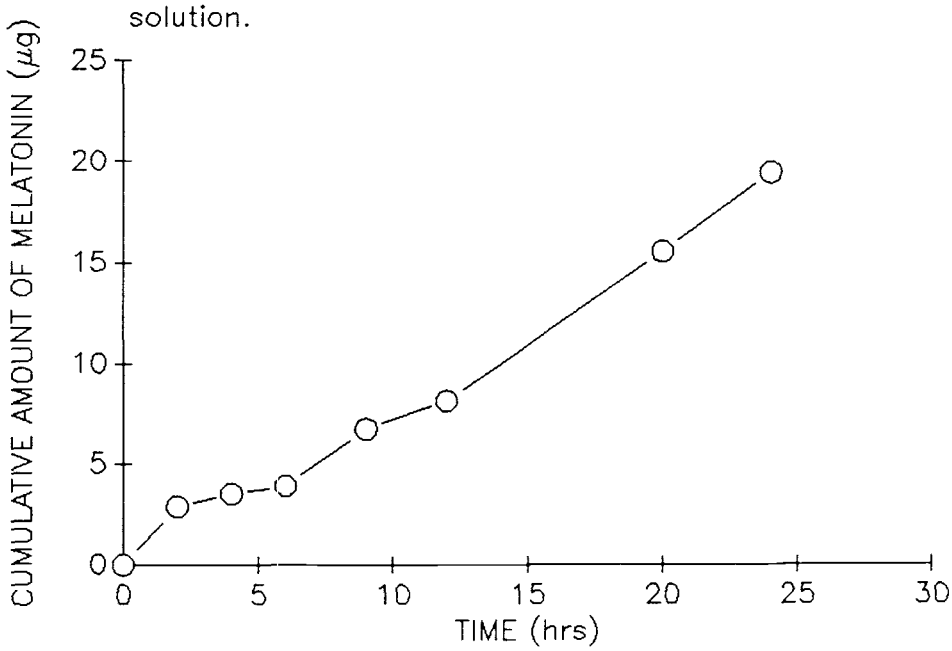


Fig. III.14 Mean diffusion profile of melatonin through hairless mouse skin from gel prepared with 40% propylene glycol/pH 6.1 buffer solution.

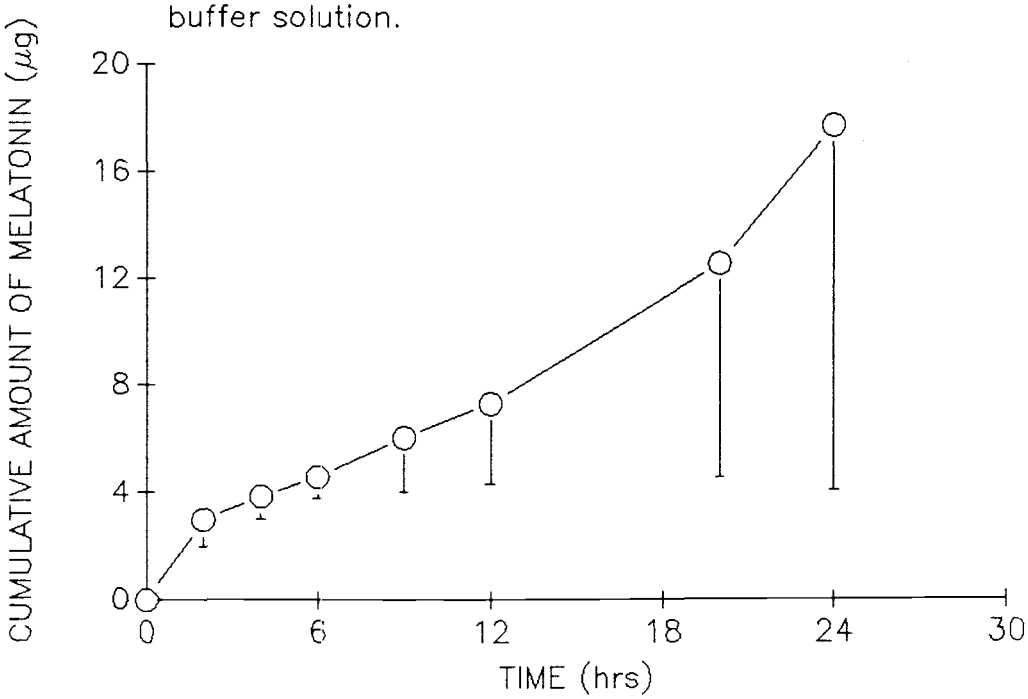


Fig. III.15 individual diffusion profile of melatonin through hairless mouse skin from gel prepared with 40% propylene glycol/pH 6.1 buffer solution.

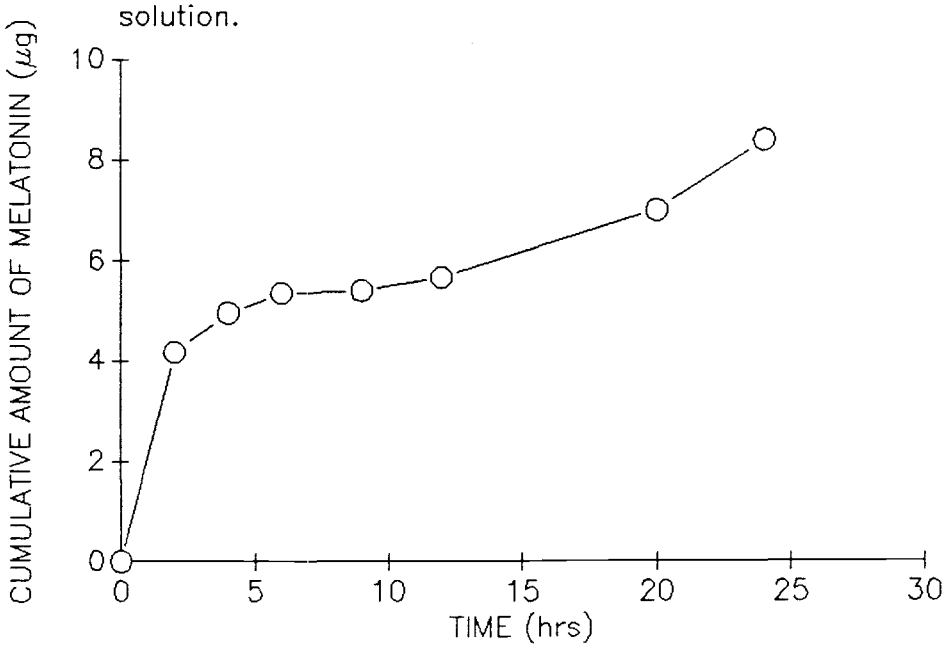


Fig. III.16 Individual diffusion profile of melatonin through hairless mouse skin from gel prepared with 40% propylene glycol/pH 6.1 buffer solution.

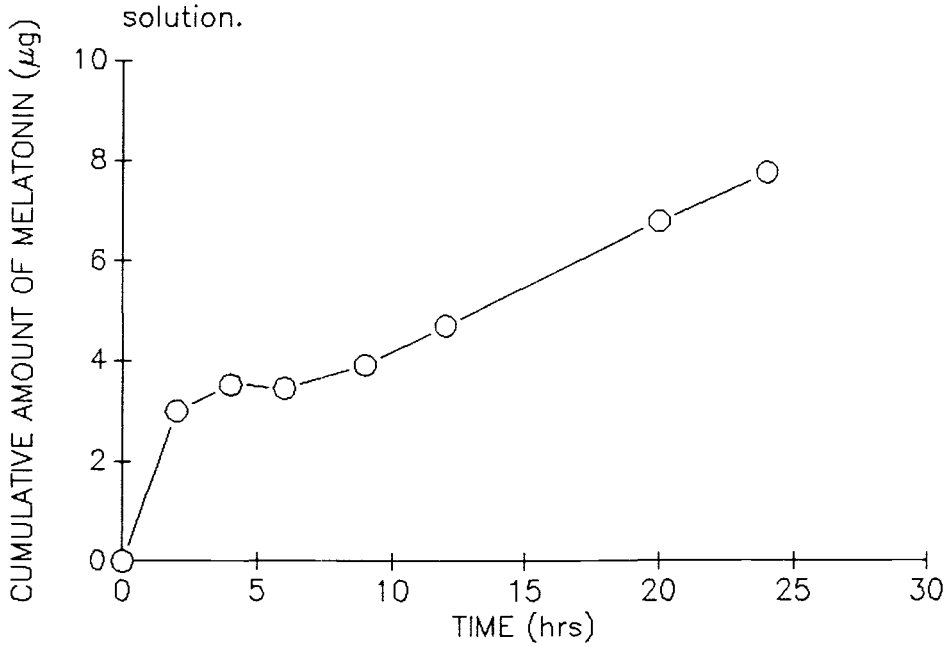


Fig. III.17 Individual diffusion profile of melatonin through hairless mouse skin from gel prepared with 40% propylene glycol/pH 6.1 buffer solution.

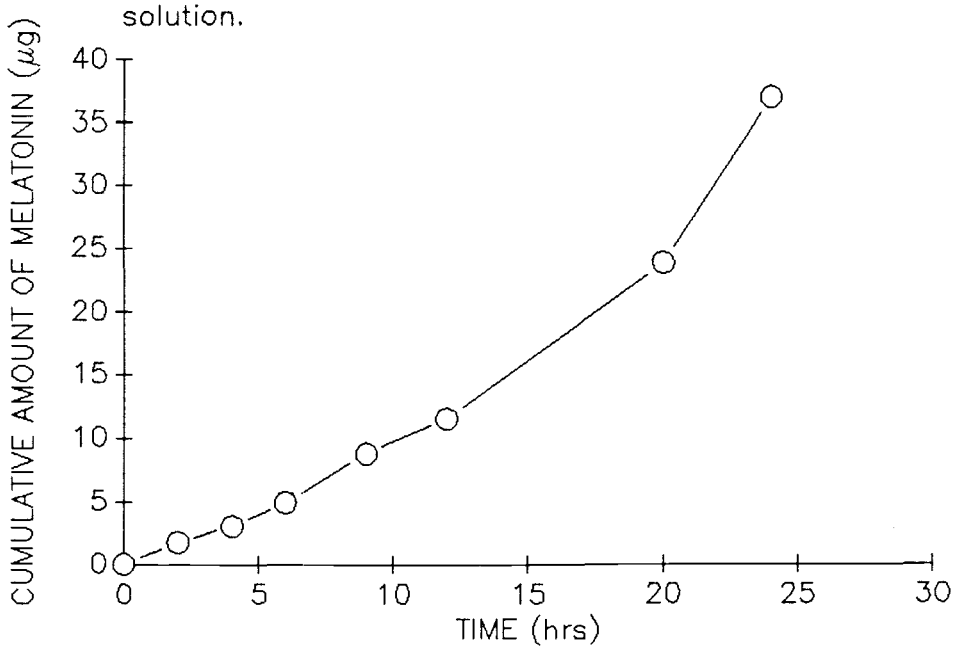


Fig. III.18 Individual diffusion profile of melatonin through hairless mouse skin from gel prepared with 65% propylene glycol/pH 6.1 buffer solution.

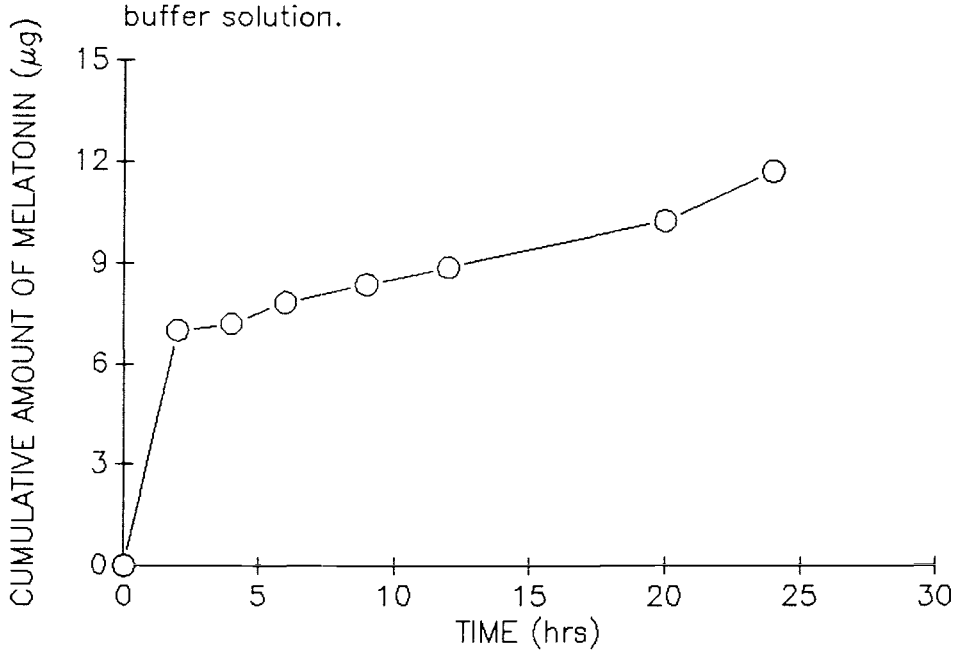


Fig. III.19 Individual diffusion profile of melatonin through hairless mouse skin (which was injured by mice fighting each other) from gel prepared with 65% propylene glycol/pH 6.1 buffer solution.

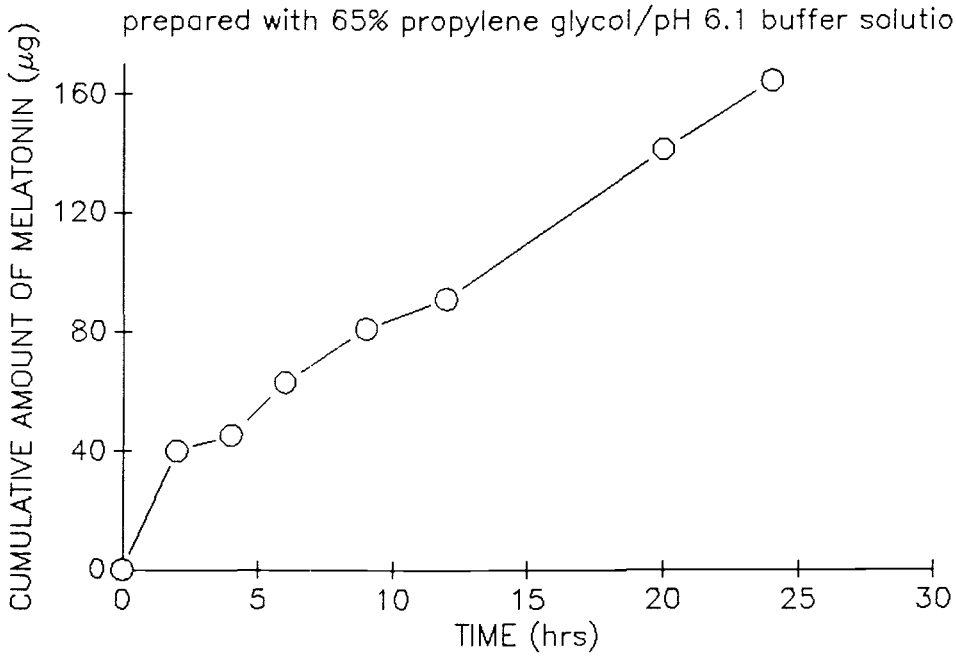
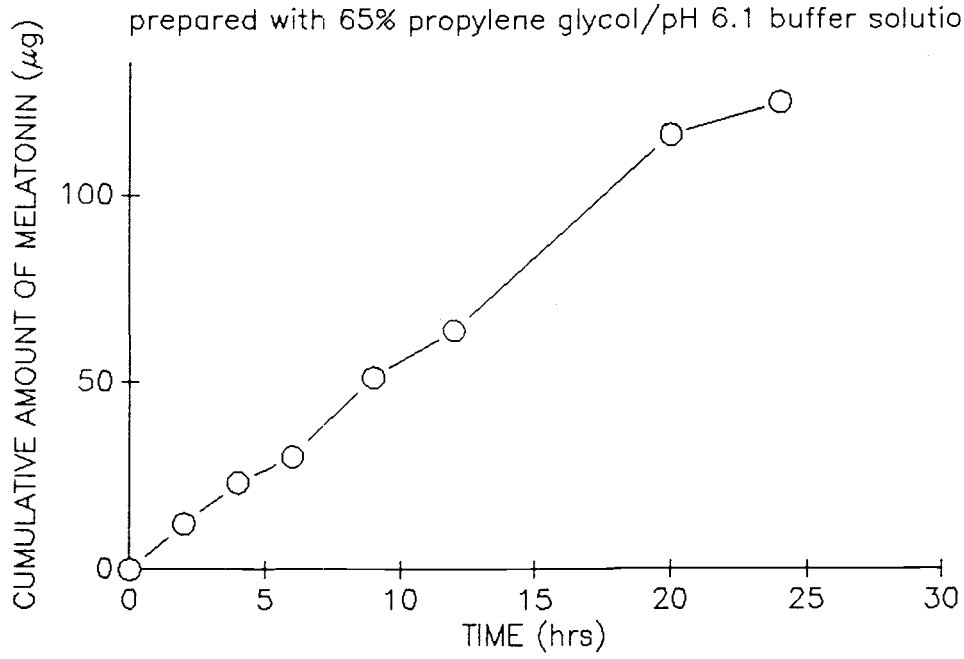


Fig. III.20 Individual diffusion profile of melatonin through hairless mouse skin (which was injured by mice fighting each other) from gel prepared with 65% propylene glycol/pH 6.1 buffer solution.



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